# METHOD 556. DETERMINATION OF CARBONYL COMPOUNDS IN DRINKING WATER BY PENTAFLUOROBENZYLHYDROXYLAMINE DERIVATIZATION AND CAPILLARY GAS CHROMATOGRAPHY WITH ELECTRON CAPTURE DETECTION

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# METHOD 556

# DETERMINATION OF CARBONYL COMPOUNDS IN DRINKING WATER BY PENTAFLUORBENZYLHYROXYLAMINE DERIVATIZATION AND CAPILLARY GAS CHROMATOGRAPHY AND ELECTRON CAPTURE DETECTION

## 1. SCOPE AND APPLICATION

1.1 This is a gas chromatographic method optimized for the determination of selected carbonyl compounds in finished drinking water and raw source water. The analytes applicable to this method are derivatized to their corresponding pentafluorobenzyl oximes. The oxime derivatives are then extracted from the water with hexane. The hexane extracts are analyzed by capillary gas chromatography with electron capture detection (GC-ECD) and quantitated using procedural standard calibration. Accuracy, precision, and method detection limit (MDL) data have been generated for the following compounds:

| Analyte | Chemical Abstract Services Registry Number |
|---|---|
| Formaldehyde | 50-00-0 |
| Acetaldehyde | 75-07-0 |
| Propanal | 123-38-6 |
| Butanal | 123-72-8 |
| Pentanal | 110-62-3 |
| Hexanal | 66-25-1 |
| Heptanal | 111-71-7 |
| Octanal | 124-13-0 |
| Nonanal | 124-19-6 |
| Decanal | 112-31-2 |
| Cyclohexanone | 108-94-1 |
| Crotonaldehyde | 123-73-9 |
| Benzaldehyde | 100-52-7 |
| Glyoxal (ethanedial) | 107-22-2 |
| Methyl glyoxal (2-oxopropanal or pyruvic aldehyde) | 78-98-8 |

1.2 This method applies to the determination of target analytes over the concentration ranges typically found in drinking water. Analyte retention times are in Section 17, Table 1. Other method performance data are presented in Section17, Tables 2-6. Experimentally determined method detection limits (MDLs) for the above listed analytes are provided in Section 17, Table 3. The MDL is defined as the statistically

calculated minimum amount that can be measured with 99% confidence that the reported value is greater than zero.[1] However, it should be noted that background levels of some method analytes (usually formaldehyde and acetaldehyde) are problematic. The minimum reporting level (MRL) for method analytes, for each analyst/laboratory that uses this method, will depend on their ability to control background levels (Sect. 4).

1.3 This method is restricted to use by or under the supervision of analysts skilled in liquid-liquid extractions, derivatization procedures and the use of GC and interpretation of gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method, using the procedures described in Section 9.

## 2.0 SUMMARY OF METHOD

2.1 A 20 mL volume of water sample is adjusted to pH 4 with potassium hydrogen phthalate (KHP) and the analytes are derivatized at 35 °C for 2 hr with 15 mg of O-(2,3,4,5,6-Pentafluorobenzyl)-hydroxylamine (PFBHA) reagent. The oxime derivatives are extracted from the water with 4 mL hexane. The extract is processed through an acidic wash step, and then analyzed by GC-ECD. The target analytes are identified and quantitated by comparison to a procedural standard (Sect. 3.9). Two chromatographic peaks will be observed for many of the target analytes. Both *(E)* and *(Z)* isomers are formed for carbonyl compounds that are asymmetrical, and that are not sterically hindered. However, the *(E)* and *(Z)* isomers may not be chromatographically resolved in a few cases. Compounds where two carbonyl groups are derivatized, such as glyoxal and methyl glyoxal, have even more possible isomers. See Section17, Table 1 and Figure 1 for the chromatographic peaks used for analyte identification.

**NOTE:** The absolute identity of the *(E)* and *(Z)* isomers was not determined during method development. Other researchers [2,3,4] have reported the first eluting peak as *(E)*, and the second peak as *(Z)*. For convenience, this method will follow this convention. Because more than 2 isomers are formed for glyoxal and methyl glyoxal, the peaks used for identification are referred to as “peak 1" and “peak 2."

2.2 All results should be confirmed on a second, dissimilar capillary GC column.

## 3. DEFINITIONS

3.1 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, 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.

3.2 FIELD REAGENT BLANK (FRB) -- An aliquot of reagent water or other blank matrix that is placed in a sample container in the laboratory and treated as a sample in all respects, including shipment to the sampling site, storage, preservation, and all analytical procedures. The purpose of the FRB is to determine if method analytes or other interferences are introduced during sample shipping or storage. For this analysis the FRB should not be opened at the sampling site.

3.3 LABORATORY FORTIFIED BLANK (LFB) -- An aliquot of reagent water or other blank matrix 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.

3.4 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.

3.5 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.

3.6 PRIMARY DILUTION STANDARD SOLUTION (PDS) -- 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.

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

3.8 QUALITY CONTROL SAMPLE (QCS) -- A solution of method analytes of known concentrations which is used to fortify an aliquot of LRB or sample matrix. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials.

3.9 PROCEDURAL STANDARD CALIBRATION -- A calibration method where aqueous calibration standards are prepared and processed (e.g. purged, extracted, and/or derivatized) in exactly the same manner as a sample. All steps in the process from addition of sampling preservatives through instrumental analyses are included in the calibration. Using procedural standard calibration compensates for any inefficiencies in the processing procedure.

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

3.11 SURROGATE ANALYTE (SUR) -- A pure analyte, which is extremely unlikely to be found in any sample, and which is added to a sample aliquot in known amount(s) before extraction or other processing and is measured with the same procedures used to measure other sample components. The purpose of the SA is to monitor method performance with each sample.

3.12 METHOD DETECTION LIMIT (MDL) -- The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero.

3.13 MATERIAL SAFETY DATA (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.

3.14 CONTINUING CALIBRATION CHECK (CCC) -- A calibration standard containing one or more method analytes, which is analyzed periodically to verify the accuracy of the existing calibration for those analytes.

3.15 MINIMUM REPORTING LEVEL (MRL) -- The minimum concentration of an analyte that should be reported. This concentration is determined by the background level of the analyte in the LRBs and the sensitivity of the method to the analyte. Ideally, the MRL will be at or near the concentration of the lowest calibration standard.

## 4. INTERFERENCES

4.1 Method interferences may be caused by contaminants in laboratory air, solvents, reagents (including reagent water), glassware, sample bottles and caps, and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in the chromatograms. All of these materials must be routinely demonstrated to be free from interferences (less than 1/2 the MRL) under the conditions of the analysis by analyzing laboratory reagent blanks as described in Section 9.3. **Subtracting blank values from sample results is not permitted.**

4.1.1 Before attempting analyses by this method, the analyst must obtain a source of reagent water free from carbonyl compounds and other interferences. The most likely interferences are the presence of formaldehyde and acetaldehyde in the reagent water. The most successful techniques for generating aldehyde free water are (1) exposure to UV light, or (2) distillation from permanganate.

4.1.2 Commercially available systems for generating reagent grade water have proved adequate, if a step involving exposure to UV light is included. For the data presented in this method, a Millipore Elix 3 reverse osmosis system followed by a Milli-Q TOC Plus polishing unit provided reagent water with background levels of l ug/L or less for each method analyte. Other researchers have reported typical blank values of 1-3 ug/L.[3,4]

4.1.3 Distillation of reagent water from acidified potassium permanganate has been reported as an effective method of eliminating background levels of aldehydes.[2] Distill 500 mL of reagent water to which 64 mg potassium permanganate and 1 mL conc. sulfuric acid have been added. In our laboratory, this procedure reduced formaldehyde levels to approximately 3 ug/L.

4.1.4 It may be necessary to purchase reagent grade water. If acceptably clean reagent grade water is purchased, care must also be taken to protect it from contamination caused by contact with laboratory air.

4.2 Formaldehyde is typically present in laboratory air and smaller amounts of other aldehydes may also be found. Care should be taken to minimize exposure of reagents and sample water with laboratory air. Because latex is a potential aldehyde contaminant source, protective gloves should not contain latex. Nitrile gloves, such as N-Dex Plus, are acceptable. Bottle caps should be made of polypropylene. Commonly used phenolic resin caps must be avoided because they can introduce formaldehyde contamination into samples.

4.3 Reagents must also be free from contamination. Many brands of solvents may contain trace amounts of carbonyl compounds.

