METHOD 8315A

DETERMINATION OF CARBONYL COMPOUNDS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

1.0 SCOPE AND APPLICATION

1.1 This method provides procedures for the determination of free carbonyl compounds in various matrices by derivatization with 2,4-dinitrophenylhydrazine (DNPH). The method utilizes high performance liquid chromatography (HPLC) with ultraviolet/visible (UV/vis) detection to identify and quantitate the target analytes. This method includes two procedures encompassing all aspects of the analysis (extraction to determination of concentration). Procedure 1 is appropriate for the analysis of aqueous, soil and waste samples and stack samples collected by Method 0011. Procedure 2 is appropriate for the analysis of indoor air samples collected by Method 0100. The list of target analytes differs by procedure. The appropriate procedure for each target analyte is listed in the table below.

Compound	CAS No.[a]	Proc. 1[b]	Proc. 2[b]
Acetaldehyde	75-07-0	X	X
Acetone	67-64-1		X
Acrolein	107-02-8		X
Benzaldehyde	100-52-7		X
Butanal (Butyraldehyde)	123-72-8	X	X
Crotonaldehyde	123-73-9	X	X
Cyclohexanone	108-94-1	X	
Decanal	112-31-2	X	
2,5-Dimethylbenzaldehyde	5779-94-2		X
Formaldehyde	50-00-0	X	X
Heptanal	111-71-7	X	
Hexanal (Hexaldehyde)	66-25-1	X	X
Isovaleraldehyde	590-86-3		X
Nonanal	124-19-6	X	
Octanal	124-13-0	X	
Pentanal (Valeraldehyde)	110-62-3	X	X
Propanal (Propionaldehyde)	123-38-6	X	X
m-Tolualdehyde	620-23-5	X	X
o-Tolualdehyde	529-20-4		X
p-Tolualdehyde	104-87-0		X

[a] Chemical Abstract Service Registry Number.

[b] The two procedures have overlapping lists of target compounds that have been evaluated using modifications of the analysis. Refer to the respective procedure number when choosing the appropriate analysis technique for a particular compound.

1.2 Method detection limits (MDL) using procedure 1 are listed in Tables 1 and 2. Sensitivity data for sampling and analysis by use of Method 0100 (procedure 2) are given in Table 3. The MDL

for a specific sample may differ from the listed value, depending upon the interferences in the sample matrix and the volume of sample used in the procedure.

1.3 The extraction procedure for solid samples is outlined in Sec. 7.1 of this method.

1.4 When this method is used to analyze unfamiliar sample matrices, compound identification should be supported by at least one additional qualitative technique. A gas chromatograph/mass spectrometer (GC/MS) may be used for the qualitative confirmation of results for the target analytes, using the extract produced by this method.

1.5 This method is restricted to use by, or under the supervision of, analysts experienced in the use of chromatography and in the interpretation of chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method.

2.0 SUMMARY OF METHOD

This method contains two procedures dealing with different sample types.

2.1 Liquid and Solid Samples (Procedure 1)

2.1.1 For wastes comprised of solids, and aqueous wastes containing greater than one percent solid material, the aqueous phase should be separated from the solid phase and stored, according to Sec. 6.2, for possible later analysis. If necessary, the particle size of the solids in the waste is reduced. The solid phase is extracted with a volume of fluid equal to 20 times the sample's weight. The extraction fluid employed is a function of the alkalinity of the solid phase of the waste. A special extractor is used when volatiles are being extracted. Following extraction, the extract is filtered through a 0.6 - 0.8 µm glass fiber filter.

2.1.2 If compatible (i.e., multiple phases will not form on combination), the initial aqueous phase of the waste is added to the aqueous extract, and these liquids are analyzed together. If incompatible, the liquids are analyzed separately and the results are mathematically combined to yield a volume-weighted average concentration.

2.1.3 A measured volume of aqueous sample (approx. 100 mL) or an appropriate amount of solids extract (approx. 25 g), is buffered to pH 3 and derivatized with 2,4-dinitrophenylhydrazine (DNPH), using either the appropriate solid-phase or a liquid-liquid extraction technique. If the solid-phase extraction (SPE) option is used, the derivatized compound is extracted using solid sorbent cartridges, then eluted with ethanol. If the liquid-liquid option is used, the derivatized compound is serially extracted three (3) times with methylene chloride. The methylene chloride extracts are concentrated using the appropriate procedure 3500 series method and exchanged with acetonitrile prior to HPLC analysis. HPLC conditions are described which permit the separation and measurement of various carbonyl compounds in the extract by absorbance detection at 360 nm.

2.1.4 If formaldehyde is the only analyte of interest, the aqueous sample and/or solid sample extract should be buffered to pH 5.0 to minimize the formation of artifact formaldehyde.

2.2 Stack Gas Samples Collected by Method 0011 (Procedure 1): The entire sample returned to the laboratory is extracted with methylene chloride and the extract is diluted or concentrated to a known volume. An aliquot of the methylene chloride extract is solvent exchanged

and concentrated or diluted as necessary. HPLC conditions are described that permit the separation and measurement of various carbonyl compounds in the extract by absorbance detection at 360 nm.

2.3 Indoor Air Samples by Method 0100 (Procedure 2): The sample cartridges are returned to the laboratory and backflushed with acetonitrile into a 5-mL volumetric flask. The eluate is diluted to volume with acetonitrile. Two aliquots of the acetonitrile extract are pipetted into two sample vials having polytetrafluoroethylene (PTFE)-lined septa. HPLC conditions are described which allow for the separation and measurement of the various carbonyl compounds in the extract by absorbance detection at 360 nm.

3.0 INTERFERENCES

3.1 Method interferences may be caused by contaminated solvents, reagents, glassware, and other sample processing hardware which can lead to discrete artifacts and/or elevated chromatogram baselines. All materials should routinely demonstrate freedom from interferences under analysis conditions by analyzing laboratory reagent blanks as described in Sec. 8.5.

3.1.1 Glassware must be scrupulously cleaned. Glassware should be rinsed as soon as possible after use with the last solvent used. This should be followed by detergent washing with hot water, and rinses with tap water and organic-free reagent water. After washing the glassware should then be drained, dried, and heated in a laboratory oven at 130°C for two to three hours before reuse. Solvent rinses with acetonitrile may be substituted for the oven heating. After drying and cooling, glassware should be stored in a clean environment to prevent accumulation of dust or other contaminants.

NOTE: Do not rinse glassware with acetone or methanol. These solvents react with DNPH to form interferences.

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

3.1.3 Polyethylene gloves must be worn when handling silica gel cartridges to reduce the possibility of contamination.

3.2 Formaldehyde contamination of the DNPH reagent is frequently encountered due to its widespread occurrence in the environment. The DNPH reagent in Procedure 2 must be purified by multiple recrystallizations in HPLC-grade acetonitrile. Recrystallization is accomplished, at 40 - 60°C, by slow evaporation of the solvent to maximize crystal size. The purified DNPH crystals are stored under HPLC-grade acetonitrile. Impurity levels of carbonyl compounds in the DNPH are determined prior to sample analysis and should be less than 25 mg/L. Refer to Appendix A for the recrystallization procedure.

3.3 Matrix interferences may be caused by contaminants co-extracted from the sample. The extent of matrix interferences will be source- and matrix- specific. If interferences occur in subsequent samples, modification of the mobile phase or some additional cleanup may be necessary.

3.4 In Procedure 1, acetaldehyde is generated during the derivatization step if ethanol is present in the sample. This background will impair the measurement of acetaldehyde levels below 0.5 ppm (500 ppb).

3.5 For Procedure 2, at the stated two column analysis conditions, the identification and quantitation of butyraldehyde may be difficult due to coelution with isobutyraldehyde and methyl ethyl ketone. Precautions should be taken and adjustment of the analysis conditions should be made to avoid potential problems.

4.0 APPARATUS AND MATERIALS

4.1 High performance liquid chromatograph (modular).

4.1.1 Pumping system - Gradient, with constant flow control capable of 1.50 mL/min.

4.1.2 High pressure injection valve with 20-µL loop.

4.1.3 Column - 250 mm x 4.6 mm ID, 5-µm particle size, C18 (Zorbax or equivalent).

4.1.4 Absorbance detector - 360 nm.

4.1.5 Strip-chart recorder compatible with detector - Use of a data system for measuring peak areas and retention times is recommended.

4.1.6 Helium - for degassing mobile phase solvents (Procedures 1 and 2).

4.1.7 Mobile phase reservoirs and suction filtration apparatus - For holding and filtering HPLC mobile phase. Filtering system should be all glass and PTFE and use a 0.22 µm polyester membrane filter.

4.1.8 Syringes - for HPLC injection loop loading, with capacity at least four times the loop volume.

4.2 Apparatus and materials for Procedure 1

4.2.1 Reaction vessel - 250-mL Florence flask.

4.2.2 Separatory funnel - 250-mL, with PTFE stopcock.

4.2.3 Kuderna-Danish (K-D) apparatus - See Method 3510 or other appropriate 3500 series method. (Other concentration apparatus may be employed if the laboratory can demonstrate equivalent performance).

4.2.4 Boiling chips - Solvent-extracted with methylene chloride, approximately 10/40 mesh (silicon carbide or equivalent).

4.2.5 pH meter - Capable of measuring to 0.01 pH units.

4.2.6 Glass fiber filter paper - 1.2 µm pore size (Fisher Grade G4 or equivalent).

4.2.7 Solid sorbent cartridges - Packed with 2 g C18 (Baker or equivalent).

4.2.8 Vacuum manifold - Capable of simultaneous extraction of up to 12 samples (Supelco or equivalent).

4.2.9 Sample reservoirs - 60-mL capacity (Supelco or equivalent).

4.2.10 Pipet - Capable of accurately delivering 0.10 mL solution.

4.2.11 Water bath - Heated, with concentric ring cover, capable of temperature control (± 2°C). The bath should be used in a hood.

