# METHOD 531.1

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

**Revision 3.0**


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## 1.0 SCOPE AND APPLICATION

1.1 This is a high performance liquid chromatographic (HPLC) method applicable to the determinations of certain N-methylcarbamoyloximes and N-methylcarbamates in ground water and finished drinking water[1]. The following compounds can be determined using this method:

| Analyte | Chemical Abstract Services Registry Number |
|---|---|
| Aldicarb | 116-06-3 |
| Aldicarb sulfone | 1646-88-4 |
| Aldicarb sulfoxide | 1646-87-3 |
| Baygon | 114-26-1 |
| Carbaryl | 63-25-2 |
| Carbofuran | 1563-66-2 |
| 3-Hydroxycarbofuran | 16655-82-6 |
| Methiocarb | 2032-65-7 |
| Methomyl | 16752-77-5 |
| Oxamyl | 23135-22-0 |

1.2 This method has been validated in a single laboratory and estimated detection limits (EDLs) have been determined for the analytes above (Section 12.0). Observed detection limits may vary between ground waters, depending upon the nature of interferences in the sample matrix and the specific instrumentation used.

1.3 This method is restricted to use by or under the supervision of analysts experienced in the use of liquid chromatography and in the interpretation of liquid chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 10.3.

1.4 When this method is used to analyze unfamiliar samples for any or all of the analytes above, analyte identifications should be confirmed by at least one additional qualitative technique.

## 2.0 SUMMARY OF METHOD

2.1 The water sample is filtered and a 400 µL aliquot is injected into a reverse phase HPLC column. Separation of the analytes is achieved using gradient elution chromatography. After elution from the HPLC column, the analytes are hydrolyzed with 0.05 N sodium hydroxide (NaOH) at 95°C. The methyl amine formed during hydrolysis is reacted with o-phthalaldehyde (OPA) and 2-mercaptoethanol to form a highly fluorescent derivative which is detected by a fluorescence detector[2].

## 3.0 DEFINITIONS

3.1 Internal Standard -- A pure analyte(s) added to a solution in known amount(s) and used to measure the relative responses of other method analytes and surrogates that are components of the same solution. The internal standard must be an analyte that is not a sample component.

3.2 Surrogate Analyte -- A pure analyte(s), which is extremely unlikely to be found in any sample, and which is added to a sample aliquot in known amount(s) before extraction and is measured with the same procedures used to measure other sample components. The purpose of a surrogate analyte is to monitor method performance with each sample.

3.3 Laboratory Duplicates (LD1 and LD2) -- Two sample aliquots taken in the analytical laboratory and analyzed separately with identical procedures. Analyses of LD1 and LD2 give a measure of the precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.

3.4 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 with laboratory procedures.

3.5 Laboratory Reagent Blank (LRB) -- An aliquot of reagent water that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.6 Field Reagent Blank (FRB) -- Reagent water placed in a sample container in the laboratory and treated as a sample in all respects, including exposure to sampling site conditions, storage, preservation and all analytical procedures. The purpose of the FRB is to determine if method analytes or other interferences are present in the field environment.

3.7 Laboratory Performance Check Solution (LPC) -- A solution of method analytes, surrogate compounds, and internal standards used to evaluate the performance of the instrument system with respect to a defined set of method criteria.

3.8 Laboratory Fortified Blank (LFB) -- An aliquot of reagent water to which known quantities of the method analytes 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 at the required method detection limit.

3.9 Laboratory Fortified Sample Matrix (LFM) -- An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is 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 LFM corrected for background concentrations.

3.10 Stock Standard Solution -- A concentrated solution containing a single certified standard that is a method analyte, or a concentrated solution of a single analyte prepared in the laboratory with an assayed reference compound. Stock standard solutions are used to prepare primary dilution standards.

3.11 Primary Dilution Standard Solution -- A solution of several analytes prepared in the laboratory from stock standard solutions and diluted as needed to prepare calibration solutions and other needed analyte solutions.

3.12 Calibration Standard (CAL) -- A solution prepared from the primary dilution standard solution and stock standard solutions of the internal standards and surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.13 Quality Control Sample (QCS) -- A sample matrix containing method analytes or a solution of method analytes in a water miscible solvent which is used to fortify reagent water or environmental samples. The QCS is obtained from a source external to the laboratory, and is used to check laboratory performance with externally prepared test materials.

## 4.0 INTERFERENCES

4.1 Method interferences may be caused by contaminants in solvents, reagents, glassware and other sample processing apparatus that lead to discrete artifacts or elevated baselines in liquid chromatograms. Specific sources of contamination have not been identified. All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 10.2.