4.4 Glassware must be scrupulously cleaned by detergent washing with hot water, and rinses with tap water and distilled water. Glassware should then be drained, dried, and heated in a laboratory oven at 130 °C for several hours before use. Solvent rinses with methanol or acetonitrile, followed by air drying, may be substituted for the oven heating. After cleaning, glassware should be stored in a clean environment to prevent any accumulation of dust or other contaminants.

4.5 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the matrix being sampled.

4.6 An interferant that elutes just prior to the acetaldehyde *(E)* isomer peak on the primary column is typically observed in chlorinated or chloraminated waters. If this peak interferes with the integration of the acetaldehyde *(E)* isomer peak, then acetaldehyde should be quantitated using only the acetaldehyde *(Z)* isomer, or from the confirmation column data.

## 5. SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. 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.[5-8]

5.2 Formaldehyde and acetaldehyde have been tentatively classified as known or suspected human or mammalian carcinogens. Glyoxal and methyl glyoxal have been shown to be mutagenic in in-vitro tests.[2]

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

6.1 SAMPLE CONTAINERS -- Grab Sample Bottle (aqueous samples) -- 30 mL amber glass, screw cap bottles and caps equipped with Teflon-faced silicone septa. Screw caps should be polypropylene. **Typical phenolic resin caps should be avoided due to the possibility of sample contamination from formaldehyde**. Prior to use, wash bottles and septa according to Section 4.4.

6.2 VIALS -- 8 mL or 12 mL vials for the acid wash step (Sect. 11.1.10), and GC autosampler vials, both types must be glass with Teflon-lined polypropylene caps.

6.3 VOLUMETRIC FLASKS -- various sizes used for preparation of standards.

6.4 BALANCE -- Analytical, capable of accurately weighing to the nearest 0.0001 g.

6.5 WATER BATH or HEATING BLOCK -- Capable of maintaining 35 ± 2 °C

6.6 GAS CHROMATOGRAPH -- Capillary Gas Chromatograph equipped with a split/splitless injector, or other injector suitable for trace analysis, and an electron capture detector.

6.6.1 Primary Column -- 30 m x 0.25mm J&W DB-5ms, 0.25 um film thickness (or equivalent). Note: The J&W DB-5 was not found to be equivalent for this application. The surrogate analyte is not resolved from octanal with the DB-5 column.

6.6.2 Confirmation Column -- 30 m x 0.25 mm Restek Rtx- 1701, 0.25 um film thickness (or equivalent)

## 7. REAGENTS AND STANDARDS

7.1 Reagent grade or better chemicals should be used in all tests. 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 ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

7.2 REAGENT WATER --. **Reagent water as free as possible from interferences and contamination is critical to the success of this method. See Section 4.1.**

7.3 ACETONITRILE -- High purity, demonstrated to be free of analytes and interferences.

7.4 HEXANE -- High purity, demonstrated to be free of analytes and interferences: B&J Brand, $GC^2$ grade or equivalent.

7.5 POTASSIUM HYDROGEN PHTHALATE (KHP) -- ACS Grade or better.

7.6 0-(2,3,5,6-PENTAFLUOROBENZYL)-HYDROXYLAMINE HYDROCHLORIDE (PFBHA) -- 98+%, Aldrich cat# 19,448-4. (Store in a desiccator - Do not refrigerate).

7.7 SULFURIC ACID -- ACS Grade or better.

7.8 COPPER SULFATE PENTAHYDRATE -- ACS Grade or better.

7.9 AMMONIUM CHLORIDE, $NH_4Cl$ or AMMONIUM SULFATE, $(NH_4)_2SO_4$.

7.10 SOLUTIONS

7.10.1 PFBHA REAGENT -- Prepare a fresh 15 mg/mL solution in reagent water daily. Prepare an amount appropriate to the number of samples to be derivatized. One mL of solution is added per sample. For example, if 14 sample vials are being extracted, prepare 15 mL of solution. For a 15 mL volume of solution, weigh 0.225 grams of PFBHA into a dry 40 mL vial, add 15 mL water and shake to dissolve.

7.10.2 0.2 N SULFURIC ACID -- Add 5 mL of concentrated sulfuric acid to 900 mL of reagent water.

7.11 STOCK STANDARD SOLUTIONS -- When a compound purity is assayed to be 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard.

7.11.1 INTERNAL STANDARD (IS) -- 1,2-DIBROMOPROPANE, 98+% purity. An alternate compound may be used as the IS at the discretion of the analyst. If an alternate is selected, an appropriate concentration will need to be determined.

7.11.1.1 INTERNAL STANDARD STOCK SOLUTION, (10,000 ug/mL) -- Accurately weigh approximately 0.1 gram to the nearest 0.0001g, into a tared 10 mL volumetric flask containing hexane up to the neck. After determining weight difference, fill to mark with hexane. Stock solutions can be used for up to 6 months when stored at -10 °C.

7.11.1.2 INTERNAL STANDARD FORTIFIED EXTRACTION SOLVENT, 400 ug/L in hexane -- This is the solvent used to extract the derivatized samples. The internal standard is added to the solvent prior to performing the extraction. The volume of this solvent to be prepared should be determined by the sample workload. The following example illustrates preparation of 1 L of fortified solvent. If fewer samples are to be analyzed each month, prepare smaller batches of working solvent. Add 40 uL of internal standard stock solution directly to 1 L of hexane in a volumetric flask. Cap flask and invert three times to ensure thorough mixing. Transfer to l L storage bottle with Teflon lined cap. This solution can be used up to 4 weeks. As a check, run a sample of this working solvent on the GC before the first extraction of aqueous samples. Have enough working solvent available to extract all calibration and aqueous samples in each extraction set. Never use two different batches of working solvent for one set of extractions.

7.11.2 SURROGATE (SUR) -- 2',4',5' -TRIFLUOROACETOPHENONE
This compound was found to be an appropriate surrogate analyte for these analyses. However, the chromatograms for this analysis are very crowded, and all possible matrix interferences cannot be anticipated. An alternate carbonyl compound may be selected as the surrogate analyte if matrix interferences or chromatographic problems are encountered. Any surrogate analyte selected must form an oxime derivative, because its purpose is to monitor the derivatization process. If an alternate surrogate is selected, its concentration may also be adjusted to meet the needs of the laboratory.

7.11.2.1 SURROGATE STOCK SOLUTION, 10,000 ug/mL -- Accurately weigh approximately 0.1 gram SUR to the nearest 0.0001g, into a 10 mL tared volumetric flask containing acetonitrile up to the neck. After determining weight difference, fill to mark with acetronitrile. Stock solutions can be used for up to 6 months when stored at -10 °C or less.

7.11.2.2 SURROGATE ADDITIVE SOLUTION, 20 ug/mL -- Dilute the surrogate stock solution to 20 ug/mL in acetonitrile. This solution can be used up to 3 months when stored at 4 °C or less.

7.11.3 STOCK STANDARD SOLUTION (SSS)

Prepare stock standard solutions for each analyte of interest at a concentration of 1 to 10 mg/mL in acetonitrile, or purchase SSSs or primary dilution standards (PDSs) from a reputable supplier. Method analytes may be obtained as neat materials or as ampulized solutions from commercial suppliers. The stock standard solutions should be stored at -10 °C or less and protected from light. Standards prepared in this manner were stable for at least 60 days. Standards may be used for longer periods of time if adequate records of stability are kept. Laboratories should use standard QC practices to determine when their standards need to be replaced.

7.11.3.1 For analytes which are solids in their pure form, prepare stock standard solutions by accurately weighing approximately 0.1 gram of pure material to the nearest 0.0001g in a 10 mL volumetric flask. Dilute to volume with acetonitrile.

7.11.3.2 Stock standard solutions for analytes which are liquid in their pure form at room temperature can be accurately prepared in the following manner.

7.11.3.2.1 Place about 9.8 mL of acetonitrile into a 10-mL volumetric flask. Allow the flask to stand, unstoppered, for about 10 min. to allow solvent film to evaporate from the inner walls of the volumetric, and weigh to the nearest 0.0001 gram.

7.11.3.2.2 Use a 100-uL syringe and immediately add 100 uL of standard material to the flask by keeping the syringe needle just above the surface of the acetonitrile. Be sure the standard material falls dropwise directly into the acetonitrile without contacting the inner wall of the volumetric.

7.11.3.2.3 Reweigh, dilute to volume, stopper, then mix by inverting several times. Calculate the concentration in milligrams per milliliter from the net gain in weight.

