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Method 531. Measurement of N-Methyl Carbamoyloximes and N-Methyl Carbamates in Drinking Water by Direct Aqueous Injection HPLC with Post Column Derivatization

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INDEX

TABLES

FIGURES

Method 531. Measurement of N-Methyl Carbamoyloximes and N-Methyl Carbamates in Drinking Water by Direct Aqueous Injection HPLC with Post Column Derivatization

1. SCOPE AND APPLICATION

1.1 This method describes a procedure for the identification and measurement of N-methyl carbamoyloximes and N-methyl carbamates in finished drinking water, raw source water, or drinking water at any treatment stage.(1,2) Single-laboratory accuracy and precision data have been determined for the following compounds.

Analyte	Chemical Abstracts Service Registry Number (CASRN)	STORET Number
Aldicarb	116-06-3	
Aldicarb sulfone	1646-88-4	
Aldicarb sulfoxide	1646-87-3	
Carbaryl	63-25-2	39750
Carbofuran	1563-66-2	81405
3-Hydroxycarbofuran	16655-82-6	
Methomyl	16752-77-5	39051
Oxamyl	23135-22-0	

Laboratories may use this method to detect and measure additional analytes after demonstrating obtains acceptable (defined in Section 10) accuracy and precision data for those analytes.

1.2 Method Detection Limits (MDLs) (3) are matrix and compound dependent. The MDL is that concentration of analyte below which there is less than a 99% confidence that the concentration is different from zero. The reagent water MDLs for the analytes given above vary from 0.5 to 1.6 µg/L. The applicable concentration range for this procedure is from the MDL to approximately 250 µg/L of undiluted sample.

1.3 This method is recommended for use by analysts experienced with high performance liquid chromatography (HPLC) and fluorescence detection techniques or by experienced technicians under the close supervision of such qualified analysts.

2. 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 0.05N sodium hydroxide at 95°C. The methyl amine formed during hydrolysis is reacted with o-phthalaldehyde (OPA) to form a highly fluorescent derivative which is detected using a fluorescence detector. (4)

3. DEFINITIONS

External standard — a known amount of a pure analyte that is analyzed under the same procedures and conditions that are used to analyze samples containing that compound.

Internal standard — a pure compound added to a sample in a known amount and used to calibrate concentration measurements of other analytes that are sample components. The internal standard must be a compound that is not a sample component.

Field duplicates — two samples taken at the same time and place under identical circumstances and treated exactly the same throughout field and laboratory procedures. Analysis of field duplicates indicates the precision associated with sample collection, preservation and storage, as well as with laboratory procedures.

Field reagent blank — reagent water placed in a sample container by the laboratory, shipped to and from the sampling site, and treated as a sample in all respects; including storage, preservation and all analytical procedures.

Laboratory control standard — a solution of analytes prepared in the laboratory by dissolving known amounts of pure compounds in a known amount of reagent water. In this method, the laboratory control standard is prepared by adding appropriate volumes of the secondary dilution standard solution to reagent water.

Laboratory duplicates — two aliquots of the same sample that are treated exactly the same throughout laboratory analytical procedures. Analysis of laboratory duplicates provides a measure of the precision associated with laboratory procedures and excludes the precision associated with sample collection, preservation or storage procedures.

Laboratory reagent blank — a 50-mL portion of acid preserved reagent water filtered, and analyzed as if it were a sample.

Performance evaluation sample — a water soluble solution of method analytes distributed by the Quality Assurance Branch (QAB), Ohio, to Environmental Monitoring and Support Laboratory, USEPA, Cincinnati, multiple laboratories for analysis. A small measured volume of the solution is added to a known volume of reagent water and analyzed using procedures identical to those used for samples. Results of analyses are

used by the QAB to determine the accuracy and precision that can be expected when a method is performed by competent analysts. Analyte true values are unknown to the analyst.

Quality control check sample — a water soluble solution containing known concentrations of analytes prepared by a laboratory other than the laboratory performing the analysis. The performing laboratory uses this solution to demonstrate that it can obtain acceptable identifications and measurements with a method. A small measured volume of the solution is added to a known volume of reagent water and analyzed with procedures identical to those used for samples. True values of analytes are known by the analyst.