4.2.12 Sample shaker - Controlled temperature incubator (± 2°C) with orbital shaking (Lab-Line Orbit Environ-Shaker Model 3527 or equivalent).

4.2.13 Syringes - 5-mL, 500-µL, 100-µL, (Luer-Lok or equivalent).

4.2.14 Syringe filters - 0.45-µm filtration disks (Gelman Acrodisc 4438 or equivalent).

4.3 Apparatus and materials for Procedure 2

4.3.1 Syringes - 10-mL, with Luer-Lok type adapter, used to backflush the sample cartridges by gravity feed.

4.3.2 Syringe rack - made of an aluminum plate with adjustable legs on all four corners. Circular holes of a diameter slightly larger than the diameter of the 10-mL syringes are drilled through the plate to allow batch processing of cartridges for cleaning, coating, and sample elution. A plate (0.16 x 36 x 53 cm) with 45 holes in a 5 x 9 matrix is recommended. See Figure 2 in Method 0100.

4.4 Volumetric flasks - 5-mL, 10-mL, and 250- or 500-mL.

4.5 Vials - 10- or 25-mL, glass with PTFE-lined screw caps or crimp tops.

4.6 Balance - Analytical, capable of accurately weighing to 0.0001 g.

4.7 Glass funnels

4.8 Polyethylene gloves - used to handle silica gel cartridges.

5.0 REAGENTS

5.1 Reagent grade inorganic chemicals shall 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.

5.2 Organic-free reagent water - Water in which an interferant is not observed at the method detection limit for the compounds of interest.

5.3 Formalin - Solution of formaldehyde (CH_2O) in organic-free reagent water, nominally 37.6 percent (w/w). Exact concentration will be determined for the stock solution in Sec. 5.7.1.1.

5.4 Aldehydes and ketones - analytical grade, used for preparation of DNPH derivative standards of target analytes other than formaldehyde. Refer to the target analyte list.

5.5 Procedure 1 reagents

5.5.1 Methylene chloride, CH_2Cl_2 - HPLC grade or equivalent.

5.5.2 Acetonitrile, CH_3CN - HPLC grade or equivalent.

5.5.3 Sodium hydroxide solutions, NaOH, 1.0 N and 5 N.

5.5.4 Sodium chloride, NaCl, saturated solution - Prepare by dissolving an excess of reagent grade solid in organic-free reagent water.

5.5.5 Sodium sulfite solution, Na_2SO_3, 0.1 M.

5.5.6 Sodium sulfate, Na_2SO_4 - granular, anhydrous.

5.5.7 Citric acid, $C_8H_8O_7$, 1.0 M solution.

5.5.8 Sodium citrate, $C_6H_5Na_3O_7 \bullet 2H_2O$, 1.0 M trisodium salt dihydrate solution.

5.5.9 Acetic acid (glacial), CH_3CO_2H.

5.5.10 Sodium acetate, CH_3CO_2Na.

5.5.11 Hydrochloric acid, HCl, 0.1 N.

5.5.12 Citrate buffer, 1 M, pH 3 - Prepare by adding 80 mL of 1 M citric acid solution to 20 mL of 1 M sodium citrate solution. Mix thoroughly. Adjust pH with NaOH or HCl as needed.

5.5.13 pH 5.0 Acetate buffer (5M) - Formaldehyde analysis only. Prepared by adding 40 mL 5M acetic acid solution to 60 mL 5M sodium acetate solution. Mix thoroughly. Adjust pH with NaOH or HCl as needed.

5.5.14 2,4-Dinitrophenylhydrazine, $[2,4\text{-}(O_2N)_2C_6H_3]NHNH_2$ (DNPH), 70% in organic-free reagent water (w/w). Prepare a 3.00 mg/mL solution by dissolving 428.7 mg of 70% (w/w) DNPH solution in 100 mL of acetonitrile.

5.5.15 Extraction fluid for Procedure 1 - Dilute 64.3 mL of 1.0 N NaOH and 5.7 mL glacial acetic acid to 900 mL with organic-free reagent water. Dilute to 1 liter with organic-free reagent water. The pH should be 4.93 ± 0.02.

5.6 Procedure 2 reagents

5.6.1 Acetonitrile, CH_3CN - HPLC grade.

5.6.2 2,4-Dinitrophenylhydrazine, $C_6H_6N_4O_4$ (DNPH) - recrystallize at least twice with HPLC-grade acetonitrile using procedure in Appendix A.

5.7 Stock standard solutions for Procedure 1

5.7.1 Stock formaldehyde (approximately 1000 mg/L) - Prepare by diluting an appropriate amount of the certified or standardized formaldehyde (approximately 265 µL) to 100 mL with organic-free reagent water. If a certified formaldehyde solution is not available

or there is any question regarding the quality of a certified solution, the solution may be standardized using the procedure in Sec. 5.7.1.1.

5.7.1.1 Standardization of formaldehyde stock solution - Transfer a 25 mL aliquot of a 0.1 M Na_2SO_3 solution to a beaker and record the pH. Add a 25.0 mL aliquot of the formaldehyde stock solution (Sec. 5.18.1) and record the pH. Titrate this mixture back to the original pH using 0.1 N HCl. The formaldehyde concentration is calculated using the following equation:

$$\text{Concentration (mg/L)} = \frac{(30.03)(\text{N HCl})(\text{mL HCl})}{0.0250 \text{ L formaldehyde}}$$

where:

N HCl	=	Normality of HCl solution used (in milli-equivalents/mL) (1 mmole of HCl = 1 milli-equivalent of HCl)
mL HCl	=	mL of standardized HCl solution used
30.03	=	Molecular of weight of formaldehyde (in mg/mmole)

5.7.2 Stock aldehyde(s) and ketone(s) - Prepare by adding an appropriate amount of the pure material to 90 mL of acetonitrile and dilute to 100 mL, to give a final concentration of 1000 mg/L.

5.8 Stock standard solutions for Procedure 2

5.8.1 Preparation of the DNPH Derivatives for HPLC analysis

5.8.1.1 To a portion of the recrystallized DNPH, add sufficient 2N HCl to obtain an approximately saturated solution. Add to this solution the target analyte in molar excess of the DNPH. Filter the DNPH derivative precipitate, wash it with 2N HCl, wash it again with water, and allow it to dry in air.

5.8.1.2 Check the purity of the DNPH derivative by melting point determination or HPLC analysis. If the impurity level is not acceptable, recrystallize the derivative in acetonitrile. Repeat the purity check and recrystallization as necessary until 99% purity is achieved.

5.8.2 Preparation of DNPH derivative standards and calibration standards for HPLC analysis.

5.8.2.1 Stock standard solutions - Prepare individual stock standard solutions for each of the target analyte DNPH derivatives by dissolving accurately weighed amounts in acetonitrile. Individual stock solutions of approximately 100 mg/L may be prepared by dissolving 0.010 g of the solid derivative in 100 mL of acetonitrile.

5.8.2.2 Secondary dilution standard(s) - Using the individual stock standard solutions, prepare secondary dilution standards in acetonitrile containing the DNPH derivatives from the target analytes mixed together. Solutions of 100 µg/L may be prepared by placing 100 µL of a 100 mg/L solution in a 100 mL volumetric flask and diluting to the mark with acetonitrile.

5.8.2.3 Calibration standards - Prepare a working calibration standard mix from the secondary dilution standard, using the mixture of DNPH derivatives at concentrations of 0.5 - 2.0 µg/L (which spans the concentration of interest for most indoor air work). The concentration of the DNPH derivative in the standard mix solutions may need to be adjusted to reflect relative concentration distribution in a real sample.

5.9 Standard storage - Store all standard solutions at 4°C in a glass vial with a PTFE-lined cap, leaving minimum headspace, and in the dark. Standards should be stable for about 6 weeks. All standards should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.

5.10 Calibration standards

Prepare calibration solutions at a minimum of 5 concentrations for each analyte of interest in organic-free reagent water (or acetonitrile for Procedure 2) from the stock standard solution. The lowest concentration of each analyte should be at, or just above, the MDLs listed in Tables 1 or 2. The other concentrations of the calibration curve should correspond to the expected range of concentrations found in real samples.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1 See the introductory material to this chapter, Organic Analytes, Sec. 4.1.

6.2 Samples must be refrigerated at 4°C. Aqueous samples must be derivatized and extracted within 3 days of sample collection. The holding times of leachates of solid samples should be kept at a minimum. All derivatized sample extracts should be analyzed within 3 days after preparation.

6.3 Samples collected by Methods 0011 or 0100 must be refrigerated at 4°C. It is recommended that samples be extracted and analyzed within 30 days of collection.

7.0 PROCEDURE

7.1 Extraction of solid samples (Procedure 1)

7.1.1 All solid samples should be made as homogeneous as possible by stirring and removal of sticks, rocks, and other extraneous material. When the sample is not dry, determine the dry weight of the sample, using a representative aliquot. If particle size reduction is necessary, proceed as per Method 1311.

7.1.1.1 Determination of dry weight - In certain cases, sample results are desired based on a dry weight basis. When such data are desired or required, a portion of sample for dry weight determination should be weighed out at the same time as the portion used for analytical determination.

WARNING: The drying oven should be contained in a hood or vented. Significant laboratory contamination may result from drying a heavily contaminated hazardous waste sample.

7.1.1.2 Immediately after weighing the sample for extraction, weigh 5 - 10 g of the sample into a tared crucible. Determine the % dry weight of the sample by drying overnight at 105°C. Allow to cool in a desiccator before weighing:

$$\% \text{ dry weight} = \frac{\text{g of dry sample}}{\text{g of sample}} \times 100$$

7.1.2 Measure 25 g of solid into a 500-mL bottle with a PTFE-lined screw cap or crimp top, and add 500 mL of extraction fluid (Sec. 5.5.15). Extract the solid by rotating the bottle at approximately 30 rpm for 18 hours. Filter the extract through glass fiber filter paper and store in sealed bottles at 4°C. Each mL of extract represents 0.050 g solid. Smaller quantities of solid sample may be used with correspondingly reduced volumes of extraction fluid maintaining the 1:20 mass to volume ratio.