4.1.1 Glassware must be scrupulously cleaned.[2] Clean all glassware as soon as possible after use by thoroughly rinsing with the last solvent used in it. Follow by washing with hot water and detergent and thorough rinsing with tap and reagent water. Drain dry, and heat in an oven or muffle furnace at 450°C for one hour. Do not heat volumetric ware. Thermally stable materials might not be eliminated by this treatment. Thorough rinsing with acetone may be substituted for the heating. After drying and cooling, seal and store glassware in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

4.1.2 The use of high purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

WARNING: When a solvent is purified, stabilizers added by the manufacturer are removed, thus potentially making the solvent hazardous. Also, when a solvent is purified, preservatives added by the mamufacturer are removed, thus potentially reducing the shelf-life.

4.2 Interfering contamination may occur when a sample containing low concentrations of analytes is analyzed immediately following a sample containing relatively high concentrations of analytes. A preventive technique is between-sample rinsing of the sample syringe and filter holder with two portions of reagent water. After analysis of a sample containing high concentrations of analytes, one or more laboratory method blanks should be analyzed.

4.3 Matrix interference may be caused by contaminants that are present in the sample. The extent of matrix interference will vary considerably from source to source, depending upon the water sampled. Positive identifications must be confirmed.

## 5.0 SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound must be treated as a potential health hazard. Accordingly, exposure to these chemicals must be reduced to the lowest possible level. 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 material safety data sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified[4-6] for the information of the analyst.

WARNING: When a solvent is purified, stabilizers added by the manufacturer are removed, thus potentially making the solvent hazardous.

**6.0** **APPARATUS AND EQUIPMENT** (All specifications are suggested. Catalog numbers are included for illustration only.)

6.1 Sampling Equipment

6.1.1 Grab sample bottle -- 60 mL screw cap vials (Pierce No. 13075 or equivalent) and caps equipped with a PTFE-faced silicone septa (Pierce No. 12722 or equivalent). Prior to use, wash vials and septa as described in Section 3.1.1.

6.2 Balance -- Analytical, capable of accurately weighing to the nearest 0.0001 g.

6.3 Filtration Apparatus

6.3.1 Macrofiltration -- To filter derivatization solutions and mobile phases used in HPLC. Recommend using 47 mm filters (Millipore Type HA, 0.45 µm for water and Millipore Type FH, 0.5 µm for organics or equivalent).

6.3.2 Microfiltration -- To filter samples prior to HPLC analysis. Use 13 mm filter holder (Millipore stainless steel XX300/200 or equivalent), and 13 mm diameter 0.2 µm polyester filters (Nuclepore 180406 or equivalent).

6.4 Syringes and Syringe Valves

6.4.1 Hypodermic syringe -- 10 mL glass, with Luer-Lok tip.

6.4.2 Syringe valve -- Three-way (Hamilton HV3-3 or equivalent).

6.4.3 Syringe needle -- 7-10 cm long, l7-gauge, blunt tip.

6.4.4 Micro syringes -- Various sizes.

6.5 Miscellaneous

6.5.1 Solution storage bottles -- Amber glass, 10-15 mL capacity with TFE-fluorocarbon-lined screw cap.

6.5.2 Helium -- For degassing solutions and solvents.

6.6 High Performance Liquid Chromatograph (HPLC)

6.6.1 HPLC system capable of injecting 200-400 µL aliquots, and performing binary linear gradients at a constant flow rate. A data system is recommended for measuring peak areas. Table 1 lists retention times observed for method analytes using the columns and analytical conditions described below.

6.6.2 Column 1 (primary column) -- 150 mm long x 3.9 mm I.D. stainless steel packed with 4 µm NovaPak C18. Mobil Phase is established at 10:90 methanol: water, hold two minutes, then program as a linear gradient to 80:20 methanol: water in 25 minutes. Alternative columns may be used in accordance with the provisions described in Section 10.4.

6.6.3 Column 2 (alternative column)* -- 250 mm long x 4.6 mm I.D. stainless steel packed with 5 µm Beckman Ultrasphere ODS. Mobile phase is established at 1.0 mL/min as a linear gradient from 15:85 methanol: water to methanol in 32 minutes. Data presented in this method were obtained using this column. *Newer manufactured columns have not been able to resolve aldicarb sulfone from oxamyl.

6.6.4 Column 3 (alternative column) -- 250 mm long x 4.6 mm I.D. stainless steel packed with 5 µm Supelco LC-1. Mobile phase is established at 1.0 mL/min as a linear gradient from 15:85 methanol: water to methanol in 32 minutes.

6.6.5 Post column reactor -- Capable of mixing reagents into the mobile phase. Reactor should be constructed using PTFE tubing and equipped with pumps to deliver 0.1-1.0 mL/min of each reagent; mixing tees; and two 1.0 mL delay coils, one thermostated at 95°C (ABI URS 051 and URA 100 or equivalent).