Stock standard solution — a concentrated solution containing a certified standard that is a method analyte, or a concentrated organic solution of an analyte prepared in the laboratory with an assayed reference compound.

Secondary dilution standard — an organic solution of analytes prepared in the laboratory from stock standard solutions and diluted as needed to prepare aqueous calibration solutions and laboratory control standards.

4. 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. 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 10.1.4.

4.1.1 Glassware must be scrupulously cleaned.(5) Clean all glassware as soon as possible after use by washing with hot water and detergent then rinsing with tap and reagent water. Drain dry. Seal and store by inverting or capping with aluminum foil in a clean environment to prevent any accumulation of dust or other contaminants.

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.

4.2 Samples may become contaminated during shipment or storage. Field reagent blanks must be analyzed to determine that sampling and storage procedures have prevented contamination.

4.3 During analysis, major contaminant sources are impurities in the mobile phase. Analyses of field reagent blanks and laboratory reagent blanks provide information about the presence of contaminants.

4.4 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 reagent blanks should be analyzed to ensure that accurate values are obtained for the next sample.

5. SAFETY

5.1 The toxicity or carcinogenicity of chemicals used in this method has not been precisely defined; each chemical should be treated as a potential health hazard, and exposure to these chemicals should be minimized. Each laboratory is responsible for maintaining awareness of OSHA regulations regarding safe handling of chemicals used in this method. Additional references to laboratory safety are cited (6-8).

6. APPARATUS AND EQUIPMENT

6.1 SAMPLE CONTAINERS — 100-mL or larger glass or plastic bottles, each equipped with a screw cap.

6.2 BALANCE — Analytical, capable of accurately weighing to the nearest 0.1 mg.

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 25 mm filter holder (Nuclepore, polycarbonate 420200 or equivalent) and 25 mm diameter 0.4 µm polycarbonate filters (Nuclepore 110607 or equivalent).

6.4 SYRINGES AND SYRINGE VALVES

6.4.1 One 10-mL glass hypodermic syringe with Luer-Lok tip.

6.4.2 One 3-way syringe valve (Hamilton HV3-3 or equivalent).

6.4.3 One 17 gauge syringe needle, seven to ten cm long, blunt tip.

6.4.4 Micro syringes, various sizes.

6.5 MISCELLANEOUS

6.5.1 Standard solution storage bottles -- 10-mL bottles equipped

with screw caps and sealed with polytetrafluoroethylene (PTFE) lined septa.

6.5.2 Helium, for degassing dissolved oxygen.

6.6 HIGH PERFORMANCE LIQUID CHROMATOGRAPH (HPLC)

6.6.1 HPLC system capable of injecting 200 to 400 µL aliquots, and performing binary linear gradients at a constant flow rate.

6.6.2 Column -- 10 cm long x 8 mm ID radially compressed HPLC column packed with 10 µm µ-Bondapak C-18 or equivalent. This column was used to generate the method performance statements in Section 13. Different HPLC columns may be used in accordance with the provisions in Section 10. Use of guard columns is highly recommended.

6.6.3 Post Column Reactor — Capable of mixing reagents into the mobile phase. Reactor to be equipped with pumps, to deliver 0.5 mL/min each reagent; mixing tees; two 1.0 mL delay coils, one thermostated at 95°C; and constructed using PTFE tubing. (Kratos URS 051 and URA 100 or equivalent). See Figure 1.

6.6.4 Fluorescence Detector — Capable of excitation at 230 nm and detecting emission energies greater than 419 nm. Fluorometers should have dispersive optics for excitation and can utilize either filter or dispersive optics at the emission detector.

6.6.5 Data System — Use of a data system to report retention times and peak areas is recommended but not required. The HPLC system must produce a strip chart recording of detector response.

7. REAGENTS AND CONSUMABLE MATERIALS

7.1 HPLC MOBILE PHASE

7.1.1 Reagent water — laboratory grade water in which an interferent is not observed at the method detection limit. Filter and degas with helium before use.

7.1.2 Organic phase — Methanol and acetonitrile at an 80:20 (v:v) composition. Prepare using HPLC grade solvents. Filter and degas with helium before use.