7.11.4 PRIMARY DILUTION STANDARD (PDS) -- The PDS for this method should include all method analytes of interest to the analyst. The PDS is prepared by combining and diluting stock standard solutions with acetonitrile to a concentration of 100 ug/mL. Store at -10 °C or less and protect from light. Standards prepared in this manner were stable for at least 60 days. Standards may be used for longer periods of time if adequate records of stability are kept. Laboratories should use standard QC practices to determine when their standards need to be replaced. This primary dilution standard is used to prepare calibration spiking solutions, which are prepared at 5 concentration levels for each analyte, and are used to spike reagent water to prepare the aqueous calibration standards.

7.11.5 CALIBRATION SPIKING SOLUTIONS -- Five calibration spiking solutions are prepared, each at a different concentration, and are used to spike reagent water to prepare the calibration standards. The calibration spiking solutions are prepared from the PDS. Store the calibration spiking solutions at -10 °C or less and protect from light. Solutions prepared in this manner were stable for at least 60 days. Solutions may be used for longer periods of time if adequate records of stability are kept. Laboratories should use standard QC practices to determine when solutions need to be replaced. An example of how the calibration spiking solutions are prepared is given in the following table. Modifications of this preparation scheme may be made to meet the needs of the laboratory.

**PREPARATION OF CALIBRATION SPIKING SOLUTIONS**

| Cal. Level | PDS Conc., ug/mL | Vol. PDS Std., uL | Final Vol., Cal Spike Sol'n, mLs | Final Conc., Cal Spike Sol'n, ug/mL |
|---|---|---|---|---|
| 1 | 100 | 250 | 5 | 5 |
| 2 | 100 | 500 | 5 | 10 |
| 3 | 100 | 1000 | 5 | 20 |
| 4 | 100 | 1500 | 5 | 30 |
| 5 | 100 | 2000 | 5 | 40 |

7.11.6 PROCEDURAL CALIBRATION STANDARDS -- A designated amount of each calibration spiking solution is spiked into five separate 20 mL aliquots of reagent water in a 30 mL sample container, to produce aqueous calibration standards. The reagent water used to make the calibration standards should contain the preservation reagents described in Section 8.1.2 (ammonium chloride or ammonium sulfate at 500 mg/L and copper sulfate pentahydrate at 500 mg/L). Aqueous calibration standards are processed and analyzed according to the procedures in Section 11. Resulting data are used to generate a calibration curve. An example of the preparation of aqueous calibration standards is given below. The lowest concentration calibration standard should be at or near (within 25% of) the MRL. Modifications of this preparation scheme may be made to meet the needs of the laboratory. Preparing aqueous calibration standards using varying volumes of one calibration spiking solution is an acceptable alternative to the example below.

**PREPARATION OF PROCEDURAL (AQUEOUS) CALIBRATION STANDARDS**

| Cal. Level | Cal. Spike Sol'n Conc., ug/mL | Vol. Cal. Spike Sol'n., uL | Final Vol., Cal Std mL | Final Conc., Cal Std ug/L |
|---|---|---|---|---|
| 1 | 5 | 20 | 20 | 5 |
| 2 | 10 | 20 | 20 | 10 |
| 3 | 20 | 20 | 20 | 20 |
| 4 | 30 | 20 | 20 | 30 |
| 5 | 40 | 20 | 20 | 40 |

## 8. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

### 8.1 SAMPLE VIAL PREPARATION

8.1.1 Grab samples must be collected in accordance with conventional sampling practices [6] using amber glass 30 mL containers with PTFE-lined screw-caps, or caps with PTFE-faced silicon septa.

8.1.2 Prior to shipment to the field, 15 mg of copper sulfate pentahydrate must be added to each bottle. This material acts as a biocide to inhibit bacteriological decay of method analytes. If samples to be collected contain free chlorine, then 15mg of ammonium chloride or ammonium sulfate must also be added to the bottle prior to sample collection. The ammonium compound will react with the free chlorine to form monochloramine, and retard the formation of additional carbonyl compounds. Add these materials as dry solids to the sample bottle. The stability of these materials in concentrated aqueous solution has not been verified.

**NOTE:** Aldehydes have been demonstrated to be extremely susceptible to microbiological decay. The use of other chlorine reducing agents such as sodium thiosulfate or ascorbic acid, has also been shown to produce invalid data. Proper sample collection and preservation is important to obtaining valid data. The data in Section17, Table 6 illustrates the importance of proper sample preservation.

### 8.2 SAMPLE COLLECTION

8.2.1 Fill sample bottles to just overflowing but take care not to flush out the sample preservation reagents. The capped sample should be head-space free.

8.2.2 When sampling from a 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 min). Collect samples from the flowing system.

8.2.3 When sampling from an open body of water, fill a 1 quart wide-mouth bottle or 1L beaker with sample from a representative area, and carefully fill sample bottles from the container.

8.2.4 After collecting the sample, cap carefully to avoid spillage, and agitate by hand for 1 min.

8.3 SAMPLE STORAGE/HOLDING TIMES

8.3.1 Samples must be iced or refrigerated at 4 °C and maintained at these conditions away from light until extraction. Samples must be extracted within 7 days of sampling. However, since aldehydes are subject to decay in stored samples, all samples should be derivatized and extracted as soon as possible.

**NOTE:** A white or blue precipitate is likely to occur. This is normal and does not indicate any problem with sample collection or storage.

8.3.2 Extracts (Sect. 11.1.11) must be stored at 4 °C or less away from light in glass vials with Teflon-lined caps. Extracts must be analyzed within 14 days of extraction.

8.4 FIELD REAGENT BLANKS -- Processing of a field reagent blank (FRB) is required along with each sample set. A sample set is composed of the samples collected from the same general sampling site at approximately the same time. Field reagent blanks are prepared at the laboratory before sample vials are sent to the field. At the laboratory, fill a sample container with reagent water (Sect. 7.2), add sample preservatives as described in Section 8.1.2, seal and ship to the sampling site along with the empty sample containers. FRBs should be confirmed to be free (less than 1/2 the MRL) of all method analytes prior to shipping them to the field. Return the FRB to the laboratory with filled sample bottles. **DO NOT OPEN THE FRB AT THE SAMPLING SITE**. If any of the analytes are detected at concentrations equal to or greater than 1/2 the MRL , then all data for the problem analyte(s) should be considered invalid for all samples in the shipping batch.

## 9. QUALITY CONTROL

9.1 Each laboratory that uses this method is required to operate a formal quality control (QC) program. Minimum QC requirements are initial demonstration of laboratory capability (which includes calculation of the MDL), analysis of laboratory reagent blanks, laboratory fortified blanks, field reagent blanks, laboratory fortified sample matrices, and QC samples. Additional QC practices are encouraged.

9.2 INITIAL DEMONSTRATION OF CAPABILITY (IDC) -- Requirements for the initial demonstration of laboratory capability are described in the following sections and summarized in Section 17, Table 7.

9.2.1 Initial demonstration of low system background. (See Sect. 9. 3)

9.2.2 Initial demonstration of precision. Prepare, derivatize, extract, and analyze 4-7 replicate LFBs fortified at 20 ug/L, or other mid-range concentration, over a period of at least 2 days. Generating the data over a longer period

of time, e.g. 4 or 5 days may produce a more realistic indication of day to day laboratory performance. The relative standard deviation (RSD) of the results of the replicate analyses must be less than 20%.

9.2.3 Initial demonstration of accuracy. Using the same set of replicate data generated for Section 9.2.2, calculate average recovery. The average recovery of the replicate values must be within ± 20% of the true value.

9.2.4 MDL[(1)] determination. Replicate analyses for this procedure should be done over at least 3 days (both the sample derivatization/extraction and the GC analyses should be done over at least 3 days). Prepare at least 7 replicate LFBs at a concentration estimated to be near the MDL. This concentration may be estimated by selecting a concentration at 2-5X the noise level. Analyze the seven replicates through all steps of Section 11. Calculate the MDL

$$MDL = St_{(n - 1,\ 1 - alpha\ =\ 0.99)}$$

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
S = standard deviation of replicate analyses.

**NOTE:** Do not subtract blank values when performing MDL calculations.

9.2.5 Minimum Reporting Level (MRL) -- Although an MDL can be calculated for analytes that commonly occur as background contaminants, the calculated MDLs should not be used as the MRL for each analyte. Method analytes that are seen in the background (typically formaldehyde, acetaldehyde) should be reported as present in field samples, only after careful evaluation of the background levels. It is recommended that a MRL be established at the mean LRB concentration + 3σ, or three times the mean LRB concentration, whichever is greater. This value should be calculated over a period of time, to reflect variability in the blank measurements. It is recommended that this value be used as a minimum reporting level in order to avoid reporting false positive results.