6.6.6 Fluorescence detector -- Capable of excitation at 330 nm (nominal) and detection of emission energies greater than 418 nm. A Schoffel Model 970 fluorescence detector was used to generate the validation data presented in this method.

## 7.0 REAGENTS AND CONSUMABLE MATERIALS

WARNING: When a solvent is purified, stabilizers added by the manufacturer are removed, thus potentially making the solvent hazardous. Also, when a solvent is purified, preservatives added by the manufacturer are removed, thus potentially reducing the shelf-life.

7.1 Reagent Water -- Reagent water is defined as water that is reasonably free of contamination that would prevent the determination of any analyte of interest. Reagent water used to generate the validation data in this method was distilled water obtained from the Magnetic Springs Water Co., 1801 Lone Eagle St., Columbus, Ohio 43228.

7.2 Methanol -- Distilled-in-glass quality or equivalent.

7.3 HPLC Mobile Phase

7.3.1 Water -- HPLC grade (available from Burdick and Jackson).

7.3.2 Methanol -- HPLC grade. Filter and degas with helium before use.

7.4 Post-Column Derivatization Solutions

7.4.1 Sodium hydroxide, 0.05 N -- Dissolve 2.0 g of sodium hydroxide (NaOH) in reagent water. Dilute to 1.0 L with reagent water. Filter and degas with helium just before use.

7.4.2 2-Mercaptoethanol (1+1) -- Mix 10.0 mL of 2-mercapto-ethanol and 10.0 mL of acetonitrile. Cap. Store in hood.

CAUTION: Stench.

7.4.3 Sodium borate (0.05 N) -- Dissolve 19.1 g of sodium borate ($Na_2B_40_7 \cdot 10H_2O$) in reagent water. Dilute to 1.0 L with reagent water. The sodium borate will completely dissolve at room temperature if prepared a day before use.

7.4.4 OPA reaction solution -- Dissolve 100 ±10 mg of o-phthalaldehyde (mp 55-58°C) in 10 mL of methanol. Add to 1.0 L of 0.05 N sodium borate. Mix, filter, and degas with helium. Add 100 µL of 2-mercaptoethanol (1+1) and mix. Make up fresh solution daily.

7.5 Monochloroacetic Acid Buffer (pH3) -- Prepare by mixing 156 mL of 2.5 M monochloroacetic acid and 100 mL 2.5 M potassium acetate.

7.6 4-Bromo-3,5-Dimethylphenyl N-Methylcarbamate (BDMC) -- 98% purity, for use as internal standard (available from Aldrich Chemical Co.).

7.7 Stock Standard Solutions (1.00 µg/µL) -- Stock standard solutions may be purchased as certified solutions or prepared from pure standard materials using the following procedure:

7.7.1 Prepare stock standard solutions by accurately weighing approximately 0.0100 g of pure material. Dissolve the material in HPLC quality methanol and dilute to volume in a 10 mL volumetric flask. Larger volumes may be used at the convenience of the analyst. If compound purity is certified at 96% or greater, the weight may be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards may be used at any concentration if they are certified by the manufacturer or by an independent source.

7.7.2 Transfer the stock standard solutions into TFE-fluorocarbon-sealed screw cap vials. Store at room temperature and protect from light.

7.7.3 Stock standard solutions should be replaced after two months or sooner if comparison with laboratory fortified blanks, or QC samples indicate a problem.

7.8 Internal Standard Solution -- Prepare an internal standard fortification solution by accurately weighing approximately 0.0010 g of pure BDMC. Dissolve the BDMC in pesticide-quality methanol and dilute to volume in a 10-mL volumetric flask. Transfer the internal standard fortification solution to a TFE-fluorocarbon-sealed screw cap bottle and store at room temperature. Addition of 5 µL of the internal standard fortification solution to 50 mL of sample results in a final internal standard concentration of 10 µg/L. Solution should be replaced when ongoing QC (Section 10.0) indicates a problem.

NOTE: BDMC has been shown to be an effective internal standard for the method analytes[1], but other compounds may be used, if the quality control requirements in Section 9.0 are met.

7.9 Laboratory Performance Check Solution -- Prepare concentrate by adding 20 µL of the 3-hydroxycarbofuran stock standard solution, 1.0 mL of the aldicarb sulfoxide stock standard solution, 200 µL of the methiocarb stock standard solution, and 1 mL of the internal standard fortification solution to a 10 mL volumetric flask. Dilute to volume with methanol. Thoroughly mix concentrate. Prepare check solution by placing 100 µL of the concentrate solution into a 100 mL volumetric flask. Dilute to volume with buffered reagent water. Transfer to a TFE-fluorocarbon-sealed screw cap bottle and store at room temperature. Solution should be replaced when ongoing QC (Section 10.0) indicates a problem.

## 8.0 SAMPLE COLLECTION, PRESERVATION AND HANDLING

8.1 Grab samples must be collected in glass containers. Conventional sampling practices[8] should be followed; however, the bottle must not be prerinsed with sample before collection.