7.2 POST COLUMN DERIVATIZATION SOLUTIONS

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

7.2.2 2-Mercaptoethanol (1+1) — Mix 10.0 mL of 2-mercapto-ethanol and 10.0 mL of acetonitrile. Cap. Store in hood. (Caution - stench)

7.2.3 Sodium borate (0.05N) — Dissolve 19.1 g of sodium borate ($Na_2 B_4O_7 \cdot 10 H_2O$) 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.

7.2.4 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 0.05N sodium borate. Mix, filter, and degas with helium. Add 100 µL of 2-mercaptoethanol (1+1) and mix. Make up fresh solution daily.

7.3 SAMPLE PRESERVATION REAGENTS

7.3.1 Sodium thiosulfate — granular.

7.3.2 Hydrochloric Acid (1+1) — Carefully add 1 volume of concentrated hydrochloric acid (HCl sp gr 1.19) to an equal volume of reagent water.

7.4 STOCK STANDARD SOLUTIONS — These solutions may be purchased as certified solutions or prepared from pure standard materials using the following procedures.

7.4.1 Accurately weigh approximately 0.0100 g of pure material. Dissolve the material in HPLC quality acetonitrile and dilute to volume in a 10-mL volumetric flask. Larger volumes may be used at the convenience of the analyst. Mix by inverting several times.

7.4.2 When the assayed compound purity is certified at 96% or greater, the weight may be used without correction to calculate the concentration of the stock standard solution.

7.4.3 Transfer the stock standard solutions into PTFE-sealed screw cap vials. Store at 4°C and protect from light. Frequently check stock standard solutions for signs of degradation or evaporation, especially just prior to preparing a calibration standard from them.

7.5 SECONDARY DILUTION STANDARD - Use stock standard solutions to prepare secondary dilution standard solutions that contain the analytes in acetonitrile. The secondary dilution standard should be prepared at a concentration such that 50 to 200 µL of the solution can be added to 25, 50 or 100 mL of reagent water to prepare aqueous calibration solutions that bracket the working concentration range. Check the secondary dilution standard solution frequently for signs of deterioration or evaporation, especially just before preparing aqueous calibration solutions.

7.6 INTERNAL STANDARD SPIKING SOLUTION — Prepare an acetonitrile solution of the internal standard at a level that gives approximately 20 µg/L when 100 µL of the solution is added to 100 mL of the sample. No universal compound can be specified for use as an internal standard. Choice of internal standard is left to the analyst.

8. SAMPLE COLLECTION, PRESERVATION AND HANDLING

8.1 Collect samples in glass or plastic containers. Conventional sampling practices (9) are to be followed; however, the bottle must not be prerinsed with sample before collection.

8.1.1 When sampling from a water tap, open the tap and allow the system to flush until the water temperature stabilizes. Adjust the flow to about 500 mL/minute and collect samples.

8.1.2 When sampling from an open body of water, fill a 1 quart wide mouth bottle or a 1 liter beaker with the sample from a representative area. Carefully fill the sample bottle to within 2 cm of the top.

8.2 SAMPLE PRESERVATION — Oxamyl, 3-hydroxycarbofuran, and carbaryl can all degrade quickly in natural waters held at room temperature (1,2). 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 auto sampler trays. Samples targeted for the analysis of these three analytes must be preserved at pH 3 in the laboratory. Aldicarb quickly oxidizes to aldicarb sulfoxide when residual chlorine is present in the sample. Acceptable storage times vary with the analyte and the preservation technique (Table 2).

8.2.1 Residual chlorine (up to 5 ppm) must be destroyed by adding 6 to 7 mg of sodium thiosulfate per 100 mL of sample. U.S. EPA methods 330.4 and 330.5 may be used to measure residual chlorine (10). Field test kits are available for this purpose.

8.2.2 After addition of preservative, seal the sample bottle, mix by inverting the sample several times, and store all samples over ice at 0 to 4°C.

8.3 FIELD REAGENT BLANKS

8.3.1 Field reagent blanks must be included in each sample set. A sample set consists of the samples collected from the same general sample site at approximately the same time. At the laboratory, fill field reagent blank sample bottles with reagent water, seal, and ship to the sampling site with the empty sample bottles. After collection, ship back to the

laboratory with the filled sample bottles. Wherever a set of samples is shipped and stored, it is accompanied by appropriate blanks.