7.2 Cleanup and separation (Procedure 1)

7.2.1 Cleanup procedures may not be necessary for a relatively clean sample matrix. The cleanup procedures recommended in this method have been used for the analysis of various sample types. If particular samples demand the use of an alternative cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of formaldehyde from a spiked sample is greater than 85%. Recovery may be lower for samples which form emulsions.

7.2.2 If the sample is not clear, or the complexity is unknown, the entire sample should be centrifuged at 2500 rpm for 10 minutes. Decant the supernatant liquid from the centrifuge bottle, and filter through glass fiber filter paper into a container which can be tightly sealed.

7.3 Derivatization (Procedure 1)

7.3.1 For aqueous samples, measure an aliquot of sample which is appropriate to the anticipated analyte concentration range (nominally 100 mL). Quantitatively transfer the sample aliquot to the reaction vessel (Sec. 4.2).

7.3.2 For solid samples, 1 to 10 mL of extract (Sec. 7.1) will usually be required. The amount used for a particular sample must be determined through preliminary experiments.

NOTE: In cases where the selected sample or extract volume is less than 100 mL, the total volume of the aqueous layer should be adjusted to 100 mL with organic-free reagent water. Record original sample volume prior to dilution.

7.3.3 Derivatization and extraction of the target analytes may be accomplished using the liquid-solid (Sec. 7.3.4) or liquid-liquid (Sec. 7.3.5) procedures.

7.3.4 Liquid-solid derivatization and extraction

7.3.4.1 For analytes other than formaldehyde, add 4 mL of citrate buffer and adjust the pH to 3.0 ± 0.1 with 6M HCl or 6M NaOH. Add 6 mL of DNPH reagent, seal the container, and place in a heated (40°C), orbital shaker (Sec. 4.2.12) for 1 hour. Adjust the agitation to produce a gentle swirling of the reaction solution.

7.3.4.2 If formaldehyde is the only analyte of interest, add 4 mL acetate buffer and adjust pH to 5.0 ± 0.1 with 6M HCl or 6M NaOH. Add 6 mL of DNPH reagent, seal

the container, and place in a heated (40°C), orbital shaker (Sec. 4.2.12) for 1 hour. Adjust the agitation to produce a gentle swirling of the reaction solution.

7.3.4.3 Assemble the vacuum manifold and connect to a water aspirator or vacuum pump. Attach a 2-g sorbent cartridge to the vacuum manifold. Condition each cartridge by passing 10 mL dilute citrate buffer (10 mL of 1 M citrate buffer dissolved in 250 mL of organic-free reagent water) through each sorbent cartridge.

7.3.4.4 Remove the reaction vessel from the shaker immediately at the end of the one hour reaction period and add 10 mL of saturated NaCl solution to the vessel.

7.3.4.5 Quantitatively transfer the reaction solution to the sorbent cartridge and apply a vacuum so that the solution is drawn through the cartridge at a rate of 3 to 5 mL/min. Continue applying the vacuum for about 1 minute after the liquid sample has passed through the cartridge.

7.3.4.6 While maintaining the vacuum conditions described in Sec. 7.3.4.4, elute each cartridge train with approximately 9 mL of acetonitrile directly into a 10 mL volumetric flask. Dilute the solution to volume with acetonitrile, mix thoroughly, and place in a tightly sealed vial until analyzed.

NOTE: Because this method uses an excess of DNPH, the cartridges will remain a yellow color after completion of Sec. 7.3.4.5. The presence of this color is not indicative of the loss of the analyte derivatives.

7.3.5 Liquid-liquid derivatization and extraction

7.3.5.1 For analytes other than formaldehyde, add 4 mL of citrate buffer and adjust the pH to 3.0 ± 0.1 with 6M HCl or 6M NaOH. Add 6 mL of DNPH reagent, seal the container, and place in a heated (40°C), orbital shaker for 1 hour. Adjust the agitation to produce a gentle swirling of the reaction solution.

7.3.5.2 If formaldehyde is the only analyte of interest, add 4 mL acetate buffer and adjust pH to 5.0 ± 0.1 with 6M HCl or 6M NaOH. Add 6 mL of DNPH reagent, seal the container, and place in a heated (40°C), orbital shaker for 1 hour. Adjust the agitation to produce a gentle swirling of the reaction solution.

7.3.5.3 Serially extract the solution with three 20 mL portions of methylene chloride using a 250 mL separatory funnel. If an emulsion forms upon extraction, remove the entire emulsion and centrifuge at 2000 rpm for 10 minutes. Separate the layers and proceed with the next extraction. Combine the methylene chloride layers in a 125-mL Erlenmeyer flask containing 5.0 grams of anhydrous sodium sulfate. Swirl contents to complete the extract drying process.

7.3.5.4 Assemble a Kuderna-Danish (K-D) concentrator by attaching a 10-mL concentrator tube to a 500-mL evaporator flask. See Sec. 4.0 of Method 3510. Pour the extract into the evaporator flask being careful to minimize transfer of sodium sulfate granules. Wash the Erlenmeyer flask with 30 mL of methylene chloride and add wash to the evaporator flask to complete quantitative transfer.

7.3.5.5 Concentrate the extract to a final volume of 5 mL, using the K-D techniques, as described in Method 3510. Exchange the solvent to acetonitrile prior to analysis.

7.4 Extraction of samples from Methods 0011 and 0100 (Procedures 1 and 2)

7.4.1 Stack gas samples collected by Method 0011 (Procedure 1)

7.4.1.1 Measure the volume of the aqueous phase of the sample prior to extraction (for moisture determination when the volume was not measured in the field). Pour the sample into a separatory funnel and drain the methylene chloride into a volumetric flask.

7.4.1.2 Extract the aqueous solution with two or three aliquots of methylene chloride. Add the methylene chloride extracts to the volumetric flask.

7.4.1.3 Fill the volumetric flask to the line with methylene chloride. Mix well and remove an aliquot.

7.4.1.4 If high concentrations of formaldehyde are present, the extract can be diluted with mobile phase, otherwise the extract solvent must be exchanged as described in Sec. 7.3.5.5. If low concentrations of formaldehyde are present, the sample should be concentrated during the solvent exchange procedure.

7.4.1.5 Store the sample at 4°C. If the extract will be stored longer than two days, it should be transferred to a vial with a PTFE-lined screw cap, or a crimp top with a PTFE-lined septum. Proceed with HPLC chromatographic analysis if further cleanup is not required.

7.4.2 Ambient air samples collected by Method 0100 (Procedure 2)

7.4.2.1 The samples will be received by the laboratory in a friction-top can containing 2 - 5 cm of granular charcoal, and should be stored in this can, in a refrigerator, until analysis. Alternatively, the samples may also be stored alone in their individual glass containers. The time between sampling and analysis should not exceed 30 days.

7.4.2.2 Remove the sample cartridge from the labeled culture tube. Connect the sample cartridge (outlet or long end during sampling) to a clean syringe.

NOTE: The liquid flow during desorption should be in the opposite direction from the air flow during sample collection (i.e, backflush the cartridge).

7.4.2.3 Place the cartridge/syringe in the syringe rack.

7.4.2.4 Backflush the cartridge (gravity feed) by passing 6 mL of acetonitrile from the syringe through the cartridge to a graduated test tube, or to a 5-mL volumetric flask.

NOTE: A dry cartridge has an acetonitrile holdup volume slightly greater than 1 mL. The eluate flow may stop before the acetonitrile in the syringe is completely drained into the cartridge because of air trapped between

the cartridge filter and the syringe Luer-Lok tip. If this happens, displace the trapped air with the acetonitrile in the syringe using a long-tip disposable Pasteur pipet.

7.4.2.5 Dilute to the 5 mL mark with acetonitrile. Label the flask with sample identification. Pipet two aliquots into sample vials having PTFE-lined septa.

7.4.2.6 Store the sample at 4°C. Proceed with HPLC chromatographic analysis of the first aliquot if further cleanup is not required. Store the second aliquot in the refrigerator until the results of the first aliquot analysis are complete and validated. The second aliquot can be used for confirmatory analysis, if necessary.

7.5 Recommended chromatographic conditions

7.5.1 Procedure 1 - For aqueous samples, soil or waste samples, and stack gas samples collected by Method 0011.

Column:	C18, 4.6 mm x 250 mm ID, 5 µm particle size
Mobile Phase Gradient:	70/30 acetonitrile/water (v/v), hold for 20 min. 70/30 acetonitrile/water to 100% acetonitrile in 15 min. 100% acetonitrile for 15 min.
Flow Rate:	1.2 mL/min
Detector:	Ultraviolet, operated at 360 nm
Injection Volume:	20 µL

7.5.2 Procedure 2 - For ambient air samples collected by Method 0100.

Column:	Two HPLC columns, 4.6 mm x 250 mm ID, (Zorbax ODS, or equivalent) in series
Mobile Phase Gradient:	60/40 CH_3CN/H_2O, hold for 0 min. 60/40 to 75/25 CH_3CN/H_2O, linearly in 30 min. 75/25 to 100% CH_3CN, linearly in 20 min. 100% CH_3CN for 5 minutes. 100% to 60/40 CH_3CN/H_2O, linearly in 1 min. 60/40 CH_3CN/H_2O for 15 minutes.
Detector:	Ultraviolet, operated at 360 nm
Flow Rate:	1.0 mL/min
Sample Injection volume:	25 µL (suggested)

NOTE: Analysts are advised to adjust their HPLC systems to optimize chromatographic conditions for their particular analytical needs. The separation of acrolein, acetone, and propionaldehyde should be a minimum criterion of the optimization in Procedure 2.

7.5.3 Filter and degas the mobile phase to remove dissolved gasses, using the following procedure:

7.5.3.1 Filter each solvent (water and acetonitrile) through a 0.22 µm polyester membrane filter, in an all glass and PTFE suction filtration apparatus.