8.2 Sample Preservation/ph Adjustment -- Oxamyl, 3-hydroxycarbofuran, aldicarb sulfoxide, and carbaryl can all degrade quickly in neutral and basic waters held at room temperature.[6,7] This short-term degradation is of concern during the time samples are being shipped and the time processed samples are held at room temperature in autosampler trays. Samples targeted for the analysis of these three analytes must be preserved at pH 3. The pH adjustment also minimizes analyte biodegradation.

8.2.1 Add 1.8 mL of monochloroacetic acid buffer to the 60 mL sample bottle. Add buffer to the sample bottle at the sampling site or in the laboratory before shipping to the sampling site.

8.2.2 If residual chlorine is present, add 80 mg of sodium thiosulfate per liter of sample to the sample bottle prior to collecting the sample.

8.2.3 After sample is collected in bottle containing buffer, seal the sample bottle and shake vigorously for one minute.

8.2.4 Samples must be iced or refrigerated at 4°C from the time of collection until storage. Samples must be stored at -10°C until analyzed. Preservation study results indicate that method analytes are stable in water samples for at least 28 days when adjusted to pH 3 and stored at -10°C. However, analyte stability may be effected by the matrix; therefore, the analyst should verify that the preservation technique is applicable to the samples under study.

## 9.0 CALIBRATION

9.1 Establish HPLC operating parameters equivalent to those indicated in Section 6.6. The HPLC system may be calibrated using either the internal standard technique (Section 9.2) or the external standard technique (Section 9.3).

9.2 Internal Standard Calibration Procedure -- The analyst must select one or more internal standards similar in analytical behavior to the analytes of interest. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. BDMC has been identified as a suitable internal standard.

9.2.1 Prepare calibration standards at a minimum of three (recommend five) concentration levels for each analyte of interest by adding volumes of one or of the more stock standards to a volumetric flask. To each calibration standard, add a known constant amount of one or more internal standards, and dilute to volume with buffered reagent water. To prepare buffered reagent water, add 10 mL of 1.0 M monochloroacetic acid buffer to 1 L of reagent water. The lowest standard should represent analyte concentrations near, but above, their respective EDLs. The remaining standards should bracket the analyte concentrations expected in the sample extracts, or should define the working range of the detector.

9.2.2 Analyze each calibration standard according to the procedure (Section 11.2). Tabulate peak height or area responses against concentration for each compound and internal standard. Calculate response factors (RF) for each analyte, surrogate and internal standard using the following equation.

$$RF = \frac{(A_s)\ (C_{is})}{(A_{is})\ (C_s)}$$

where: $A_s$ = Response for the analyte to be measured.
$A_{is}$ = Response for the internal standard.
$C_{is}$ = Concentration of the internal standard µg/L).
$C_s$ = Concentration of the analyte to be measured µg/L).

9.2.3 If the RF value over the working range is constant (20% RSD or less) the average RF can be used for calculations. Alternatively, the results can be used to plot a calibration curve of response ratios ($As/A_{is}$) vs. $C_s$.

9.2.4 The working calibration curve or RF must be verified on each working shift by the measurement of one or more calibration standards. If the response for any analyte varies from the predicted response by more than ±20%, the test must be repeated using a fresh calibration standard. If the repetition also fails, a new calibration curve must be generated for that analyte using freshly prepared standards.

9.2.5 Single-point calibration is a viable alternative to a calibration curve. Prepare single point standards from the secondary dilution standards. The single point standards should be prepared at a concentration that deviates from the sample extract response by no more than 20%.

9.2.6 Verify calibration standards periodically, recommend at least quarterly, by analyzing a standard prepared from reference material obtained from an independent source. Results from these analyses must be within the limits used to routinely check calibration.

9.3 External Standard Calibration Procedure

9.3.1 Prepare calibration standards at a minimum of three (recommend five) concentration levels for each analyte of interest by adding volumes of one or more stock standards to a volumetric flask. Dilute to volume with buffered reagent water. The lowest standard should represent analyte concentrations near, but above, the respective EDLs. The remaining standards should bracket the analyte concentrations expected in the sample extracts, or should define the working range of the detector.

9.3.2 Starting with the standard of lowest concentration, analyze each calibration standard according to Section 11.2 and tabulate responses (peak height or area) versus the concentration in the standard. The results can be used to prepare a calibration curve for each compound. Alternatively, if the ratio of response to concentration (calibration factor) is a constant over the working range <20% relative standard

deviation), linearity through the origin can be assumed and the average ratio or calibration factor can be used in place of a calibration curve.