8.3.2 If used in the field, the reducing agent is added to the blanks after receipt in the laboratory.

9. CALIBRATION

9.1 INITIAL CALIBRATION

9.1.1 CALIBRATION SOLUTIONS

9.1.1.1 At least three calibration solutions, containing each analyte, are needed. One calibration solution should contain each analyte at a concentration approaching; but greater than, the method detection limit (Table 1) for that compound. The other two solutions should contain analytes at concentrations that bracket the range expected in samples. For example, if the method detection limit for a particular analyte is 1.0 µg/L, and a sample is expected to contain approximately 10 µg/L is analyzed, aqueous solutions of standards should be prepared at concentrations of 2.0 µg/L, 10 µg/L, and 25 µg/L.

9.1.1.2 To prepare calibration solutions, add appropriate volumes (µL) of the secondary dilution standard solution to aliquots of reagent water at pH=3. Add the internal standard to give solutions at constant concentration in internal standard.

9.1.2 Analyze duplicate aliquots of each calibration solution using procedures identical to those used to analyze samples.

9.1.2.1 If the external standard technique is being used, prepare a concentration calibration curve for each analyte by plotting integrated area or peak height of the analyte as a function of the aqueous concentration (µg/L is equivalent to ng/mL). If the ratio of area to concentration or peak height to concentration of an analyte is constant throughout the concentration range (each point on the calibration curve is between 0.9 to 1.1 times the average ratio), the average ratio may be used instead of a calibration curve.

9.1.2.2 If the internal standard technique is being used, calculate the response to each compound relative to the internal standard. Calculate the response factor (RF) with the equation,

$$RF = \frac{A_x \cdot Q_s}{A_s \cdot Q_x},$$

where: A_x = the area or peak height of the analyte standard;
A_s = the area or peak height of the internal standard;
Q_s = concentration of internal standard; and
Q_x = concentration of analyte standard.

RF is a unitless number; units used to express concentration of analyte and internal standard must be equivalent. If the RF of an analyte is constant throughout the concentration range (each RF is between 0.9 to 1.1 times the average RF), the average RF may be used. For an analyte with non-linear RF, a calibration curve of $Area_x/Area_s$ plotted versus Q_x may be used to determine an analyte concentration.

9.2 DAILY CALIBRATION — Check calibration data each day by measurement of one or more laboratory control standards or calibration solutions. If the response for any analyte falls outside of 0.85 to 1.15 times the expected response, prepare and analyze a fresh calibration solution to determine if the problem is being caused by deterioration of the calibration solution. When the internal standard technique is being used, verify each day that response factors have not changed. If the RF falls outside of 0.85 to 1.15 times the expected RF, prepare and analyze new standard solutions to determine new response factors.

10. QUALITY CONTROL

10.1 Minimum quality control requirements consist of:

10.1.1 Initial demonstration of laboratory analytical capability (accuracy and precision procedures, Sect. 10.2 and 10.3),

10.1.2 Analysis of a laboratory control standard near the beginning of each 8-h work period,

10.1.3 Analysis of a field reagent blank along with each sample set,

10.1.4 Analysis of a laboratory reagent blank when the field reagent blank contains analytes at concentrations above the method detection limits,

10.1.5 Quarterly analysis of a quality control check sample, (if available for analytes of concern), and

10.1.6 Continued maintenance of performance records to define the quality of generated data.

10.2 ACCURACY — Determine accuracy, by analyzing four aliquots of a quality control (QC) check sample containing known amounts of analytes of concern. QC check samples for some, but not all listed analytes, are currently available from the U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Quality Assurance Branch, Cincinnati, Ohio 45268; alternatively, certified standard solutions may be purchased from commercial vendors.

10.2.1 Calculate accuracy using either the external or internal standard procedure. The concentration measured in the QC sample solution is expressed as a percentage (P) of the true value for the QC sample.

$$P = \frac{\bar{C}_{QC}}{T_{QC}} \cdot 100 \quad ,$$

where:

$\bar{C}_{QC}$ = the mean concentration of the QC check sample determined using an independent external or internal standard calibration, and

T_{QC} = the true concentration of the QC check sample.