7.5.3.2 Degas each filtered solution by purging with helium for 10 - 15 minutes (100 mL/min) or by heating to 60°C for 5 - 10 minutes in an Erlenmeyer flask covered with a watch glass. A constant back pressure restrictor (350 kPa) or 15 - 30 cm of 0.25 mm ID PTFE tubing should be placed after the detector to eliminate further mobile phase outgassing.

7.5.3.3 Place the mobile phase components in their respective HPLC solvent reservoirs, and program the gradient system according to the conditions listed in Sec. 7.5.2. Allow the system to pump for 20 - 30 minutes at a flow rate of 1.0 mL/min with the initial solvent mixture ratio (60%/40% CH_3CN/H_2O). Display the detector output on a strip chart recorder or similar output device to establish a stable baseline.

7.6 Calibration

7.6.1 Establish liquid chromatographic operating conditions to produce a retention time similar to that indicated in Table 1 for the liquid-solid derivatization and extraction or in Table 2 for liquid-liquid derivatization and extraction. For determination of retention time windows, see Sec. 7.5 of Method 8000. Suggested chromatographic conditions are provided in Sec. 7.5.

7.6.2 Process each calibration standard solution through derivatization and extraction, using the same procedure employed for sample processing (Secs. 7.3.4 or 7.3.5).

7.6.3 Analyze a solvent blank to ensure that the system is clean and interference free.

NOTE: The samples and standards must be allowed to come to ambient temperature before analysis.

7.6.4 Analyze each processed calibration standard using the chromatographic conditions listed in Sec. 7.5, and tabulate peak area against calibration solution concentration in µg/L.

7.6.5 Tabulate the peak area along with standard concentration injected to determine the calibration factor (CF) for the analyte at each concentration (see Sec. 7.8.1 for equations). The percent relative standard deviation (%RSD) of the mean CF of the calibration standards should be $\leq$ 20 percent or a system check will have to be performed. If a calibration check after the system check does not meet the criteria, a recalibration will have to be performed. If the recalibration does not meet the established criteria, new calibration standards must be made.

7.6.6 The working calibration curve must be verified each day, before and after analyses are performed, by analyzing one or more calibration standards. The calibration factor obtained should fall within ± 15 percent of the initially established calibration factor or a system check will have to be performed. If a calibration check after the system check does not meet the criteria, the system must be recalibrated.

7.6.7 After 10 sample runs, or less, one of the calibration standards must be reanalyzed to ensure that the DNPH derivative calibration factors remain within ± 15% of the original calibration factors.

7.7 Sample analysis

7.7.1 Analyze samples by HPLC, using conditions established in Sec. 7.5. For Procedure 1 analytes, Tables 1 and 2 list the retention times and MDLs that were obtained under these conditions. For Procedure 2 analytes, refer to Figure 3 for the sample chromatogram.

7.7.2 If the peak area exceeds the linear range of the calibration curve, a smaller sample injection volume should be used. Alternatively, the final solution may be diluted with acetonitrile and reanalyzed.

7.7.3 After elution of the target analytes, calculate the concentration of analytes found in the samples using the equations found in Sec. 7.8 or the specific sampling method used.

7.7.4 If the peak area measurement is prevented by the presence of observed interferences, further cleanup is required.

7.8 Calculations

7.8.1 Calculate each calibration factor, mean calibration factor, standard deviation,and percent relative standard deviation as follows:

$$CF = \frac{\text{Peak Area of the Compound in the Standard}}{\text{Concentration of the Compound Injected (in ug/L)}}$$

$$\text{mean CF} = \overline{CF} = \frac{\sum_{i=1}^{n} CF_i}{n}$$

$$SD = \sqrt{\frac{\sum_{i=1}^{n} (CF_i - \overline{CF})^2}{n-1}} \qquad RSD = \frac{SD}{\overline{CF}} \times 100$$

where:

$\overline{CF}$ = Mean calibration factor using the 5 calibration concentrations.
CF_i = Calibration factor for calibration standard i (i = 1-5).
RSD = Relative standard deviation of the calibration factors.
n = Number of calibration standards.

7.8.2 Calculate the concentrations in liquid samples as follows:

$$\text{Concentration of aldehydes (µg/L)} = \frac{\text{(Area of sample peak)} \times 100}{\overline{CF} \times V_s}$$

where:

$\overline{CF}$ = Mean calibration factor the analyte.
V_s = Number of mL of sample (unitless).

7.8.3 Calculate the concentration in solid samples as follows:

$$\text{Concentration of aldehydes } (\mu g/g) = \frac{(\text{Area of sample peak}) \times 100}{\overline{CF} \times V_{ex}}$$

where:

$\overline{CF}$ = Mean calibration factor the analyte.
V_{ex} = Number of mL extraction fluid aliquot (unitless).

7.8.4 Calculate the concentration of formaldehyde in stack gas samples (Method 0011) as follows: (Procedure 1)

7.8.4.1 Calculation of total formaldehyde

To determine the total formaldehyde in mg, use the following equation:

$$\text{Total mg formaldehyde} = C_d \times V \times DF \times \frac{(\text{g/mole formaldehyde})}{(\text{g/mole DNPH derivative})} \times 10^{-3} \text{ L/mL}$$

where:

C_d = measured concentration of DNPH-formaldehyde derivative, mg/L
V = organic extract volume, mL
DF = dilution factor

7.8.4.2 Formaldehyde concentration in stack gas

Determine the formaldehyde concentration in the stack gas using the following equation:

$$C_f \text{ in mg/m}^3 = \frac{K \times (\text{total formaldehyde in mg})}{V_{m(std)}}$$

where:

K = 35.31 ft^3/m^3, if $V_{m(std)}$ is expressed in English units
= 1.00 m^3/m^3, if $V_{m(std)}$ is expressed in metric units
$V_{m(std)}$ = volume of gas sample as measured by dry gas meter, corrected to standard conditions, dscm (dscf)

7.8.5 Calculation of the concentration of formaldehyde and other carbonyls from indoor air sampling by Method 0100. (Procedure 2)

7.8.5.1 The concentration of target analyte "a," in air at standard conditions (25°C and 101.3 kPa), $Conc_{std}$ in ng/L, may be calculated using the following equation:

$$Conc_{a,std} \text{ in ng/L} = \frac{(Area_a)(Vol_a)(MW_a)(1000 \text{ ng/ug})}{(\overline{RF})(MW_d)(V_{TotStd})(1000 \text{ mL/L})} \times DF$$

where:

$Area_a$ = Area of the sample peak for analyte "a"

$\overline{CF}$ = Mean calibration factor for analyte "a" from the calibration in µg/L. (See Sec. 7.8.1)

Vol_a = Total volume of the sample cartridge eluate (mL)

MW_a = Molecular weight of analyte "a" in g/mole

MW_d = Molecular weight of the DNPH derivative of analyte "a" in g/mole

V_{TotStd} = Total volume of air sampled converted to standard conditions in liters (L). (To calculate the concentration at sampling conditions use V_{tot}.) (See Sec. 9.0 of Method 0100)

DF = Dilution Factor for the sample cartridge eluate, if any. If there is no dilution, DF = 1

7.8.5.2 The target analyte "a" concentration at standard conditions may be converted to parts per billion by volume, $Conc_a$ in ppbv, using the following equation:

$$Conc_a \text{ in ppbv} = \frac{(Conc_{a,std})(22.4)}{(MW_a)}$$

where:

$Conc_{a,std}$ = Concentration of "a," at standard conditions, in ng/L

22.4 = Ideal gas law volume (22.4 nL of gas = 1 nmole, at standard conditions)

MW_a = Molecular weight of analyte "a" in g/mole (or ng/nmole)

8.0 QUALITY CONTROL

8.1 Refer to Chapter One and Method 8000 for specific quality control (QC) procedures. Each laboratory should maintain a formal quality assurance program. The laboratory should also maintain records to document the quality of the data generated.

8.2 Quality control procedures necessary to evaluate the HPLC system operation are found in Method 8000, Sec. 7.0 and include evaluation of retention time windows, calibration verification and chromatographic analysis of samples.

8.3 Initial Demonstration of Proficiency - Each laboratory must demonstrate initial proficiency with each sample preparation and determinative method combination it utilizes, by generating data of acceptable accuracy and precision for target analytes in a clean matrix. The laboratory must also

repeat the following operations whenever new staff are trained or significant changes in instrumentation are made. See Method 8000, Sec. 8.0 for information on how to accomplish this demonstration.

8.4 Sample Quality Control for Preparation and Analysis - The laboratory must also have procedures for documenting the effect of the matrix on method performance (precision, accuracy, and detection limit). At a minimum, this includes the analysis of QC samples including a method blank, a matrix spike, a duplicate, and a laboratory control sample (LCS) in each analytical batch.

8.4.1 Documenting the effect of the matrix should include the analysis of at least one matrix spike and one duplicate unspiked sample or one matrix spike/matrix spike duplicate pair. The decision on whether to prepare and analyze duplicate samples or a matrix spike/matrix spike duplicate must be based on a knowledge of the samples in the sample batch. If samples are expected to contain target analytes, then laboratories may use one matrix spike and a duplicate analysis of an unspiked field sample. If samples are not expected to contain target analytes, laboratories should use a matrix spike and matrix spike duplicate pair. Each batch of samples collected by Method 0011 will include a sample for use as a Matrix Spike Sample. Because of the high cost of obtaining Method 0011 samples and the inadvisability of splitting the samples, it will be acceptable to use the average value of the other samples in the batch in place of analysis of an additional unspiked matrix duplicate.

8.4.2 A Laboratory Control Sample (LCS) should be included with each analytical batch. The LCS consists of an aliquot of a clean (control) matrix similar to the sample matrix and of the same weight or volume. The LCS is spiked with the same analytes at the same concentrations as the matrix spike. When the results of the matrix spike analysis indicate a potential problem due to the sample matrix itself, the LCS results are used to verify that the laboratory can perform the analysis in a clean matrix.