9.3 LABORATORY REAGENTS BLANKS (LRB) -- 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, 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. Because background contamination is a

significant problem for several method analytes, it is highly recommended that the analyst maintain a historical record of LRB data. If target analytes are detected in the LRB at concentrations equal to or greater than 1/2 the MRL (Sect. 9.2.5), then all data for the problem analyte(s) should be considered invalid for all samples in the extraction batch.

9.4 CONTINUING CALIBRATION CHECK/LABORATORY FORTIFIED BLANK -- Since this methodology is based on procedural standard calibration, a LFB and the calibration check sample (CCC) are prepared and analyzed in the same manner. Laboratory fortified blank QC requirements are therefore omitted. Calibration procedure options and the QC acceptance criteria associated with them are fully described in Sect 10.3. Please refer to that section for these criteria.

9.5 INTERNAL STANDARD--The analyst must monitor the IS response peak area of all injections during each analysis day. A mean IS response is determined from the five point calibration curve. The IS response for any chromatographic run should not deviate from this mean IS response by more than 30%. If a deviation greater than 30% occurs with an individual extract inject a second aliquot of that extract.

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

9.5.2 If a deviation of greater than 30% is obtained for the reinjected extract, the analyst should check the calibration by analyzing the most recently acceptable calibration standard. If the calibration standard fails the criteria of Section 9.5, recalibration is in order per Section 10. If the calibration standard is acceptable, extraction of the sample should be repeated provided the sample is still within the holding time. Otherwise, report results obtained from the reinjected extract, but annotate as suspect.

9.6 SURROGATE RECOVERY--The surrogate standard is fortified into the aqueous portion of all calibration standards, samples, FRBs and LRBs. The surrogate is a means of assessing method performance from derivatization to final chromatographic measurement.

9.6.1 When surrogate recovery from a sample, blank, or CCC is <70% or >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 extract.

9.6.2 If the extract reanalysis meets the surrogate recovery criterion, report only data for the reanalyzed extract.

9.6.3 If the extract reanalysis fails the 70-130% recovery criterion, the analyst should check the calibration by analyzing the most recently acceptable calibration standard. If the calibration standard fails the criteria of Section 9.6.1, recalibration is in order per Section 10. If the calibration standard is acceptable, it may be necessary to extract another aliquot of sample if sample holding time has not been exceeded. If the sample reextract also fails the recovery criterion, report all data for that sample as suspect.

9.7 LABORATORY FORTIFIED SAMPLE MATRIX (LFM)

9.7.1 Within each analysis set, a minimum of one field sample is fortified as a LFM for every 20 samples analyzed. The LFM is prepared by spiking a sample with an appropriate amount of the calibration standard. The concentrations 5, 10, and 20 ug/L are suggested spiking concentrations. Select the spiking concentration that is closest to, and at least twice the matrix background concentration. Use historical data or rotate through the designated concentrations to select a fortifying concentration. Selecting a duplicate vial of a sample that has already been analyzed, aids in the selection of appropriate spiking levels.

9.7.2 Calculate the percent recovery (R) for each analyte, after correcting the measured concentration, A, from the fortified sample for the background concentration, B, measured in the unfortified sample, i.e.,

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

where C is the fortified concentration. Compare these values to control limits appropriate for reagent water data collected in the same fashion.

9.7.3 Recoveries may exhibit a matrix dependence. For samples fortified at or above their native concentration, recoveries should range between 70 - 130%. If the accuracy of any analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control, the accuracy problem encountered with the fortified 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. Repeated failure to meet the suggested recovery criteria indicates potential problems with the extraction procedure and should be investigated.

9.8 FIELD DUPLICATES -- Within each analysis batch, a minimum of one field sample should be analyzed in duplicate. Duplicate sample analyses serve as a check on sampling and laboratory precision.

9.8.1 Calculate the relative percent difference (RPD) for duplicate measurements (FD1 and FD2) as shown below.

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

9.8.2 Relative percent differences for laboratory duplicates should fall in the range of ± 30 %. Greater variability may be observed for target analytes with concentrations near their MRL.

9.9 QUALITY CONTROL SAMPLE (QCS) -- At least quarterly, analyze a QCS from an external source. If measured analyte concentrations are not of acceptable accuracy (60-140% of the expected value), check the entire analytical procedure to locate and correct the problem source.

9.10 ASSESSING*(Z/E*) RATIOS -- In addition to monitoring analyte response from CCC/LFB, the ratio of the peak areas of each isomer pair should be monitored. When samples and standards are processed and analyzed by exactly the same procedure, the ratio of the *(Z/E)* isomers produced by each method analyte will be reproducible. This information can be used as a QC check to avoid biased results caused by an interferant with one isomer of the pair. Calculate and record the ratio of the peak area of the first eluting isomer (designated *(E)*) to the second eluting isomer (designated *(Z)*). This ratio will be used in data evaluation Section 12.4.

## 10. CALIBRATION AND STANDARDIZATION

10.1 Demonstration and documentation of acceptable initial calibration is required before any samples are analyzed, and is required intermittently throughout sample analysis. After initial calibration is successful, the analyst may choose one of two options for maintaining on-going calibration. The first option is to verify the initial calibration daily using a minimum of 2 calibration standards. The other option is daily calibration of the method with all 5 calibration standards. These options are further described in Section 10.3.

10.2 INITIAL CALIBRATION CURVE

10.2.1 Establish GC operating parameters equivalent to the suggested specifications in Section 17, Table 1. The GC system must be calibrated using the internal standard (IS) technique. Other GC columns or GC conditions may be used if equivalent or better performance can be demonstrated.

10.2.2 Five calibration standards are recommended to calibrate over the range of approximately 2-40 ug/L. The lowest level standard will depend upon the level of blank contamination for each analyte (Sect. 7.11.6).

10.2.3 Prepare each calibration standard by the procedural standard calibration method. Method analytes are fortified into reagent water and carried through the entire extraction and derivatization procedure described in Section 11.

10.2.4 Inject 1 uL of each calibration standard extract into the GC and tabulate peak area response and concentration for each analyte and the internal standard. **NOTE:** The formaldehyde peak will be much larger (for the same concentration) than the other analyte peaks. The formaldehyde peak may need to be attenuated on some instruments/data systems to avoid signal saturation.

10.2.5 *(Z/E)* ISOMERS -- Two isomers, referred to as *(E)* and *(Z)*, are formed for most asymmetrical carbonyl compounds derivatized with PFBHA. Chromatographic resolution is usually obtained with the columns suggested in Section 6.6 for acetaldehyde, propanal, butanal, pentanal, hexanal, heptanal, octanal, and crotonaldehyde (see chromatograms in Fig. 1 and Fig 2). With dicarbonyl species such as glyoxal and methyl glyoxal, *(E)* and *(Z)* isomerism occurs from oxime formation with both carbonyl groups, increasing the number of isomers. The demonstration data included in this method has used two distinct isomer peaks each for glyoxal and methyl glyoxal. Use one of the following methods for both calibration and quantitation of each method analyte.

(a) Use the sum of the isomer peak areas for each constituent for both calibration and quantitation.

(b) Use the peak area of each individual isomer to independently calculate a concentration for each isomer. Then average the amount of the two isomers to report one value for the analyte.

10.2.6 Generate a calibration curve for each analyte by plotting the area ratios ($A_a/A_{is}$) against the concentration ratios ($C_a/C_{is}$) of the five calibration standards where:

$A_a$ is the peak area of the analyte (or analyte isomer pair),
$A_{is}$ is the peak area of the internal standard,
$C_a$ is the concentration of the analyte, and
$C_{is}$ is the concentration of the internal standard.

10.2.7 This curve must always be forced through zero and can be defined as either first or second order. Forcing zero allows for a better estimate of the background level of method analytes.

10.2.8 A data system is recommended to collect the chromatographic data, to calculate relative response factors, and calculate either linear or second order calibration curves.

10.2.9 VERIFICATION OF CALIBRATION STANDARD MATERIALS -- Analyze a LFB prepared from standard materials from a source other than those used to prepare the initial calibration curve (Sect. 3.8, QCS). Calculate the concentration of this QCS from the calibration curve. The calculated concentration of the QCS must agree within 60-140% of its true value. This step verifies the validity of calibration standard materials and the calibration curve prior to sample analyses.

10.3 OPTIONS FOR ON-GOING CALIBRATION

The time, temperature, pH, and PFBHA concentration will all affect the rate, efficiency and reproducibility of the derivatization reaction. It is critical that those parameters be controlled. Calibration frequency will depend upon the laboratory's ability to control these parameters so that continuing calibration check standard criteria can be met. Some laboratories may find it more productive to prepare and analyze a calibration curve with each batch of samples. A batch of samples for this methodology should not exceed 20 samples, including field samples, FRBs, laboratory duplicates, and fortified sample matrices.