NOTE: The internal standard concentration and the volume injected must be constant for calibration solutions and all samples for which the calibration solutions are used.

10.2.2 For each analyte, the mean accuracy should be in the range of 80 to 110% (1,2). For some listed analytes, this may not be feasible for low concentration measurements.

10.3 PRECISION

10.3.1 Calculate the method precision of each analyte as the standard deviation (s, in µg/L) of the four measured values obtained in the accuracy calculations:

$$S^2 = \frac{1}{n-1}\left[\sum_{i=1}^{n} X_i^2 - \left(\sum_{i=1}^{n} X_i\right)^2 \Big/ n\right]$$

$$S = (S^2)^{1/2}$$

where n = number of measurements for each analyte, and
X_i = individual measured value.

10.3.2 Calculate the dispersion of the measured values for each analyte as the percent relative standard deviation (RSD):

$$RSD = \frac{s}{\bar{C}} \cdot 100 \quad ,$$

where s = standard deviation, and
$\bar{C}$ = mean observed concentration.

10.3.3 Adequate precision is obtained if the relative standard deviation is $\leq$ 15%. (1,2)

10.4 LABORATORY CONTROL STANDARD — To demonstrate that the current calibration curve is valid, analyze a laboratory control standard at the beginning of each 8-h work period.

10.4.1 For each analyte to be measured, select a concentration representative of its occurrence in drinking water samples.

10.4.2 Prepare the laboratory control standard by adding 50 to 500 µL of the secondary dilution standard to 50 mL of reagent water at pH = 3.

10.4.3 Add an appropriate volume of the internal standard spiking solution and analyze using the same procedures (Sect. 11) used for samples.

10.4.4 Determine calibration acceptability and appropriate remedial actions, if needed. (For the external standard technique, see Sect. 9.1.2.1; for the internal standard technique, see Sect. 9.1.2.2.)

10.5 MONITORING THE INTERNAL STANDARD — All samples and laboratory control standards are at equal concentrations of the internal standard. The response of that compound is used to monitor system performance. If for any sample, the response varies more than ±15% from that observed in the previous sample or laboratory control standard, do not report analyte concentrtations for that sample. Take remedial action to solve the system performance problem and reanalyze the sample.

10.6 FIELD REAGENT BLANKS — Analyze a field reagent blank along with each sample set. If a field reagent blank contains analytes at concentrations above the method detection limits, analyze a laboratory reagent blank. If one or more analytes that are not detected at concentrations above method detection limits in the laboratory reagent blank are detected in significant amounts in the field blank, sampling or storage procedures have not prevented sample contamination, and the appropriate analyte measurement(s) must be discarded.

10.7 QUALITY CONTROL CHECK SAMPLES — At least quarterly, analyze a quality control check sample obtained from the U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Quality Assurance Branch, Cincinnati, Ohio. Quality control check

samples currently are available for some but not all listed analytes. If measured analyte concentrations are not within ±20% of true values, check the entire analytical procedure to locate and correct the problem source.

10.8 Additional QC procedures may be necessary, depending on the purpose of the analysis performed with this method.

10.8.1 Laboratory Duplicates — Determine the precision associated with laboratory techniques by analyzing two aliquots (Sect. 11.1.2) of a sample in which some analytes were detected in measurable quantities. Calculate the range (R) of concentrations measured for each duplicate pair:

$$R = C_1 - C_2,$$

where C_1 represents the larger and,
C_2 represents the smaller of the two measurements.

Calculate percent relative range (RR) of duplicate analyses using the formula:

$$RR = \frac{R}{\bar{C}} \cdot 100 \quad ,$$

where R = range of concentrations measured, and

$\bar{C}$ = mean concentration measured.

Generally, if RR is greater than 30%, precision is inadequate, and laboratory techniques must be improved.

10.8.2 Field Duplicates — Analyze in duplicate, 10% of samples in which some analytes were detected in measurable quantities to indicate precision limitations imposed by sampling, transport and storage techniques as well as laboratory techniques. If acceptable results are obtained from analysis of field duplicates, analysis of laboratory duplicates is usually not necessary.