8.4.3 Refer to Table 4 for QC acceptance limits derived from the interlaboratory method validation study on Method 8315.

8.4.4 See Method 8000, Sec. 8.0 for the details on carrying out sample quality control procedures for preparation and analysis.

8.5 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Whenever possible, the laboratory should analyze standard reference materials and participate in relevant performance evaluation studies.

9.0 METHOD PERFORMANCE

9.1 The MDLs for Procedure 1 listed in Table 1 were obtained using organic-free reagent water and liquid-solid extraction. The MDLs for Procedure 1 listed in Table 2 were obtained using organic-free reagent water and methylene chloride extraction. Results reported in Tables 1 and 2 were achieved using fortified reagent water volumes of 100 mL. Lower detection limits may be obtained using larger sample volumes.

9.1.1 Procedure 1 of this method has been tested for linearity of recovery from spiked organic-free reagent water and has been demonstrated to be applicable over the range 50-1000 µg/L .

9.1.2 To generate the MDL and precision and accuracy data reported in this section, analytes were segregated into two spiking groups, A and B. Chromatograms using liquid-solid and liquid-liquid extraction are presented in Figures 1 (a and b) and 2 (a and b), respectively.

9.2 The sensitivity of Procedure 2 sampling (Method 0100) and analysis is listed in Table 3.

9.3 Method 8315, Procedure 1, was tested by 12 laboratories using organic-free reagent water and ground waters spiked at six concentrations over the range 30-2200 µg/L. Method accuracy and precision were found to be directly related to the concentration of the analyte and independent of the sample matrix. Mean recovery weighted linear regression equations, calculated as a function of spike concentration, as well as overall and single-analyst precision regression equations, calculated as functions of mean recovery, are presented in Table 5. These equations can be used to estimate mean recovery and precision at any concentration value within the range tested.

10.0 REFERENCES

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

11.0 SAFETY

11.1 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.

11.2 Formaldehyde has been tentatively classified as a known or suspected, human or mammalian carcinogen.

TABLE 1

PROCEDURE 1 - METHOD DETECTION LIMITS[a] USING
LIQUID-SOLID EXTRACTION

Analyte	Retention Time (minutes)	MDL (µg/L)[a]
Formaldehyde	5.3	6.2
Acetaldehyde	7.4	43.7[b]
Propanal	11.7	11.0
Crotonaldehyde	16.1	5.9
Butanal	18.1	6.3
Cyclohexanone	27.6	5.8
Pentanal	28.4	15.3
Hexanal	34.1	10.7
Heptanal	35.0	10.0
Octanal	40.1	6.9
Nonanal	40.4	13.6
Decanal	44.1	4.4

[a] The method detection limit (MDL) is defined in Chapter One. With the exception of acetaldehyde, all reported MDLs are based upon analyses of 6 to 8 replicate blanks spiked at 25 µg/L.

[b] The reported MDL is based upon analyses of three replicate blanks fortified at 250 µg/L.

TABLE 2

PROCEDURE 1 - METHOD DETECTION LIMITS[a] USING
LIQUID-LIQUID EXTRACTION

Analyte	Retention Time (minutes)	MDL (µg/L)[a]
Formaldehyde	5.3	23.2
Acetaldehyde	7.4	110.2[b]
Propanal	11.7	8.4
Crotonaldehyde	16.1	5.9
Butanal	18.1	7.8
Cyclohexanone	27.6	6.9
Pentanal	28.4	13.4
Hexanal	34.1	12.4
Heptanal	35.0	6.6
Octanal	40.1	9.9
Nonanal	40.4	7.4
Decanal	44.1	13.1

[a] The method detection limit (MDL) is defined in Chapter One. With the exception of acetaldehyde, all reported MDLs are based upon analyses of 6 to 8 replicate blanks spiked at 25 µg/L.

[b] The reported MDL is based upon analyses of three replicate blanks fortified at 250 µg/L.

TABLE 3

PROCEDURE 2 - SENSITIVITY (ppb, v/v) OF SAMPLING AND ANALYSIS FOR CARBONYL COMPOUNDS IN AMBIENT AIR USING AN ADSORBENT CARTRIDGE FOLLOWED BY GRADIENT HPLC[a]

	Sample Volume (L)[b]									
Compound	10	20	30	40	50	100	200	300	400	500
Acetaldehyde	1.36	0.68	0.45	0.34	0.27	0.14	0.07	0.05	0.03	0.03
Acetone	1.28	0.64	0.43	0.32	0.26	0.13	0.06	0.04	0.03	0.03
Acrolein	1.29	0.65	0.43	0.32	0.26	0.13	0.06	0.04	0.03	0.03
Benzaldehyde	1.07	0.53	0.36	0.27	0.21	0.11	0.05	0.04	0.03	0.02
Butyraldehyde	1.21	0.61	0.40	0.30	0.24	0.12	0.06	0.04	0.03	0.02
Crotonaldehyde	1.22	0.61	0.41	0.31	0.24	0.12	0.06	0.04	0.03	0.02
2,5-Dimethyl-benzaldehyde	0.97	0.49	0.32	0.24	0.19	0.10	0.05	0.03	0.02	0.02
Formaldehyde	1.45	0.73	0.48	0.36	0.29	0.15	0.07	0.05	0.04	0.03
Hexanal	1.09	0.55	0.36	0.27	0.22	0.11	0.05	0.04	0.03	0.02
Isovaleraldehyde	1.15	0.57	0.38	0.29	0.23	0.11	0.06	0.04	0.03	0.02
Propionaldehyde	1.28	0.64	0.43	0.32	0.26	0.13	0.06	0.04	0.03	0.03
m-Tolualdehyde	1.02	0.51	0.34	0.25	0.20	0.10	0.05	0.03	0.03	0.02
o-Tolualdehyde	1.02	0.51	0.34	0.25	0.20	0.10	0.05	0.03	0.03	0.02
p-Tolualdehyde	1.02	0.51	0.34	0.25	0.20	0.10	0.05	0.03	0.03	0.02
Valeraldehyde	1.15	0.57	0.38	0.29	0.23	0.11	0.06	0.04	0.03	0.02

[a] The ppb values are measured at 1 atm and 25°C. The sample cartridge is eluted with 5 mL acetonitrile and 25 µL is injected into the HPLC. The maximum sampling flow through a DNPH-coated Sep-PAK is about 1.5 L/minute.

[b] A sample volume of 1000 L was also analyzed. The results show a sensitivity of 0.01 ppb for all the target analytes.

TABLE 4

MULTILABORATORY PERFORMANCE DATA

Analyte	Spike Concentration[a]	x[b]	S_R[c]	Acceptance Limits, %[d]
Formaldehyde	160	154	30.5	39 - 153
Propanal	160	148	22.4	50 - 134
Crotonaldehyde	160	160	34.8	35 - 165
Butanal	160	151	22.7	52 - 137
Cyclohexanone	160	169	39.2	32 - 179
Hexanal	160	151	34.6	30 - 159
Octanal	160	145	40.1	15 - 166
Decanal	160	153	40.0	21 - 171

[a] Spike concentration, µg/L.

[b] Mean recovery calculated using the organic-free reagent water, mean recovery, linear regression equation, µg/L.

[c] Overall standard deviation calculated using the organic-free reagent water, overall standard deviation linear regression equation, µg/L.

[d] 99% confidence limits calculated as $(x \pm 3S_R)100$/spike concentration.

TABLE 5

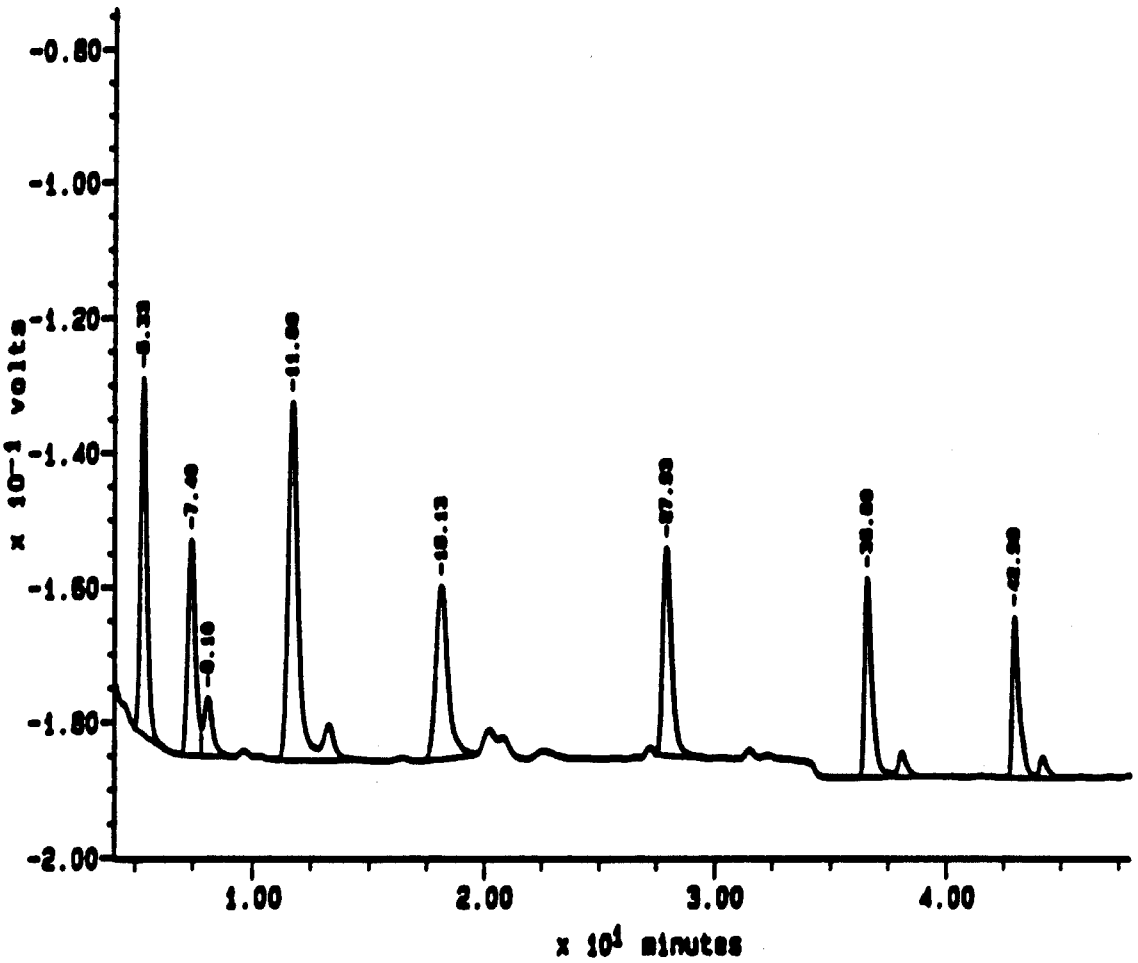
WEIGHTED LINEAR REGRESSION EQUATIONS FOR MEAN RECOVERY AND PRECISION (µg/L)