10.3.1 CONTINUING CALIBRATION CHECK (CCC) OPTION--The analyst must periodically verify calibration during the analysis of samples in order to ensure accuracy of analytical results. Prepare a minimum of one low-level (suggested concentration 2-5 ug/L) and one mid-level (suggested concentration 10-30 ug/L) calibration standard with each batch of samples. Verify calibration using these two standards, prior to analyzing any of the sample extracts from the batch. In addition, reanalyze one of these two standard extracts after every tenth sample extract, and after the last sample in an analysis batch to ensure instrument stability throughout the analysis batch. Recovery must be within 70-130% of the true value for the mid-level standard, and within 50-150% of the true value for the low-level standard.

10.3.2 DAILY CALIBRATION OPTION -- The analyst may choose to create a new calibration curve for each batch of samples by preparing and analyzing a standard at all five calibration concentrations, with each batch of samples. If this option is selected, the calibration standard extracts should be analyzed prior to the analysis of sample extracts. To ensure that sensitivity

and performance of the method has not changed significantly between sample batches , or changed since the IDC, the following performance check is required. The response (peak area) of the internal standard, surrogate and each method analyte in the mid-level standard (suggested concentration 10-30 ug/L), must be within 50-150% of the mean peak area for that analyte in the initial demonstration of precision replicates (Sect. 9.2.2). One of the calibration standard extracts must be reanalyzed after every tenth sample extract, and after the last sample in an analysis batch to ensure instrument stability throughout the analysis batch. Recovery must be within 70 to 130% of the true value for mid- and high-level calibration standards, and within 50-150% of the true value for the low-level standard (suggested concentration 2-5 ug/L).

## 11. **PROCEDURE**

11.1 SAMPLE EXTRACTION -- Once samples have been opened, process the samples straight through to step 11.1.11. There is no known "safe" stopping point once sample processing has begun. Samples are derivatized and extracted in the sample bottle in which they were collected. Transferring the sample to another container for derivatization and extraction has been shown to cause a loss of method analytes.

11.1.1 Remove the samples from storage and allow them to equilibrate to room temperature.

11.1.2 Remove 10 mL of sample and discard. Mark the level of the remaining sample volume on the outside of the bottle, for later sample volume determination.

11.1.3 Add 200 mg KHP to the sample for pH adjustment.

11.1.4 Add 20 uL surrogate solution (Sect 7.11.2.2).

11.1.5 Add 1 mL of freshly prepared PFBHA Reagent as per Section 7.10.1. Cap and swirl gently to mix.

11.1.6 Place all samples in a constant-temperature water bath set at 35 ± 2 $^{o}$C for 2 hrs. Remove vials and cool to room temperature for 10 min.

11.1.7 To each vial add 0.05 mL (approximate 2 to 4 drops) of concentrated sulfuric acid. This prevents the extraction of excess reagent, which will cause chromatographic interferences.

11.1.8 Add 4 mL of hexane that contains the internal standard (as per Sect. 7.11.1.2).

11.1.9 Shake manually for 3 min. Let stand for approximately 5 min to permit phases to separate.

11.1.10 Draw off hexane layer (top layer) using a clean disposable Pasteur pipet for each sample into a smaller 8 mL vial containing 3 mL 0.2 N sulfuric acid. Shake for 30 sec and let stand for 5 min for phase separation. **NOTE:** This acid wash step further reduces the reagent and other interferants from the final extract. **Do not skip this step.**

11.1.11 Draw off top hexane layer using another clean disposable pipet for each sample and place in two 1.8 mL autosampler vials per sample. Store extra autosampler vials as a backup extract. Extracts may be stored for up to 14 days at 4 °C.

11.1.12 Sample Volume Determination -- Discard remaining water sample and hexane in each sample bottle. Fill with water to the level indicated by the mark made in Section 11.1.2. Pour the water into a 25 mL graduated cylinder and measure the volume to the nearest mL. Record the sample volume for each sample.

Alternately, if a laboratory has control over the brand and style of the sample bottles being used, the exact volume of a number of bottles from the same manufacturer and lot may be measured, and the average bottle volume minus 10 mL may be used as the sample volume for all samples using the same lot of sample bottles. A minimum of 10 % of the sample bottles obtained from the same manufacturer, from the same lot should be measured.

11.2 GAS CHROMATOGRAPHY

11.2.1 Analyze the extracts by GC/ECD. Table 1 (Sect. 17) summarizes recommended GC operating conditions and retention times observed using this method. Figure 1 illustrates the performance of the recommended primary column with the method analytes. Figure 2 illustrates the performance of the recommended confirmation column with the method analytes. Other GC columns or chromatographic conditions may be used if the requirements of Section 9 are met.

11.2.2 The width of the retention time window used to make identifications should be based on measurements of actual retention time variations of standards over the course of time. Plus or minus three times the standard deviation of the retention time for a compound can be used to calculate a suggested window size; however the experience of the analyst should weigh heavily in the interpretation of chromatograms.

11.2.3 If an analyte peak area exceeds the range of the calibration curve, the extract may be diluted with the hexane extraction solvent (that contains the internal standard) and reanalyzed. Incorporate the dilution factor into final concentration calculations. The analyst must not extrapolate beyond the calibration range established.

## 12. DATA ANALYSIS AND CALCULATIONS

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 (or isomer peaks) in a calibration standard or the laboratory fortified blank.

12.2 Calculate the analyte concentrations using the first or second order calibration curves generated as described in Section 10.

12.3 For any analytes that are found, adjust the calculated concentration to reflect the true sample volume determined in Section 11.1.12.

12.4 Prior to reporting the data, the chromatogram should be reviewed for any incorrect peak identification or poor integration. If a confirmation column has been used, all identifications should be verified using the retention time data from that analysis. In addition, the *(Z/E)* isomer ratio should be within 50% of the ratio observed in standards. If the *(Z/E)* ratio does not meet these criteria, it is likely that an interferant occurred at the retention time of one of the isomer peaks. In this case, the amount indicated by the lower of the 2 isomer peaks should be reported. (This may require that the analyst recalculate the analyte amount using individual isomer peaks for quantitation.) If one peak of the isomeric pair is missing, the identification is not confirmed and should not be reported.

12.5 Analyte concentrations are reported in ug/L.

## 13. METHOD PERFORMANCE

13.1 Precision and accuracy data are presented in Section 17. Data are presented for three water matrices: reagent water (Table 2), chlorinated “finished” surface water (Table 4) , and untreated “raw” surface water (Table 5). These data, as well as the MDL data in Table 3, were generated in two laboratories. Data in Table 2 and column A of Table 3, were generated in one laboratory, while data in column B of Table 3 and in Tables 4 and 5 were generated in a second laboratory. Method performance in both laboratories was similar.

13.2 DERIVATIZATION PARAMETERS -- This method is a procedural standard method that will generate accurate and precise results when used as written. The time, temperature, pH, and PFBHA concentration will all affect the rate, efficiency

and reproducibility of the derivatization reaction. It is critical that those parameters be controlled. Calibration frequency will depend upon the laboratory's ability to control these parameters. Some laboratories may need to prepare and analyze a calibration curve with each batch of samples. Of all the method analytes, glyoxal, methyl glyoxal, benzaldehyde, crotonaldehyde and cyclohexanone are the most difficult to derivatize. Poor sensitivity for any of these compounds indicates that there may be a problem with the reaction conditions. Measurements of nonanal, decanal, glyoxal and methyl glyoxal appear to be less precise than the measurement of other analytes.

13.3 The importance of low background levels of formaldehyde and acetaldehyde cannot be overemphasized. Some laboratories or reagent waters may also contain background amounts of other method analytes. Care must be taken to avoid reporting false positive results that result from background contamination.

13.4 The importance of proper sample collection and preservation also cannot be overemphasized. Holding time studies in various matrices showed better than 70% recovery of all method analytes when samples were collected, preserved, and stored according to Section 8, and analyzed within 7 days. There were variations in the recovery of analytes from fortified samples from different matrices. Therefore, it is strongly recommended that samples be analyzed as soon as possible after collection. The data in Section 17, Table 6 illustrates the dramatic difference between a preserved and a non-preserved sample.

13.5 Data for crotonaldehyde is not listed in Section 17, Tables 4-6, because it was not included in the standard mixtures being used at the time that those data were collected. However, crotonaldehyde was included in many other studies not presented here, and its performance was similar to other method analytes.