9.3.3 The working calibration curve or calibration factor must be verified on each working day by the measurement of a minimum of two calibration check standards, one at the beginning and one at the end of the analysis day. These check standards should be at two different concentration levels to verify the concentration curve. For extended periods of analysis (greater than eight hours), it is strongly recommended that check standards be interspersed with samples at regular intervals during the course of the analyses. If the response for any analyte varies from the predicted response by more than ±20%, the test must be repeated using a fresh calibration standard. If the results still do not agree, generate a new calibration curve or use a single point calibration standard as described in Section 9.3.3.

9.3.4 Single-point calibration is a viable alternative to a calibration curve. Prepare single point standards from the secondary dilution standards. The single point standards should be prepared at a concentration that deviates from the sample extract response by no more than 20%.

9.3.5 Verify calibration standards periodically, recommend at least quarterly, by analyzing a standard prepared from reference material obtained from an independent source. Results from these analyses must be within the limits used to routinely check calibration.

## 10.0 QUALITY CONTROL

10.1 Minimum quality control (QC) requirements are initial demonstration of laboratory capability, monitoring internal standard peak area or height in each sample and blank (when internal standard calibration procedures are being employed), analysis of laboratory reagent blanks, laboratory fortified samples, laboratory fortified blanks and QC samples.

10.2 Laboratory Reagent Blanks (LRB) -- Before processing any samples, the analyst must demonstrate that all glassware and reagent interferences are under control. Each time a set of samples is extracted or reagents are changed, a LRB must be analyzed. If within the retention time window of any analyte of interest 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.

10.3 Initial Demonstration of Capability

10.3.1 Select a representative concentration (about 10 times EDL) for each analyte. Prepare a sample concentrate (in methanol) containing each analyte at 1000 times selected concentration. With a syringe, add 50 µL of the concentrate to each of at least four 50 mL aliquots of reagent

water, and analyze each aliquot according to procedures beginning in Section 11.0.

10.3.2 For each analyte the recovery value for all four of these samples must fall in the range of R ±30% (or within R $\pm 3S_R$ if broader) using the values for R and $S_R$ for reagent water in Table 2. For those compounds that meet the acceptance criteria, performance is judged acceptable and sample analysis may begin. For those compounds that fail these criteria, this procedure must be repeated using four fresh samples until satisfactory performance has been demonstrated.

10.3.3 The initial demonstration of capability is used primarily to preclude a laboratory from analyzing unknown samples via a new, unfamiliar method prior to obtaining some experience with it. It is expected that as laboratory personnel gain experience with this method the quality of data will improve beyond those required here.

10.4 The analyst is permitted to modify HPLC columns, HPLC conditions, internal standards or detectors to improve separations or lower analytical costs. Each time such method modifications are made, the analyst must repeat the procedures in Section 10.3.

10.5 Assessing the Internal Standard

10.5.1 When using the internal standard calibration procedure, the analyst is expected to monitor the IS response (peak area or peak height) of all samples during each analysis day. The IS response for any sample chromatogram should not deviate from the daily calibration check standard's IS response by more than 30%.

10.5.2 If >30% deviation occurs with an individual extract, optimize instrument performance and inject a second aliquot.

10.5.2.1 If the reinjected aliquot produces an acceptable internal standard response, report results for that aliquot.

10.5.2.2 If a deviation of greater than 30% is obtained for the reinjected extract, analysis of the sample should be repeated beginning with Section 11.0, provided the samples is still available. Otherwise, report results obtained from the reinjected extract, but annotate as suspect.

10.5.3 If consecutive samples fail the IS response acceptance criterion, immediately analyze a calibration check standard.

10.5.3.1 If the check standard provides a response factor (RF) within 20% of the predicted value, then follow procedures itemized in Section 10.5.2 for each sample failing the IS response criterion.

10.5.3.2 If the check standard provides a response factor which deviates more than 20% of the predicted value, then the analyst must recalibrate, as specified in Section 9.0.

10.6 Assessing Laboratory Performance -- Laboratory Fortified Blanks (LFB)

10.6.1 The laboratory must analyze at least one LFB sample with every 20 samples or one per sample set (all samples analyzed within a 24-hour period) whichever is greater. The fortification concentration of each analyte in the LFB should be 10 times EDL or the MCL, whichever is less. Calculate accuracy as percent recovery ($X_i$). If the recovery of any analyte falls outside the control limits (see Section 10.7.2), that analyte is judged out of control, and the source of the problem must be identified and resolved before continuing analyses.

10.6.2 Until sufficient data become available from within their own laboratory, usually a minimum of results from 20-30 analyses, the laboratory should assess laboratory performance against the control limits in Section 10.3.2 that are derived from the data in Table 2. When sufficient internal performance data becomes available, develop control limits from the mean percent recovery ($\bar{X}$) and standard deviation (S) of the percent recovery. These data are used to establish upper and lower control limits as follows:

UPPER CONTROL LIMIT = $\bar{X}$ + 3S
LOWER CONTROL LIMIT = $\bar{X}$ - 3S

After each 5-10 new recovery measurements, new control limits should be calculated using only the most recent 20-30 data points. These calculated control limits should never exceed those established in Section 10.3.2.