10.8.3 Matrix Effects Determination — Dose and analyze 5% of the samples to determine matrix effects. Because analytes may be present in the unspiked aliquots, analysis of one or more unspiked aliquots is necessary to determine the initial concentrations, which are then subtracted from the concentrations measured in spiked aliquots. For each analyte, the amount added to determine matrix effects should exceed twice the amount measured in unspiked aliquots. Analysis of dosed samples over time will indicate if the storage and preservation procedures are adequate for the matrix.

11. PROCEDURE

11.1 ANALYSIS PROCEDURES

11.1.1 Adjust the pH of the sample or standard to pH of 3 ± 0.2 by the dropwise addition of hydrochloric acid (1+1). Fill a 50 mL volumetric flask to the mark with the sample. Add the constant amount of internal standard, if internal standards are used (1 µL per mL of sample). Mix by inverting the flask several times. If 1-Naphthol begins to appear in the chromatograms of carbaryl standards, hydrolysis is occurring and is an indication that the pH of the standard is not low enough.

11.1.2 Sample filtration — 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 to 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 5 mL through the filter and collect the remaining 5 mL for analysis. Rinse the syringe with reagent water. Discard the filter.

11.1.3 Sample injection — If a constant volume injection loop is being used, be sure to overfill the loop. Inject a constant volume (between 200 to 400 µL) of the filtered sample or calibration standard into the HPLC system. Begin the 10 minute linear gradient. Initial conditions are 80% water, 4% acetonitrile, 16% methanol (i.e. 80% water and 20% of a 20:80 mix of acetonitrile/methanol). Final conditions are 30% water, 14% acetonitrile, 56% methanol (i.e. 30% water and 70% of a 20:80 mix of acetonitrile/methanol).

11.1.4 Data Acquisition — Acquire data until the last analyte of interest elutes. If any peak overloads the photomultiplier or exceeds the working range of the calibration solutions, dilute an aliquot of the sample and reanalyze beginning at Section 11.1.2.

11.1.5 Equilibration to Initial Conditions — Return the mobile phase to initial composition. Pump at initial conditions until the baseline has flattened. Proceed with the next analysis.

11.2 IDENTIFICATION PROCEDURES — Analytes are identified by comparing the retention times of the unknowns to the retention times of authentic standards.

11.2.1 Qualitative identification is achieved if the HPLC retention time of the analyte in the sample is within 6 seconds of that observed for the same analyte in the calibration standard. (See Table 1.)

11.3 CONFIRMATORY PROCEDURES

11.3.1 Analyze using an HPLC column of different selectivity. Trimethyl silyl reverse phase columns invert the elution order of aldicarb sulfone and aldicarb sulfoxide when the mobile phase is changed from water/acetonitrile to water/methanol.(1)

11.3.2 Fortify with the analyte of interest and reanalyze. The presence of the analyte is not confirmed if the native compound and fortified analyte do not coelute.

11.3.3 Moving belt liquid chromatography/mass spectrometer,(11) thermospray LC/MS (12) and capillary column GC/MS (13) can be used to confirm the qualitative and quantitative identifications.

12. CALCULATIONS

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

$$C_x = \frac{A_x \cdot Q_s}{A_s \cdot RF} ,$$

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. PRECISION AND ACCURACY

13.1 The single laboratory accuracy and precision data in Table 1 were collected using a nominal spike level of 2.5 µg/L, a 400 µL injection volume and a three point calibration curve at nominal values of 2.5, 5, and 10 µg/L. Peak height calculations were used rather than electronic integration. Similar data have been obtained in two other independent laboratories.(1,2)

13.2 The MDLs were calculated using the following equation.

$$MDL = t_{(n-1,\ 1-\alpha=0.99)} * s$$

where:

$t_{(n-1,\ 1-\alpha=0.99)}$ is the students' t value for $\alpha=0.01$, and $n=7$ replicates and

s is the standard deviation (in µg/L) for the seven replicate analyses.