## 14. POLLUTION PREVENTION

14.1 This method uses a micro-extraction procedure which requires very small quantities of organic solvents.

14.2 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 N.W., Washington, D.C. 20036.

## 15. WASTE MANAGEMENT

15.1 Due to the nature of this method tHere is little need for waste management. Only small volumes of solvents are used. The matrices of concern are finished drinking water or source 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 14.2.

## 16. REFERENCES


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

### TABLE 1. CHROMATOGRAPHIC CONDITIONS AND RETENTION DATA FOR ANALYTE DERIVATIVES

| | Column A | | Column B | |
|---|---|---|---|---|
| ANALYTE | RT (min) | Peak #, Fig 1 | RT (min) | Peak #, Fig.2 |
| Formaldehyde | 10.69 | 2 | 13.35 | 2 |
| Acetaldehyde (E) | 14.23 | 3 | 16.71 | 3 |
| Acetaldehyde (Z) | 14.49 | 4 | 17.00 | 4 |
| Propanal (E) | 17.36 | 5 | 19.59 | 5 |
| Propanal (Z) | 17.62 | 6 | 19.86 | 6 |
| Butanal (E) | 20.58 | 7 | 22.62 | 7 |
| Butanal (Z) | 20.81 | 8 | 22.88 | 8 |
| Crotonaldehyde (E) | 22.67 | 9 | 24.79 | not shown |
| Crotonaldehyde (Z) | 23.00 | 10 | 25.31 | not shown |
| Pentanal (E) | 23.82 | 11 | 25.54 | 9 |
| Pentanal (Z) | 24.01 | 12 | 25.70 | 10 |
| Hexanal (E) | 26.99 | 13 | 28.74 | 11 |
| Hexanal (Z) | 27.16 | 14 | 28.93 | 12 |
| Cyclohexanone | 29.42 | 15 | 31.40 | 13 |
| Heptanal (E) | 30.05 | 16 | 31.64 | 14 |
| Heptanal (Z) | 30.16 | 17 | 31.78 | 15 |
| Octanal (E) | 33.01 | 19 | 34.45 | 16 |
| Octanal (Z) | 33.09 | 20 | 34.57 | 18 |
| Benzaldehyde | 33.88 | 21 | 36.74 | 19 |
| Nonanal (E) | 35.89 | 22 | 37.18 | 20 |
| Decanal | 38.63 | 23 | 39.80 | 21 |

| | Column A | | Column B | |
|---|---|---|---|---|
| ANALYTE | RT (min) | Peak #, Fig 1 | RT (min) | Peak #, Fig.2 |
| Glyoxal (peak1) | 40.87 | 24 | 43.74 | 22 |
| Glyoxal (peak 2) | 41.09 | 25 | 43.86 | 23 |
| Methyl glyoxal (peak 1) | 41.22 | 26 | 43.86 | 24 |
| Methyl glyoxal (peak 2) | 41.88 | 27 | 44.38 | 25 |
| 1.2 dibromopropane (Internal standard) | 6.14 | 1 | 7.84 | 1 |
| 2',4',5' trifluoro-acetophenone (Surrogate) | 32.84 | 18 | 34.45 | 17 |

Column A- DB-5ms, 30 m x 0.25mm i.d., 0.25 um film thickness, injector temp. 220 °C, head pressure 15 psi, detector temp. 300 °C, splitless injection, 1 min split delay. Temperature program: 50 °C for 1 min, program at 4 °C/min to 220 °C, program at 20 °C/min to 250 °C and hold at 250 °C for 10 min. (Figure 1)

Column B- Rtx-1701, 30 m x 0.25 mm i.d., 0.25 um film thickness, injector temp. 180 °C, head pressure 15 psi, detector temp. 300 °C,splitless injection, 1 min split delay. Temperature program: 50 °C for 1 min, program at 4 °C/min to 220 °C, program at 20 °C/min to 250 °C and hold at 250 °C for 10 min. (Figure 2)

Carrier gas- Helium

Detector gas- P5 Argon/Methane

**TABLE 2. PRECISION AND ACCURACY IN REAGENT WATER (n=8)** [a]

| ANALYTE | Fortified Conc. (ug/L) | Mean Conc. Measured (ug/L) | Standard Deviation | Relative Std Dev (%) | Mean Accuracy (%) |
|---|---|---|---|---|---|
| Formaldehyde | 20.0 | 19.5 | 0.792 | 4.0 | 98 |
| Acetaldehyde | 20.0 | 19.2 | 0.784 | 4.1 | 96 |
| Propanal | 20.0 | 19.5 | 0.873 | 4.5 | 97 |
| Butanal | 20.0 | 19.8 | 1.12 | 5.6 | 99 |
| Crotonaldehyde | 20.0 | 19.8 | 1.11 | 5.6 | 99 |
| Pentanal | 20.0 | 20.0 | 1.10 | 5.5 | 100 |
| Hexanal | 20.0 | 19.7 | 1.25 | 6.4 | 98 |
| Cyclohexanone | 20.0 | 19.2 | 0.729 | 3.8 | 96 |
| Heptanal | 20.0 | 19.2 | 1.37 | 7.1 | 98 |
| Octanal | 20.0 | 19.1 | 1.32 | 6.9 | 95 |
| Benzaldehyde | 20.0 | 19.1 | 0.362 | 1.9 | 95 |
| Nonanal | 20.0 | 18.8 | 1.09 | 5.8 | 94 |
| Decanal | 20.0 | 18.7 | 1.20 | 6.4 | 93 |
| Glyoxal | 20.0 | 18.4 | 0.571 | 3.1 | 92 |
| Methyl glyoxal | 20.0 | 17.9 | 1.36 | 7.6 | 90 |

a- Analyzed over 2 days on the primary chromatographic column (DB-5ms).

**TABLE 3. METHOD DETECTION LIMITS IN REAGENT WATER (n=8)**

| ANALYTE | Fortified Conc. (ug/L) | MDL(ug/L) Column A | MDL(ug/L) Column B |
|---|---|---|---|
| Formaldehyde | 2.0 | 0.36 | 0.11 |
| Acetaldehyde | 2.0 | 0.21 | 0.14 |
| Propanal | 2.0 | 0.41 | 0.06 |
| Butanal | 2.0 | 0.35 | 0.12 |
| Crotonaldehyde | 2.0 | 0.28 | 0.09 |
| Pentanal | 2.0 | 0.47 | 0.17 |
| Hexanal | 2.0 | 0.42 | 0.35 |
| Cyclohexanone | 2.0 | 0.29 | 0.10 |
| Heptanal | 2.0 | 0.43 | 0.71 |
| Octanal | 2.0 | 0.60 | 0.12 |
| Benzaldehyde | 2.0 | 0.31 | 0.06 |
| Nonanal | 2.0 | 0.74 | 0.40 |
| Decanal | 2.0 | 1.0 | 0.82 |
| Glyoxal | 2.0 | 0.59 | 0.21 |
| Methyl glyoxal | 2.0 | 0.81 | 0.22 |

Column A= DB-5ms

Column B= Rtx-1701

**TABLE 4. PRECISION AND ACCURACY IN CHLORINATED TAP WATER(n=4)** [a]

| ANALYTE | Fortified Conc. (ug/L) | Mean Conc. Measured (ug/L) | Standard Deviation | Relative Std Dev (%) | Mean Accuracy (%) |
|---|---|---|---|---|---|
| Formaldehyde | 20.0 | 21.6 | 0.197 | 0.9 | 108 |
| Acetaldehyde* | 20.0 | 19.6 | 0.263 | 1.3 | 98 |
| Propanal | 20.0 | 19.5 | 0.178 | 0.9 | 98 |
| Butanal | 20.0 | 20.2 | 0.254 | 1.3 | 101 |
| Pentanal | 20.0 | 20.3 | 0.318 | 1.6 | 101 |
| Hexanal | 20.0 | 20.2 | 0.382 | 1.9 | 101 |
| Cyclohexanone | 20.0 | 20.7 | 0.247 | 1.2 | 103 |
| Heptanal | 20.0 | 20.5 | 3.073 | 2.5 | 103 |
| Octanal | 20.0 | 20.3 | 0.979 | 4.8 | 102 |
| Benzaldehyde | 20.0 | 21.2 | 0.432 | 2.0 | 106 |
| Nonanal | 20.0 | 20.0 | 0.828 | 4.2 | 100 |
| Decanal | 20.0 | 19.8 | 1.203 | 6.1 | 99 |
| Glyoxal | 20.0 | 22.3 | 0.783 | 3.5 | 112 |
| Methyl glyoxal | 20.0 | 21.0 | 0.775 | 3.7 | 105 |

a- Analyzed on the primary chromatographic column (DB-5ms).
*- Values taken from the confirmation column.