10.6.3 It is recommended that the laboratory periodically determine and document its detection limit capabilities for analytes of interest.

10.6.4 At least quarterly, analyze a QC sample from an outside source.

10.6.5 Laboratories are encouraged to participate in external performance evaluation studies such as the laboratory certification programs offered by many states or the studies conducted by U.S. EPA. Performance evaluation studies serve as independent checks on the analyst's performance.

10.7 Assessing Analyte Recovery -- Laboratory Fortified Sample Matrix

10.7.1 The laboratory must add a known concentration to a minimum of 5% of the routine samples or one sample concentration per set, whichever is greater. The concentration should not be less then the background concentration of the sample selected for fortification. Ideally, the concentration should be the same as that used for the laboratory fortified blank (Section 10.6). Over time, samples from all routine sample sources should be fortified.

10.7.2 Calculate the percent recovery, P of the concentration for each analyte, after correcting the analytical result, X, from the fortified sample for the background concentration, b, measured in the unfortified sample, i.e.,:

$P = 100 (X - b) /$ fortifying concentration,

and compare these values to control limits appropriate for reagent water data collected in the same fashion. If the analyzed unfortified sample is found to contain **NO** background concentrations, and the added concentrations are those specified in Section 10.7, then the appropriate control limits would be the acceptance limits in Section 10.7. If, on the other hand, the analyzed unfortified sample is found to contain background concentration, b, estimate the standard deviation at the background concentration, $s_b$, using regressions or comparable background data and, similarly, estimate the mean, $\bar{X}_a$, and standard deviation, $s_a$, of analytical results at the total concentration after fortifying. Then the appropriate percentage control limits would be $\bar{P} \pm 3s_p$, where:

$\bar{P} = 100\bar{X} /$ (b + fortifying concentration)

and $s_p = 100 (s_a^2 + s_b^2)^{1/2} /$ fortifying concentration

For example, if the background concentration for Analyte A was found to be 1 µg/L and the added amount was also 1 µg/L, and upon analysis the laboratory fortified sample measured 1.6 µ/L, then the calculated P for this sample would be (1.6 µg/L minus 1.0 µg/L)/ 1 µg/L or 60%. This calculated P is compared to control limits derived from prior reagent water data. Assume it is known that analysis of an interference free sample at 1 µg/L yields an s of 0.12 µg/L and similar analysis at 2.0 µg/L yields $\bar{X}$ and s of 2.01 µg/L and 0.20 µg/L, respectively. The appropriate limits to judge the reasonableness of the percent recovery, 60%, obtained on the fortified matrix sample is computed as follows:

$[100\ (2.01\ \mu g/L) / 2.0\ \mu g/L]$

$\pm 3\ (100)\ [(0.12\ \mu g/L)^2 + (0.20\ \mu g/L)^2]^{1/2}/1.0\ \mu g/L =$

$100.5\% \pm 300\ (0.233) =$

$100.5\% \pm 70\%$ or 30% to 170 recovery of the added analyte.

10.7.3 If the recovery of any such analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control (Section 10.6), the recovery problem encountered with the dosed sample is judged to be matrix related, not system related. 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.

10.8 Assessing Instrument System -- Laboratory Performance Check Sample (LPC) - Instrument performance should be monitored on a daily basis by analysis of the LPC sample. The LPC sample contains compounds designed to indicate appropriate instrument sensitivity, column performance (primary column) and chromatographic performance. LPC sample components and performance criteria are listed in Table 3. Inability to demonstrate acceptable instrument performance indicates the need for reevaluation of the instrument system. The sensitivity requirements are set based on the EDLs published in this method. If laboratory EDLs differ from those listed in this method, concentrations of the instrument QC standard compounds must be adjusted to be compatible with the laboratory EDLs.

10.9 The laboratory may adopt additional quality control practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. For example, field or laboratory duplicates may be analyzed to assess the precision of the environmental measurements or field reagent blanks may be used to assess contamination of samples under site conditions, transportation and storage.

## 11.0 PROCEDURE

11.1 pH Adjustment and Filtration

11.1.1 Add preservative to any samples not previously preserved (Section 8.0). Adjust the pH of the sample or standard to pH 3 ±0.2 by adding 1.5 mL of 2.5 M monochloroacetic acid buffer to each 50 mL of sample. This step should not be necessary if sample pH was adjusted during sample collection as a preservation precaution. Fill a 50 mL volumetric flask to the mark with the sample. Add 5 µL of the internal standard fortification solution (if the internal standard calibration procedure is being employed) and mix by inverting the flask several times.