Analyte	Applicable Conc. Range		Organic-free Reagent Water	Ground Water
Formaldehyde	39.2 - 2450	X	0.909C + 8.79	0.870C + 14.84
		s_R	0.185X + 1.98[a]	0.177X + 13.85
		s_r	0.093X + 5.79	0.108X + 6.24
Propanal	31.9 - 2000	X	0.858C + 10.49	0.892C + 22.22
		s_R	0.140X + 1.63	0.180X + 12.37
		s_r	0.056X + 2.76	0.146X + 2.08[a]
Crotonaldehyde	32.4 - 2030	X	0.975C + 4.36	0.971C + 2.94
		s_R	0.185X + 5.15	0.157X + 6.09
		s_r	0.096X + 1.85	0.119X - 2.27
Butanal	35.4 - 2220	X	0.902C + 6.65	0.925C + 12.71
		s_R	0.149X + 0.21	0.140X + 6.89
		s_r	0.086X - 0.71	0.108X - 1.63[a]
Cyclohexanone	31.6 - 1970	X	0.962C + 14.97	0.946C + 28.95
		s_R	0.204X + 4.73[a]	0.345X + 5.02
		s_r	0.187X + 3.46	0.123X + 7.64
Hexanal	34.1- 2130	X	0.844C + 15.81	0.926C + 9.16
		s_R	0.169X + 9.07	0.132X + 8.31
		s_r	0.098X + 0.37[a]	0.074X - 0.40[a]
Octanal	32.9 - 2050	X	0.856C + 7.88	0.914C + 13.09
		s_R	0.200X + 11.17	0.097X + 12.41
		s_r	0.092X + 1.71[a]	0.039X + 1.14
Decanal	33.2 - 2080	X	0.883C + 12.00	0.908C + 6.46
		s_R	0.225X + 5.52	0.153X + 2.23
		s_r	0.088X + 2.28[a]	0.052X + 0.37

[a] Variance is not constant over concentration range.
X Mean recovery, µg/L, exclusive of outliers.
s_R Overall standard deviation, µg/L, exclusive of outliers.
s_r Single-analyst standard deviation, µg/L, exclusive of outliers.

FIGURE 1a

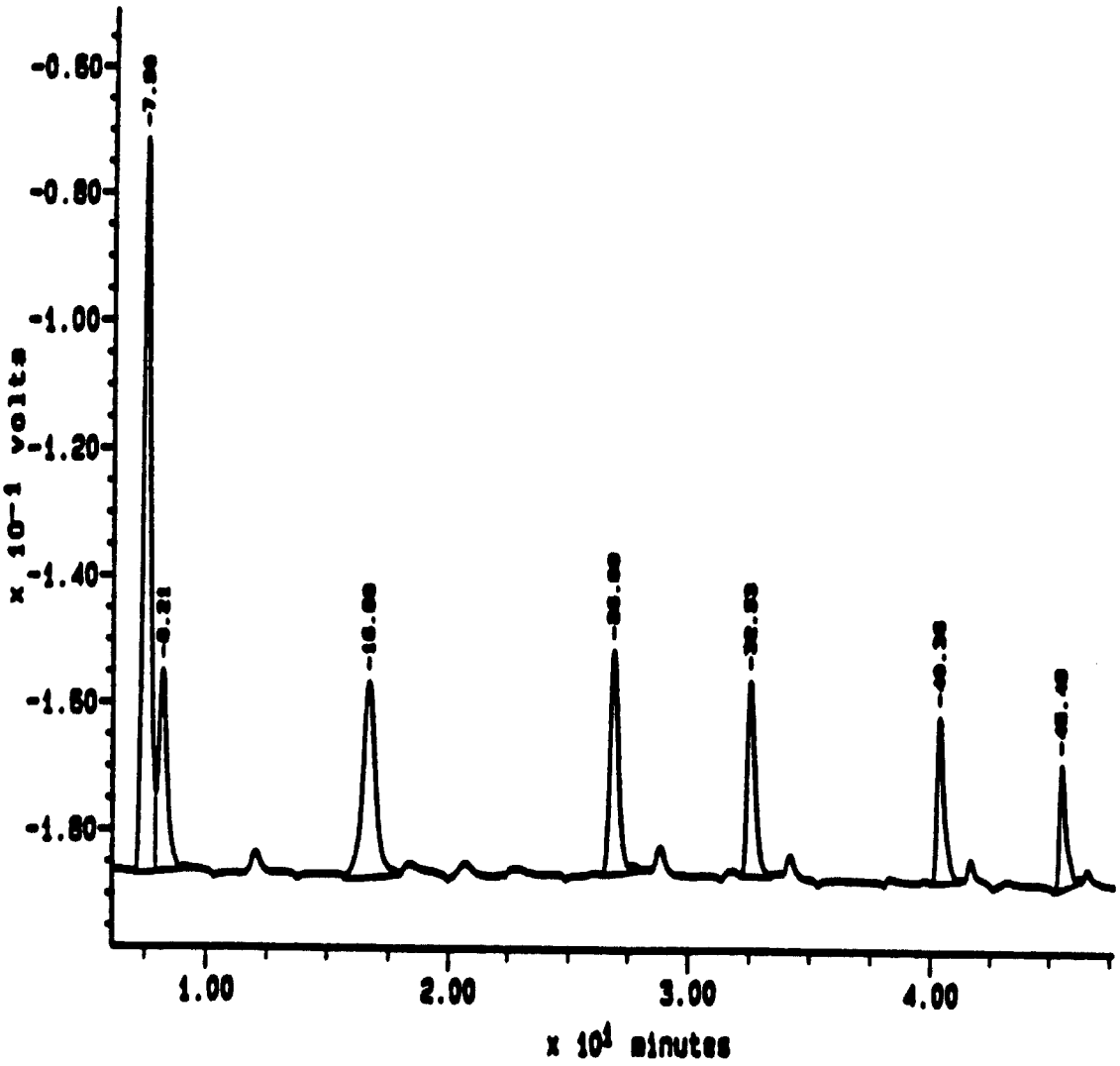
PROCEDURE 1
LIQUID-SOLID PROCEDURAL STANDARD OF GROUP A ANALYTES AT 625 µg/L



Retention Time (min)	Analyte Derivative
5.33	Formaldehyde
11.68	Propanal
18.13	Butanal
27.93	Cyclohexanone
36.60	Heptanal
42.99	Nonanal

FIGURE 1b

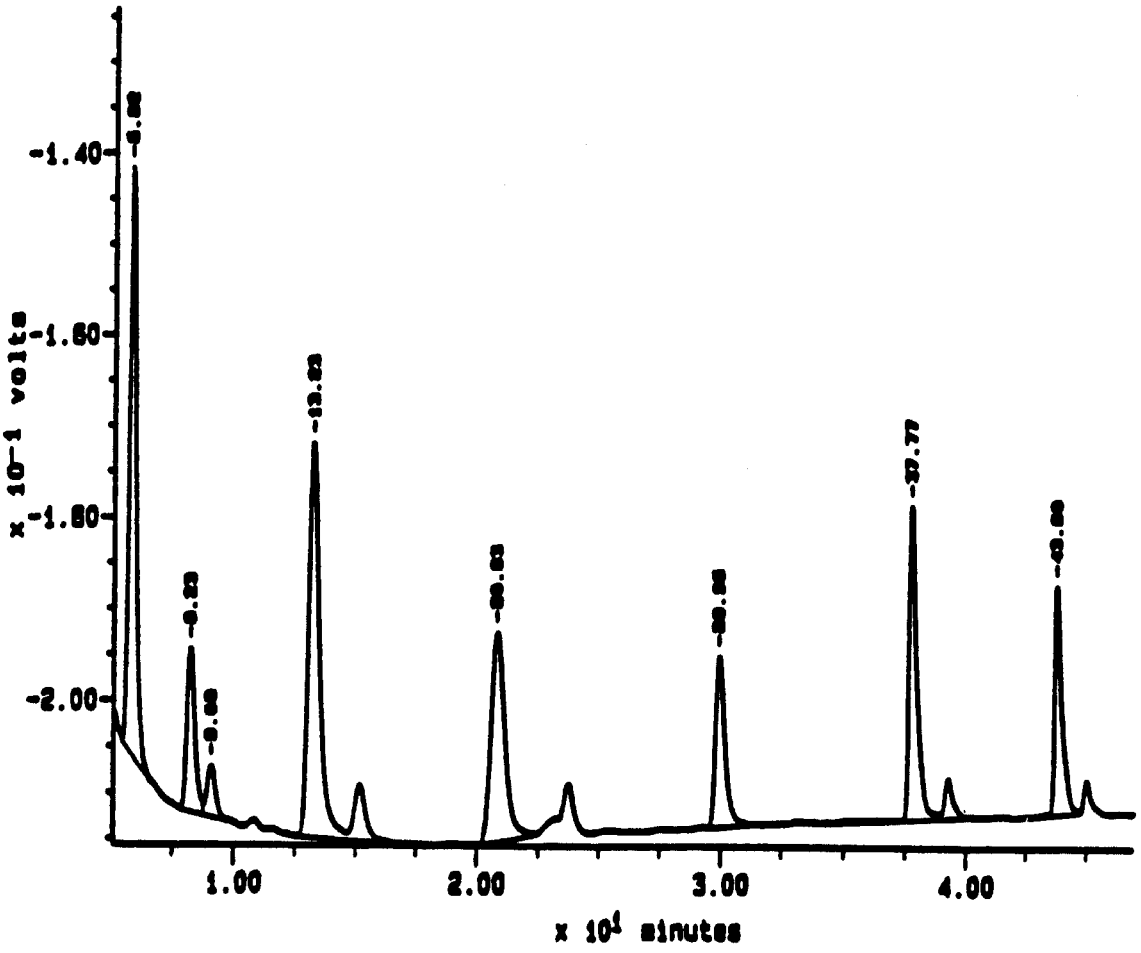
PROCEDURE 1
LIQUID-SOLID PROCEDURAL STANDARD OF GROUP B ANALYTES AT 625 µg/L