**TABLE 5. PRECISION AND ACCURACY IN UNTREATED SURFACE WATER (n=4)** [a]

| ANALYTE | Fortified Conc. (ug/L) | Mean Conc. Measured (ug/L) | Standard Deviation | Relative Std Dev (%) | Mean Accuracy (%) |
|---|---|---|---|---|---|
| Formaldehyde | 20.0 | 19.4 | 0.941 | 4.8 | 97 |
| Acetaldehyde* | 20.0 | 20.0 | 0.891 | 4.4 | 100 |
| Propanal | 20.0 | 19.5 | 0.966 | 5.0 | 97 |
| Butanal | 20.0 | 18.9 | 0.965 | 5.1 | 94 |
| Pentanal | 20.0 | 18.7 | 1.00 | 5.4 | 93 |
| Hexanal | 20.0 | 18.2 | 1.080 | 6.0 | 91 |
| Cyclohexanone | 20.0 | 17.3 | 0.943 | 5.4 | 87 |
| Heptanal | 20.0 | 18.0 | 0.995 | 5.5 | 90 |
| Octanal | 20.0 | 18.3 | 1.017 | 5.6 | 92 |
| Benzaldehyde | 20.0 | 17.2 | 1.118 | 6.5 | 86 |
| Nonanal | 20.0 | 18.4 | 0.881 | 4.8 | 92 |
| Decanal | 20.0 | 18.1 | 0.908 | 5.0 | 90 |
| Glyoxal | 20.0 | 20.5 | 1.10 | 5.4 | 102 |
| Methyl glyoxal | 20.0 | 23.3 | 0.799 | 3.4 | 116 |

a- Analyzed on the primary chromatographic column (DB-5ms).
*- Values taken from the confirmation column.

**TABLE 6. HOLDING TIME DATA FOR SAMPLES FROM AN UNTREATED SURFACE WATER SOURCE, FORTIFIED WITH METHOD ANALYTES AT 20 ug/L, WITH AND WITHOUT COPPER SULFATE BIOCIDE**

| | % Recovery without Copper Sulfate | | | | % Recovery with Copper Sulfate | | | |
|---|---|---|---|---|---|---|---|---|
| **Analyte** | Day 0 | Day 6 | Day 14 | Day 21 | Day 0 | Day 6 | Day 14 | Day 21 |
| Formaldehyde | 104 | 144 | 569 | 619 | 105 | 105 | 109 | 106 |
| Acetaldehyde | 96 | 23 | 21 | 24 | 98 | 99 | 103 | 98 |
| Propanal | 94 | 21 | 19 | 22 | 98 | 98 | 103 | 96 |
| Butanal | 92 | 20 | 18 | 21 | 99 | 98 | 102 | 91 |
| Pentanal | 87 | 19 | 16 | 21 | 96 | 98 | 100 | 94 |
| Hexanal | 83 | 21 | 17 | 22 | 93 | 97 | 100 | 92 |
| Cyclohexanone | 94 | 99 | 84 | 78 | 96 | 101 | 98 | 94 |
| Heptanal | 83 | 20 | 16 | 17 | 96 | 94 | 97 | 91 |
| Octanal | 82 | 18 | <10 | 11 | 99 | 96 | 96 | 93 |
| Benzaldehyde | 94 | 83 | 74 | 79 | 98 | 100 | 104 | 92 |
| Nonanal | 72 | 15 | <10 | <10 | 104 | 98 | 92 | 84 |
| Decanal | 50 | <10 | <10 | <10 | 107 | 101 | 93 | 82 |
| Glyoxal | 103 | 98 | 37 | <10 | 106 | 108 | 106 | 90 |
| Methyl glyoxal | 108 | 68 | <10 | <10 | 111 | 105 | 94 | 73 |

- All samples were stored headspace free at 4 °C.
- Values at all time points are the mean of 5 replicate analyses. RSDs for replicate analyses of samples containing copper sulfate were <10%. RSDs for unpreserved samples were higher due to the degradation process.

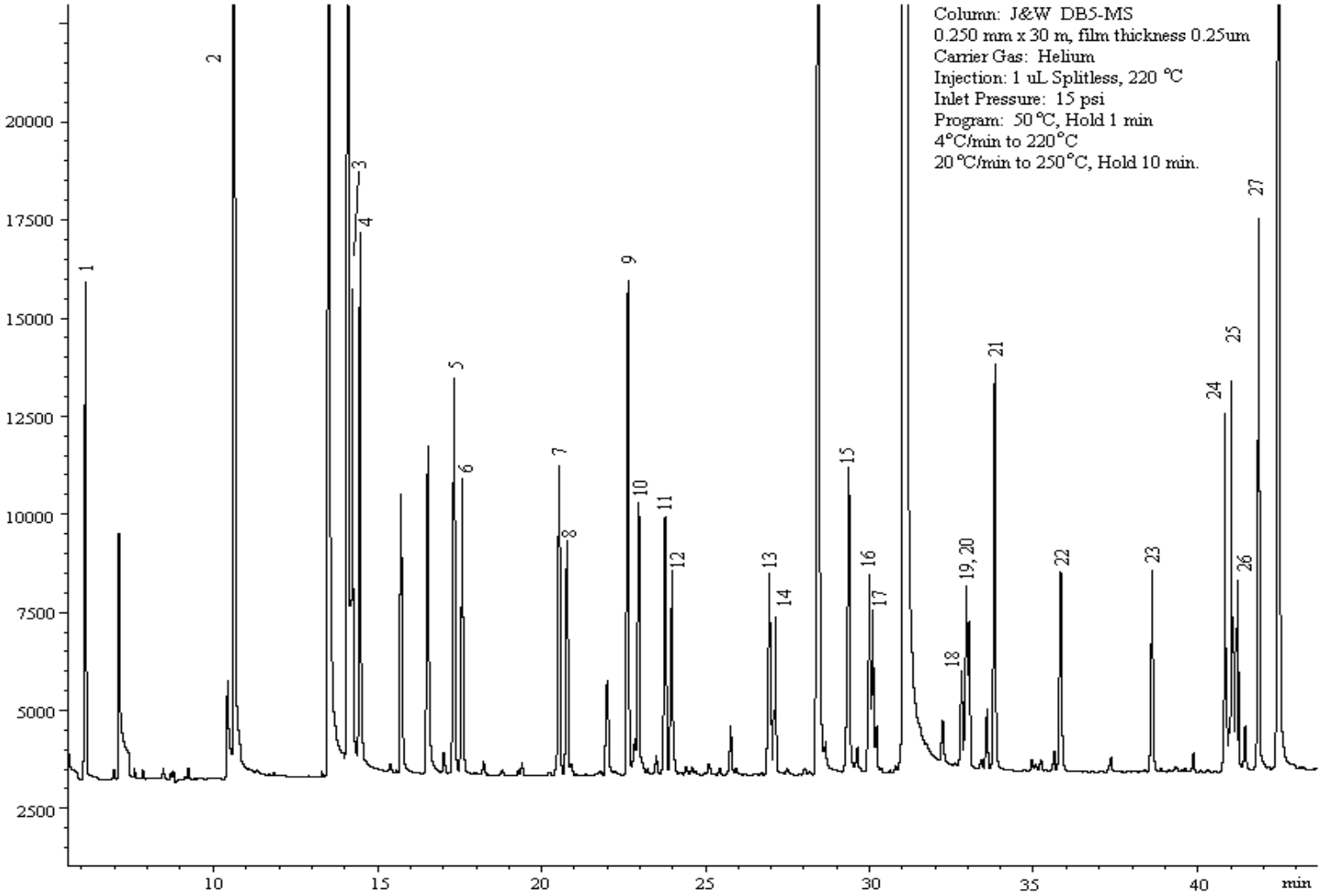
**TABLE 7. INITIAL DEMONSTRATION OF CAPABILITY REQUIREMENTS**

| Reference | Requirement | Specification and Frequency | Acceptance Criteria |
|---|---|---|---|
| **Sect. 9.3** | Initial Demonstration of Low System Background | Analyze method blank and determine that all target analytes are below 1/2 the MRL prior to performing IDC | The LRB concentration must be ≤ 1/2 of the intended MRL |
| **Sect 9.2.2** | Initial Demonstration of Precision (IDP) | Analyze 4-7 replicate LRBs fortified at 20.0 ug/L (or mid cal.) on at least 2 different days | RSD must be ≤ 20 % |
| **Sect. 9.2.3** | Initial Demonstration of Accuracy | Calculate average recovery of IDP replicates | Mean recovery ± 20% of true value |
| **Sect. 9.2.4** | Method Detection Limit (MDL) Determination | a) select a fortifying level at 2 - 5 x the noise level<br>b) analyze 7 replicates in reagent water taken thru all steps<br>c) calculate MDL via equation - do not subtract blank<br>d) replicate extractions and analyses must be conducted over at least 3 days | |
| **Sect. 9.2.5** | Minimum Reporting Levels (MRLs) | MRLs should be established for all analytes during IDC, and be updated as additional LRB data is available. | Establish the MRL for each analyte, as the LRB concentration + 3σ or 3 times the mean LRB concentration, whichever is greater. |