11.1.2 Affix the three-way valve to a 10 mL syringe. Place a clean filter in the filter holder and affix the filter holder and the 7-10 cm syringe needle to the syringe valve. Rinse the needle and syringe with reagent water. Prewet the filter by passing 5 mL of reagent water through the filter. Empty the syringe and check for leaks. Draw 10 mL of sample into the syringe and expel through the filter. Draw another 10 mL of sample into the syringe, expel through the filter, and collect the last 5 mL for analysis. Rinse the syringe with reagent water. Discard the filter.

11.2 Liquid Chromatography

11.2.1 Section 6.6 summarizes the recommended operating conditions for the liquid chromatograph. Table 1 lists retention times observed using this method. Other HPLC columns, chromatographic conditions, or detectors may be used if the requirements of Section 10.4 are met.

11.2.2 Calibrate the system daily as described in Section 10.0. The standards and samples must be in pH 3 buffered water.

11.2.3 Inject 400 µL of the sample. Record the volume injected and the resulting peak size in area units.

11.2.4 If the response for the peak exceeds the working range of the system, dilute the sample with pH 3 buffered reagent water and reanalyze.

11.3 Identification of Analytes

11.3.1 Identify a sample component by comparison of its retention time to the retention time of a reference chromatogram. If the retention time of an unknown compound corresponds, within limits, to the retention time of a standard compound, then identification is considered positive.

11.3.2 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time can be used to calculate a suggested window size for a compound. However, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

11.3.3 Identification requires expert judgement when sample components are not resolved chromatographically. When peaks obviously represent more than one sample component (i.e., broadened peak with shoulder(s) or valley between two or more maxima), or any time doubt exists over the identification of a peak on a chromatogram, appropriate alternate techniques, to help confirm peak identification, need to be employed. For example, more positive identification may be made by the use of an alternative detector which operates on a chemical/physical principle different from that originally used; e.g.,

mass spectrometry, or the use of a second chromatography column. A suggested alternative column is described in Section 6.6.3.

## 12.0 CALCULATIONS

Determine the concentration of individual compounds in the sample using the following equation:

$$C_X = \frac{(A_X)\ (Q_s)}{(A_s)\ (RF)}$$

where: $C_X$ = analyte concentration in micrograms per liter.
$A_x$ = response of the sample analyte.
$A_s$ = response of the standard (either internal or external), in units consistent with those used for the analyte response.
RF = response factor (with an external standard, RF = 1, because the standard is the same compound as the measured analyte).
$Q_s$ = concentration of internal standard present or concentration of external standard that produced $A_s$, in micrograms per liter.

## 13.0 PRECISION AND ACCURACY

13.1 In a single laboratory, analyte recoveries from reagent water were determined at five concentration levels. Results were used to determine analyte EDLs and demonstrate method range.[1] Analyte recoveries and standard deviation about the percent recoveries at one concentration are given in Table 2.

13.2 In a single laboratory, analyte recoveries from two standard synthetic ground waters were determined at one concentration level. Results were used to demonstrate applicability of the method to different ground water matrices.[1] Analyte recoveries from the two synthetic matrices are given in Table 2.

## 14.0 REFERENCES


1. National Pesticide Survey Method No. 5., "Measurement of N-Methylcarbamoyloximes and N-Methylcarbamates in Groundwater by HPL with Post Column Derivatization."

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, 10, 1049, 1977.

3. ASTM Annual Book of Standards, Part 11, Volume 11.02, D3694-82, "Standard Practice for Preparation of Sample Containers and for Preservation," American Society for Testing and Materials, Philadelphia, PA, p. 86, 1986.

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

5. "OSHA Safety and Health Standards, General Industry," (29 CFR 1910), Occupational Safety and Health Administration, OSHA 2206, (Revised, January 1976).

6. "Safety in Academic Chemistry Laboratories," American Chemical Society Publication, Committee on Chemical Safety, 3rd Edition, 1979.

7. Foerst, D.L. and Moye, H.A. "Aldicarb in Drinking Water via Direct Aqueous Injection HPLC with Post Column Derivatization," Proceedings of the 12th annual AWWA Water Quality Technology Conference, 1984.

8. Hill, K.M., Hollowell, R.H., and DalCortevo, L.A. "Determination of N-Methylcarbamate Pesticides in Well Water by Liquid Chromatography and Post Column Fluorescence Derivatization," Anal. Chem, 56, 2465 (1984).

9. ASTM Annual Book of Standards, Part 11, Volume 11.01, D3370-82, "Standard Practice for Sampling Water," American Society for Testing and Materials, Philadelphia, PA, p. 130, 1986.