Retention Time (min)	Analyte Derivative
7.50	Acetaldehyde
16.68	Crotonaldehyde
26.88	Pentanal
32.53	Hexanal
40.36	Octanal
45.49	Decanal

FIGURE 2a

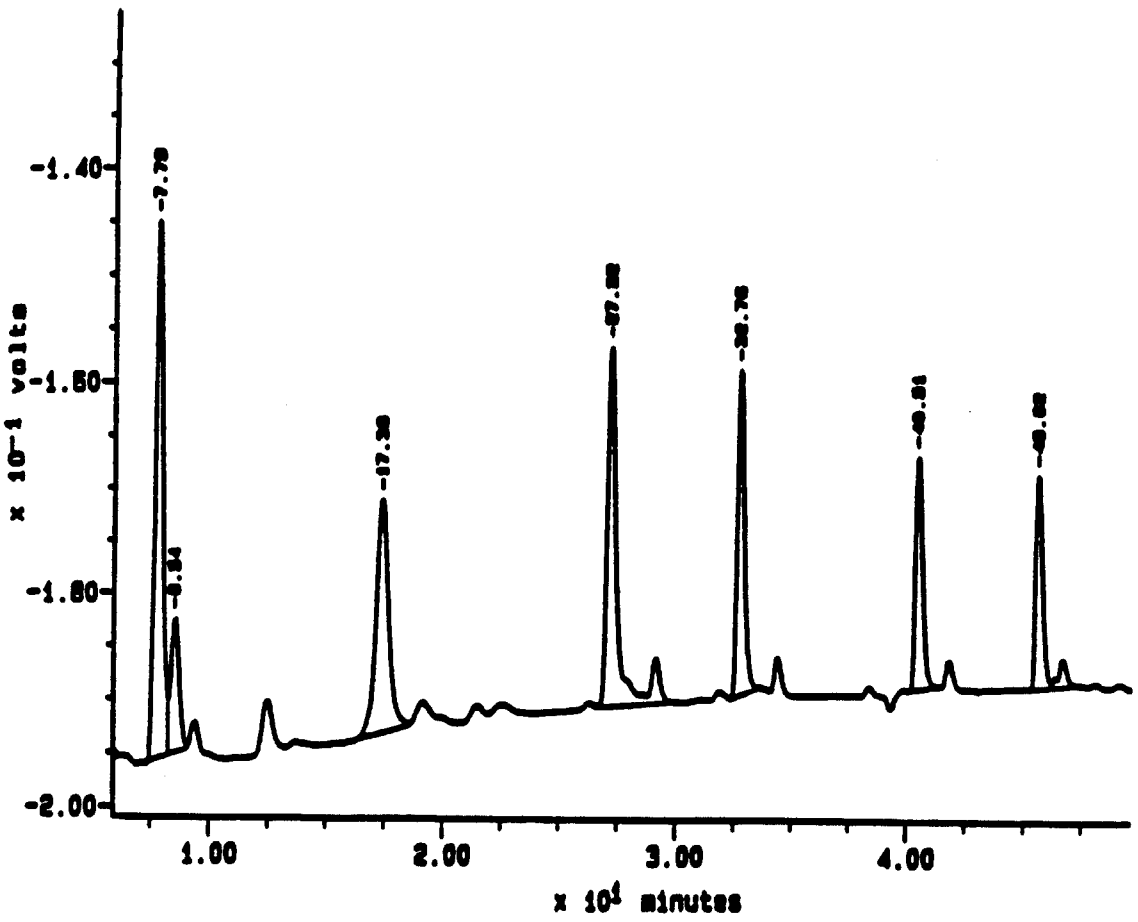
PROCEDURE 1
LIQUID-LIQUID PROCEDURAL STANDARD OF GROUP A ANALYTES AT 625 µg/L



Retention Time (min)	Analyte Derivative
5.82	Formaldehyde
13.23	Propanal
20.83	Butanal
29.95	Cyclohexanone
37.77	Heptanal
43.80	Nonanal

FIGURE 2b

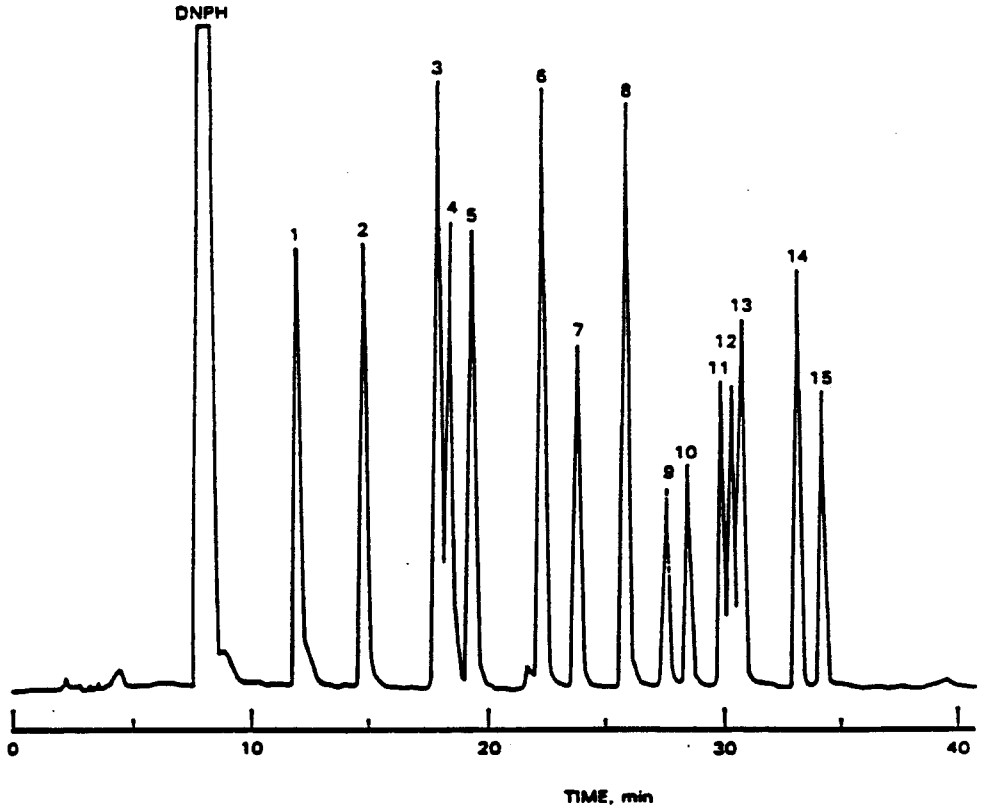
PROCEDURE 1
LIQUID-LIQUID PROCEDURAL STANDARD OF GROUP B ANALYTES AT 625 µg/L



Retention Time (min)	Analyte Derivative
7.79	Acetaldehyde
17.38	Crotonaldehyde
27.22	Pentanal
32.76	Hexanal
40.51	Octanal
45.62	Decanal