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Table 1. Single Operator Accuracy and Precision Data in Acid Preserved Reagent Water

Analyte	Retention Time[a] (min)	Retention Window[b] (sec)	Spike Level (µg/L)	Observed Concentration[c]	Standard Deviation (µg/L)	Relative Standard Deviation (%)	Accuracy (%)	Method Detection Limit (µg/L)
Aldicarb sulfoxide	5.40	±0.07	2.40	2.40	0.22	9.4	100	0.8[d]
Aldicarb sulfone	6.05	±0.08	2.56	2.61	0.16	6.1	102	0.5
Oxamyl	6.45	±0.06	2.91	2.65	0.50	18.9	91	1.6
Methomyl	7.34	±0.06	2.78	2.47	0.21	8.5	89	0.7[d]
3-Hydroxycarbofuran	9.08	±0.06	2.41	2.39	0.50	21	99	1.6
Aldicarb	11.43	±0.06	2.55	2.85	0.35	12.2	112	1.3[d]
Carbofuran	12.54	±0.04	3.20	2.81	0.28	9.8	88	0.9
Carbaryl	13.08	±0.06	2.57	1.98	0.22	11.1	77	0.7
1-Naphthol	13.57	±0.09	--	--	--	--	--	--

[a]See Section 11.1.3 for chromatographic conditions, dead volume time is 1.84 min.
[b]99% Confidence Limit of retention time, 20 runs over 16 hours.
[c]400 µL injection, seven replicates. 5 hours storage time.
[d]MDL base in pooled value see Reference 1.

Table 2. Acceptable Storage Time for Selected Method 531 Analytes[a]

Analyte	Reagent Water Recovery Time in Days pH = 7	Reagent Water Recovery Time in Days pH = 3	Raw Source Water Recovery Time in Days pH = 7	Raw Source Water Recovery Time in Days pH = 3
Aldicarb Sulforide	70	18	35	40
Aldicarb Sulfone	70	41	19	70
Oxamyl	53	65	3	70
Methomyl	70	62	70	70

a All samples stored at 5°C. Acceptable indicates time to show a 15% loss of analyte. See Reference 1.