**TABLE 1. RETENTION TIMES FOR METHOD ANALYTES**

| Analyte | Retention Time[a] (minutes) | | |
|---|---|---|---|
| | Primary[1] | Alternative[2] | Alternative[3] |
| Aldicarb sulfoxide | 6.80 | 15.0 | 17.5 |
| Aldicarb sulfone | 7.77 | 15.2 | 12.2 |
| Oxamyl | 8.20 | 17.4 | 14.6 |
| Methomyl | 8.94 | 18.4 | 14.8 |
| 3-Hydroxycarbofuran | 13.65 | 23.3 | 19 |
| Aldicarb | 16.35 | 27.0 | 21.4 |
| Baygon | 18.86 | 29.3 | 24.4 |
| Carbofuran | 19.17 | 29.6 | 23.4 |
| Carbaryl | 20.29 | 30.8 | 25.4 |
| Methiocarb | 24.74 | 34.9 | 28.6 |
| BDMC | 25.28 | | 35.5 |

[a]Columns and analytical conditions are described in Sections 6.6.2 and 6.6.3.
[1]Waters NovaPak C18.
[2]Beckman Ultrasphere ODS.
[3]Supelco LC-1.

**TABLE 2. SINGLE LABORATORY ACCURACY, PRECISION AND ESTIMATED DETECTION LIMITS (EDLS) FOR ANALYTES FROM REAGENT WATER AND SYNTHETIC GROUND WATERS[a]**

| Analyte | EDL[b] µg/L | Concentration Level µg/L | Reagent Water | | Synthetic Water 1[e] | | Synthetic Water 2[f] | |
|---|---|---|---|---|---|---|---|---|
| | | | R[c] | $S_R$[d] | R | $S_R$ | R | $S_R$ |
| Aldicarb | 1.0 | 5 | 115 | 3.5 | 106 | 3.2 | 102 | 8.2 |
| Aldicarb sulfone | 2.0 | 10 | 101 | 4.0 | 98 | 3.9 | 95 | 9.5 |
| Aldicarb sulfoxide | 2.0 | 10 | 97 | 4.9 | 105 | 4.2 | 94 | 10.3 |
| Baygon | 1.0 | 5 | 106 | 3.2 | 96 | 4.8 | 97 | 5.8 |
| Carbaryl | 2.0 | 10 | 97 | 5.8 | 94 | 4.7 | 104 | 10.4 |
| Carbofuran | 1.5 | 7.5 | 102 | 5.1 | 102 | 3.1 | 100 | 7.0 |
| 3-Hydroxycarbofuran | 2.0 | 10 | 102 | 4.1 | 98 | 4.9 | 101 | 10.1 |
| Methiocarb | 4.0 | 20 | 94 | 1.9 | 102 | 4.1 | 112 | 3.4 |
| Methomyl | 0.5 | 2.5 | 105 | 4.2 | 98 | 3.9 | 105 | 9.5 |
| Oxamyl | 2.0 | 10 | 100 | 4.0 | 97 | 2.9 | 102 | 10.2 |

[a]Data corrected for amount detected in blank and represent the mean of seven to eight samples.

[b]EDL = Estimated detection limit; defined as either MDL (Appendix B to 40 CFR Part 136 - Definition and Procedure for the Determination of the Method Detection Limit - Revision 1.11) or a level of compound in a sample yielding a peak in the final extract with signal-to-noise ratio of approximately 5, whichever value is higher. The concentration level used in determining the EDL is not the same as the concentration level presented in this table.

[c]R = Average percent recovery.

[d]$S_R$ = Standard deviation of the percent recovery.

[e]Corrected for amount found in blank; Absopure Nature Artesian Spring Water Obtained from the Absopure Water Company in Plymouth, Michigan.

[f]Corrected for amount found in blank; reagent water fortified with fulvic acid at the 1 mg/L concentration level. A well-characterized fulvic acid, available from the International Humic Substances Society (associated with the United States Geological Survey in Denver, Colorado) was used.

**TABLE 3. LABORATORY PERFORMANCE CHECK SOLUTION**

| Test | Analyte | Conc, µg/mL | Requirements |
|---|---|---|---|
| Sensitivity | 3-Hydroxycarbofuran | 2 | Detection of analyte; S/N >3 |
| Chromatographic performance | Aldicarb sulfoxide | 100 | 0.80 < PGF <1.20 |
| Column performance | Methiocarb | 20 | Resolution >0.7[b] |
| | 4-Bromo-3,5-dimethylphenyl N-methylcarbamate (IS) | 10 | |

[a]PGF - peak Gaussian factor. Calculated using the equation:

$$PGF = \frac{1.83 \times W\ (1/2)}{W\ (1/10)}$$

where: W(1/2) is the peak width at half height and W(1/10) is the peak width at tenth height.

[b]Resolution between the two peaks as defined by the equation:

$$R = \frac{t}{W}$$

where: t is the difference in elution times between the two peaks and W is the average peak width, at the baseline, of the two peaks.