**TABLE 8. QUALITY CONTROL REQUIREMENTS (SUMMARY)**

| Reference | Requirement | Specification and Frequency | Acceptance Criteria |
|---|---|---|---|
| **Sect. 10.2** | Initial Calibration | Use internal standard technique to generate curve with five standards that span the approximate range of 2-40 ug/L. First or second order curves must be forced through zero. Either sum E/Z isomer areas or average the amount of each isomer. Calculate E/Z ratios for analytes. Run QCS. | QCS must agree within 60 -140 %.<br><br>Lowest concentration should be near MRL. |
| **Sect. 9.3** | Laboratory Reagent Blank (LRB) | Include LRB with each extraction batch (up to 20 samples). Analyze prior to analyzing samples and determine to be free of interferences. | All analytes < 1/2 MRL |
| **Sect. 10.3.1** | Continuing Calibration Check (CCC) Option | Verify initial calibration by running CCCs prior to analyzing samples, after 10 samples, and after the last sample. | Recovery for mid-level CCC must be 70-130% of the true value, recovery for low level must be 50-150% of the true value. |
| **Sect. 10.3.2** | Daily Calibration Option | Calibrate daily, but verify that sensitivity and performance have not changed significantly since IDC. | Peak areas for IS, SUR, and method analytes for mid-level CAL std must be +/- 50% of the peak areas obtained for that CAL std during IDC. |
| **Sect. 8.4** | Field Reagent Blanks (FRB) | 1 per shipping batch | All analytes < 1/2 MRL |
| **Sect. 9.5** | Internal Standard (IS) | 1,2-Dibromopropane is added to all samples, blanks and standards | IS area counts must be 70 - 130% of the average initial calibration area counts |

| Reference | Requirement | Specification and Frequency | Acceptance Criteria |
|---|---|---|---|
| **Sect. 9.6** | Surrogate Standard (SUR) | 2',4',5' -Trifluoroacetophenone is added samples, blanks and standards | Surrogate recovery must be 70 - 130 % of the true value. |
| **Sect. 9.7** | Laboratory Fortified Sample Matrix (LFM) | Fortify at least one sample per analysis batch (20 samples or less) at a concentration close to that in the native sample. | Recoveries not within 70-130% may indicate matrix effect |
| **Sect. 9.8** | Field Duplicates | Extract and analyze at least one duplicate sample with every analysis batch (20 samples or less) | Suggested RPD ≤ 30 % |
| **Sect. 9.9** | Quality Control Sample (QCS) | Analyze a QCS at least quarterly from an external/second source. | QCS must agree within 60 -140 %. |
| **Sect.9.10, Sect. 10.2.5 and Sect. 12.4** | E/Z Isomer Ratio Agreement | Calculate the E/Z isomer ratio for target analytes and compare to E/Z ratio in initial calibration | E/Z ratio in standards, blanks, and samples must be within ± 50% of E/Z ratio in initial calibration.<br><br>Do not report value if one isomer is missing. |
| **Sect. 8.3.1** | Sample Holding Time | Properly preserved samples may be stored in the dark at 4 °C for 7 days. | Do not report data for samples that have exceeded their holding time, or that have not been properly preserved or stored. |
| **Sect. 8.3.2** | Extract Holding Time | Extracts may be stored in the dark at 4 °C for 14 days. | Do not report data for extracts that have exceeded their holding time. |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Carrier Gas: Helium
Injection: 1 uL Splitless, 220 °C
Inlet Pressure: 15 psi
Program: 50 °C, Hold 1 min
4°C/min to 220°C
20 °C/min to 250°C, Hold 10 min.
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Figure 1

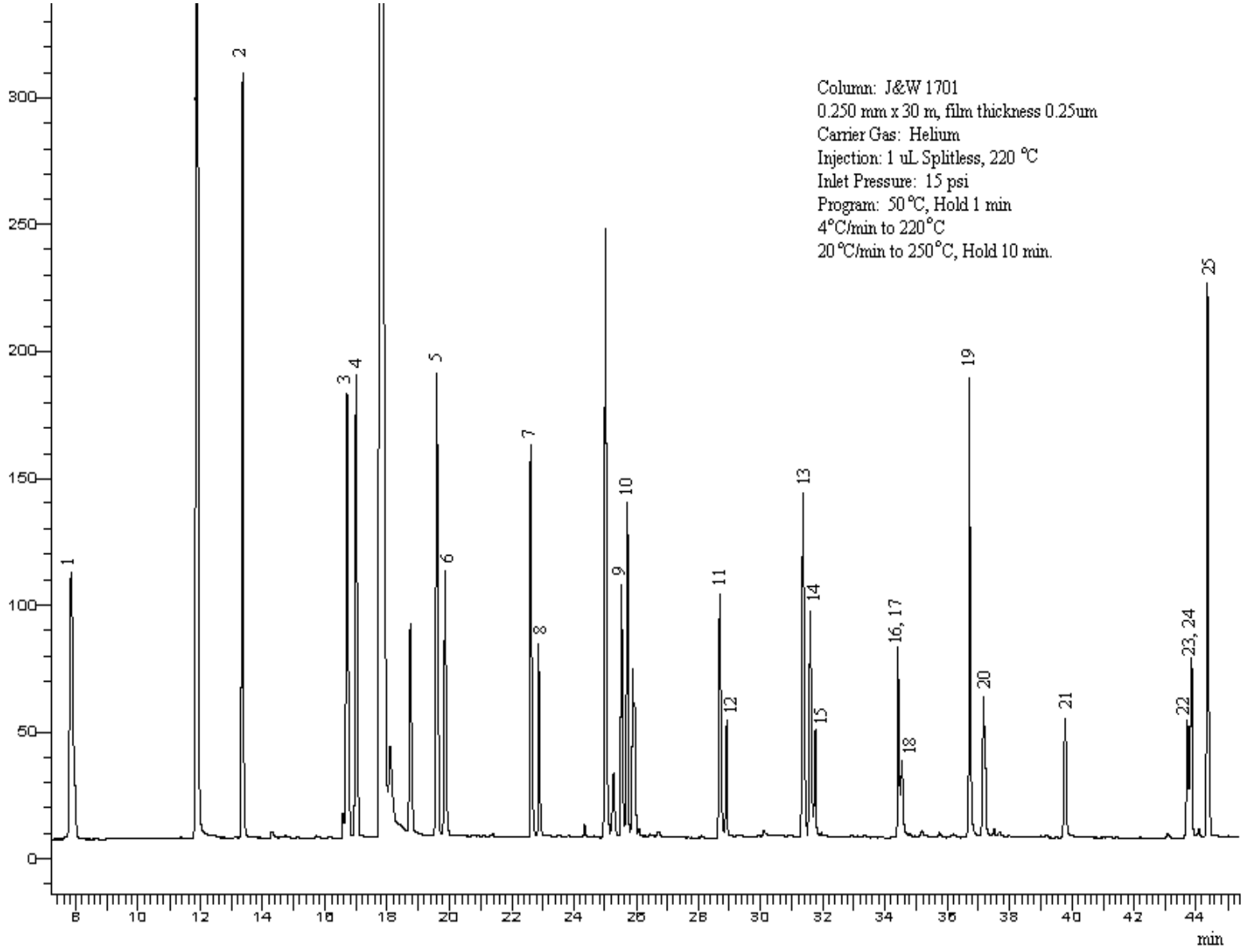
Column: J&W 1701
0.250 mm x 30 m, film thickness 0.25um
Carrier Gas: Helium
Injection: 1 uL Splitless, 220 °C
Inlet Pressure: 15 psi
Program: 50 °C, Hold 1 min
4°C/min to 220°C
20 °C/min to 250°C, Hold 10 min.
min

Figure 2