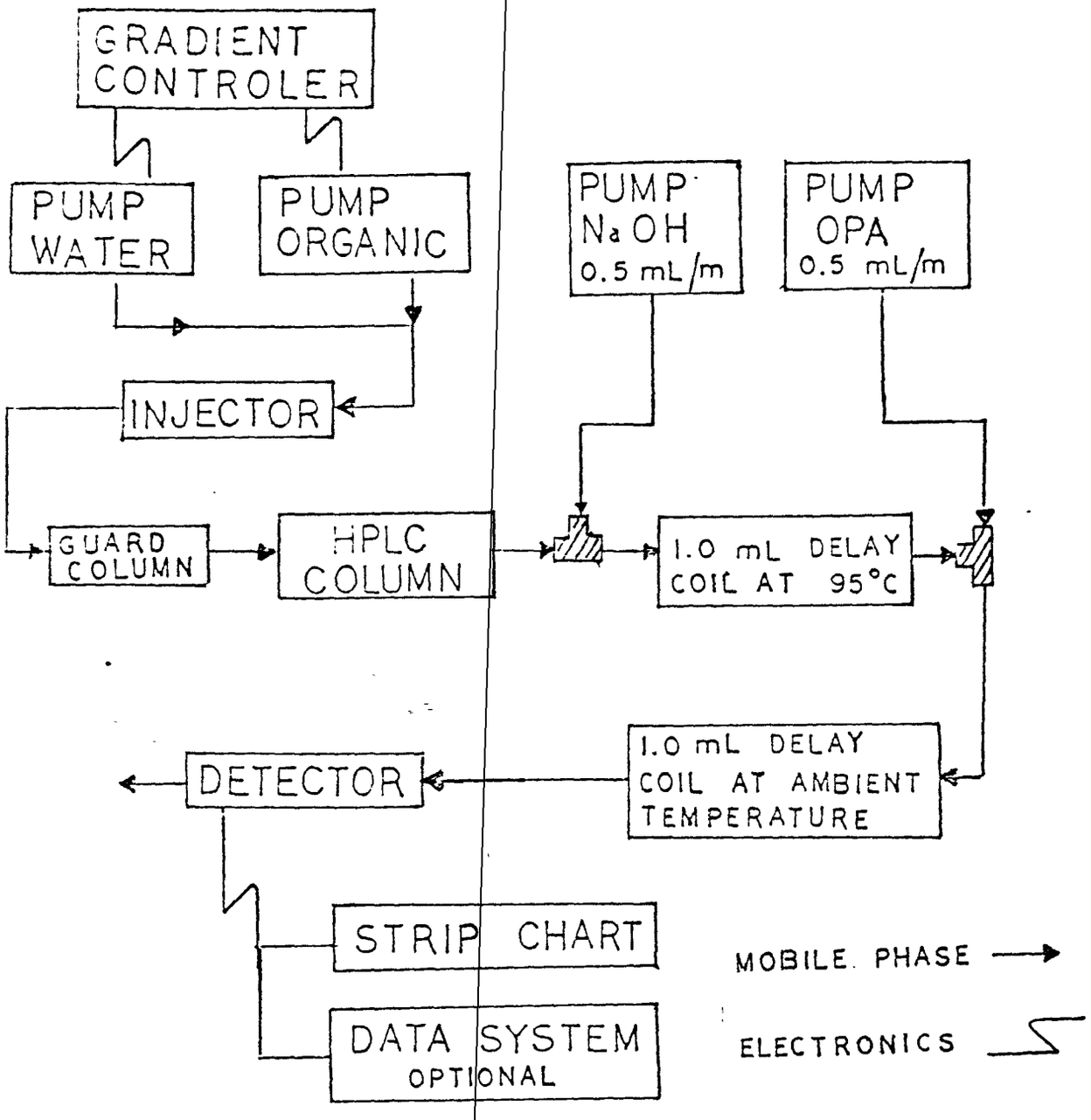

Figure 1. Block Diagram of HPLC System

COLUMN – 8 mm ID X 10 cm C-18 Rad Pak

GRADIENT – from 80:4:16 (water: acetonitrile:methanol) to 30:14:56 (W:A:M) in 15 minutes

DETECTOR – Fluoresence, 235 nm/ex 419 nm/em

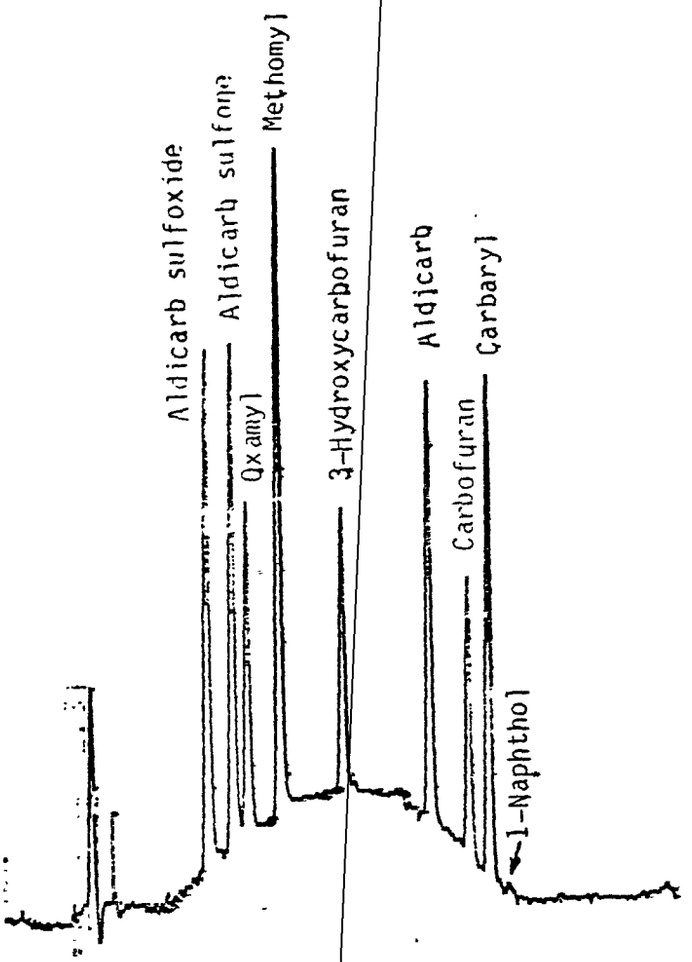

Figure 2. HPLC Chromatogram of N-Methyl Carbamoyloximes and N-Methyl Carbamates