FIGURE 3

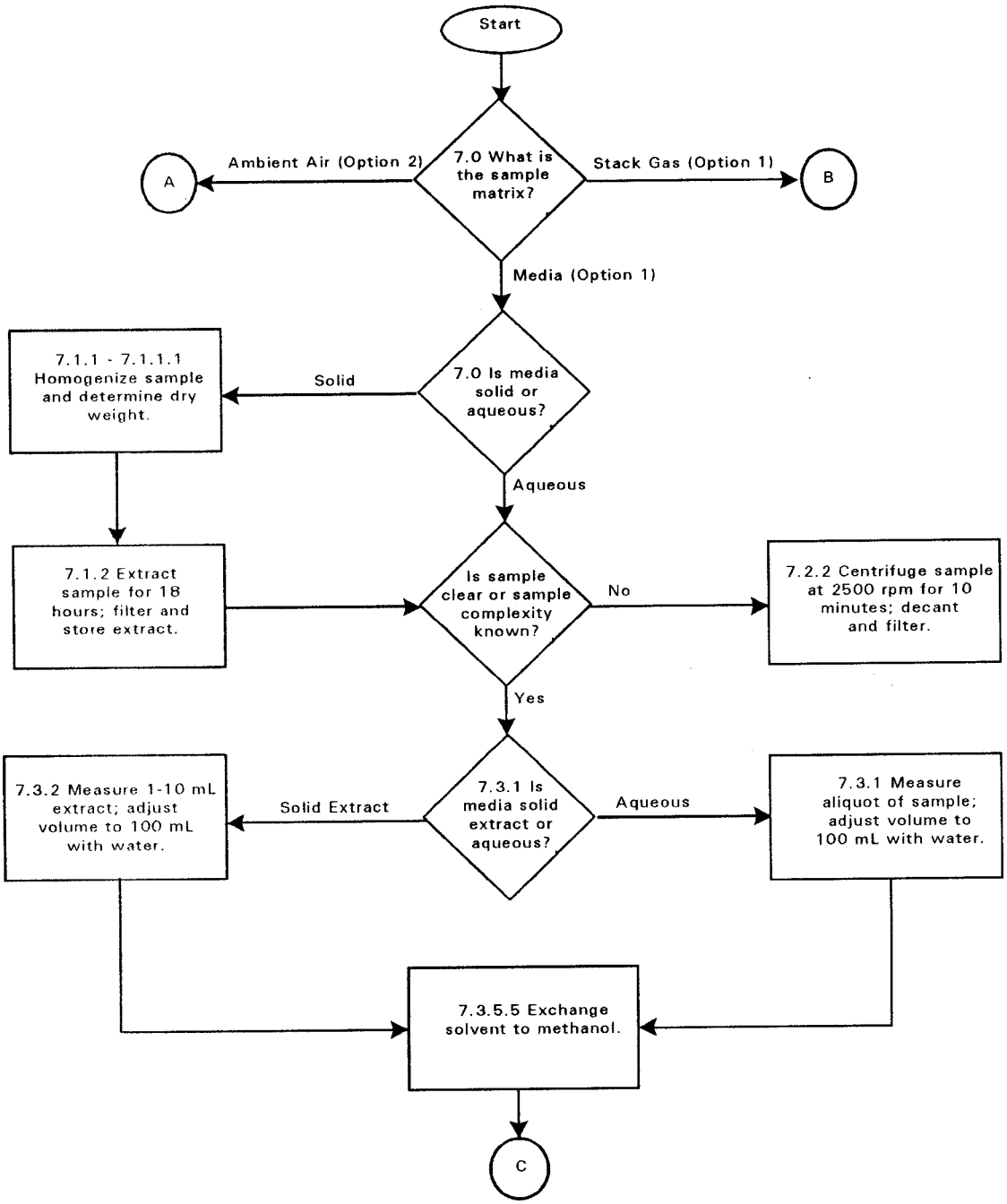
PROCEDURE 2
CHROMATOGRAPHIC SEPARATION OF THE DNPH DERIVATIVES OF
15 CARBONYL COMPOUNDS

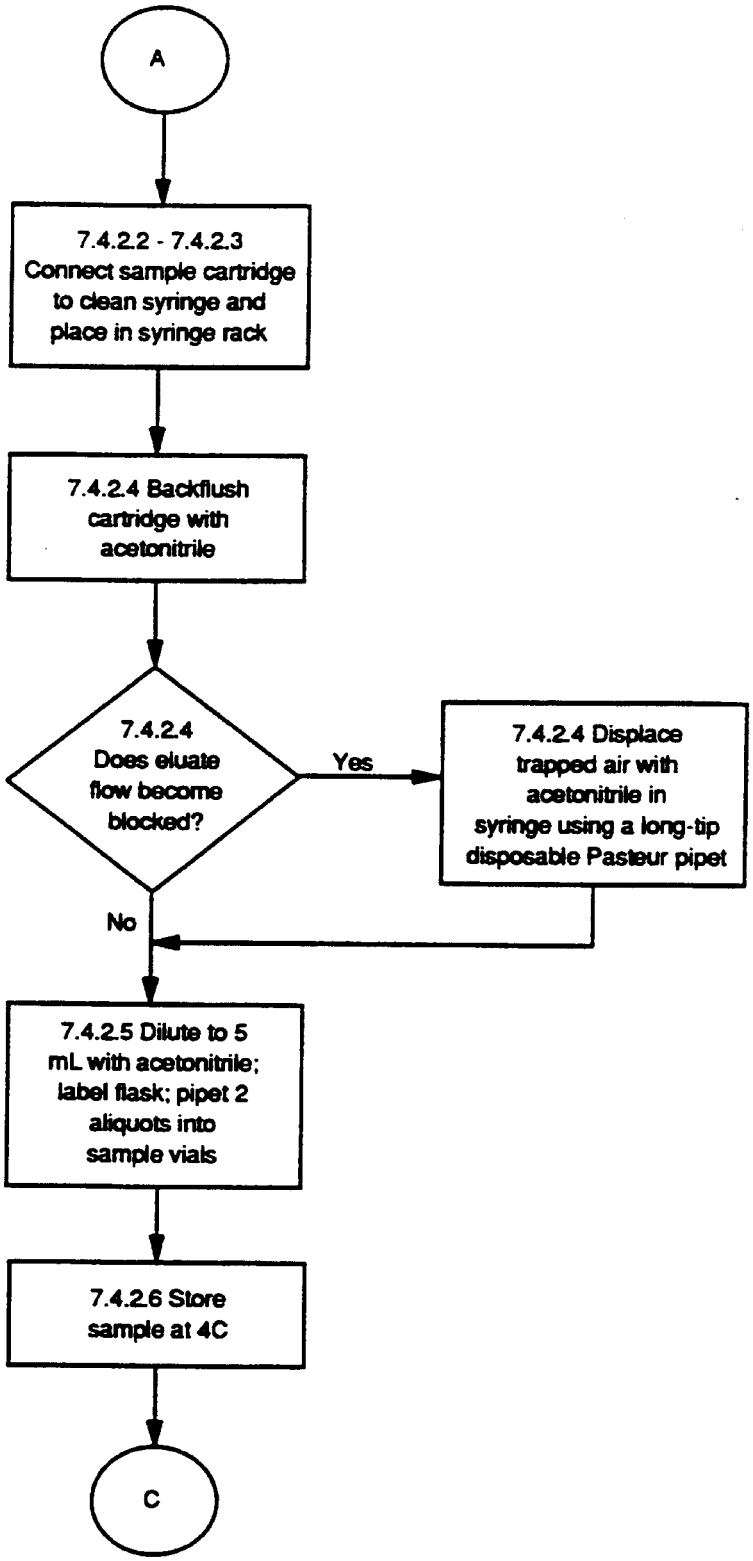


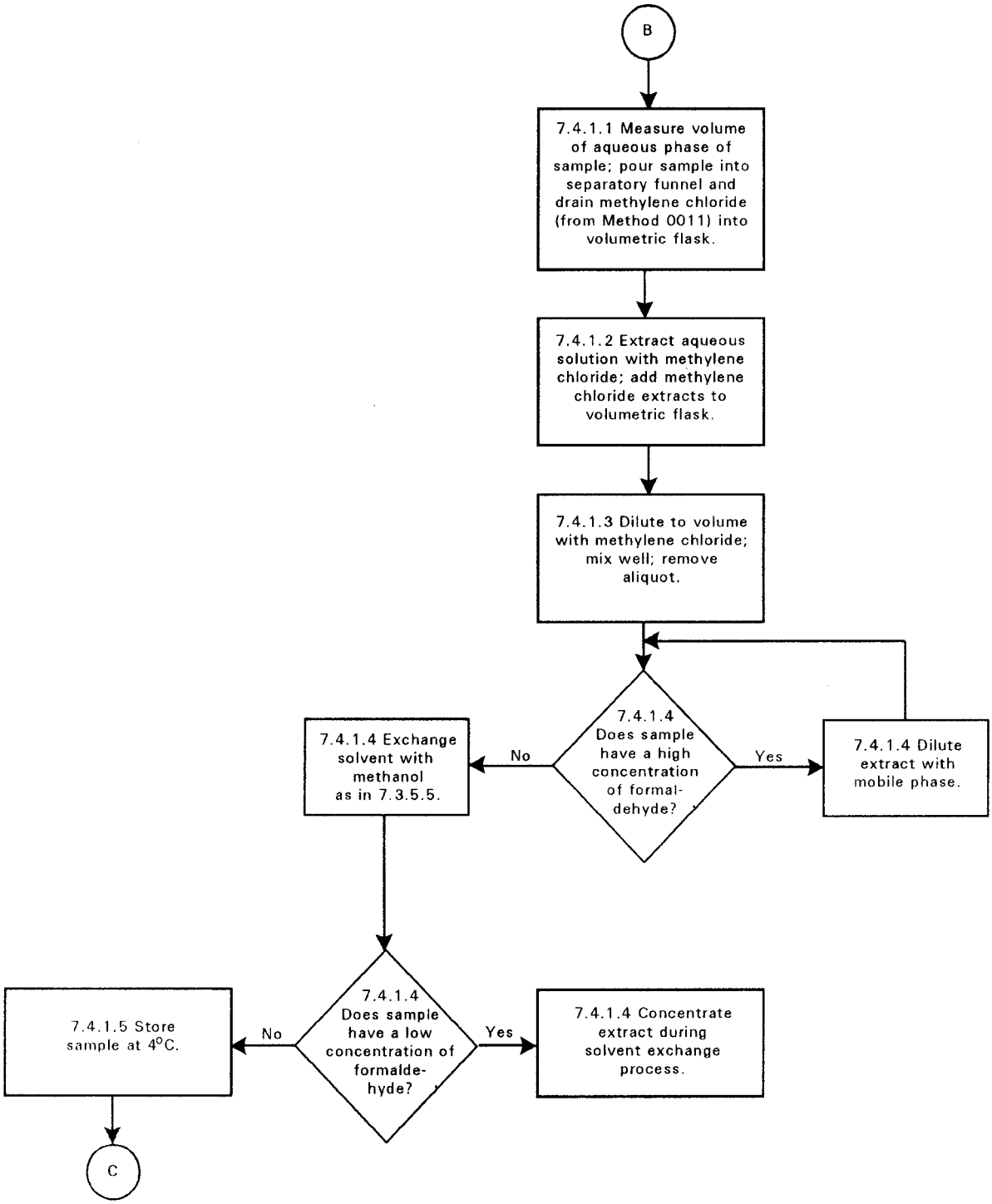
Peak Identification

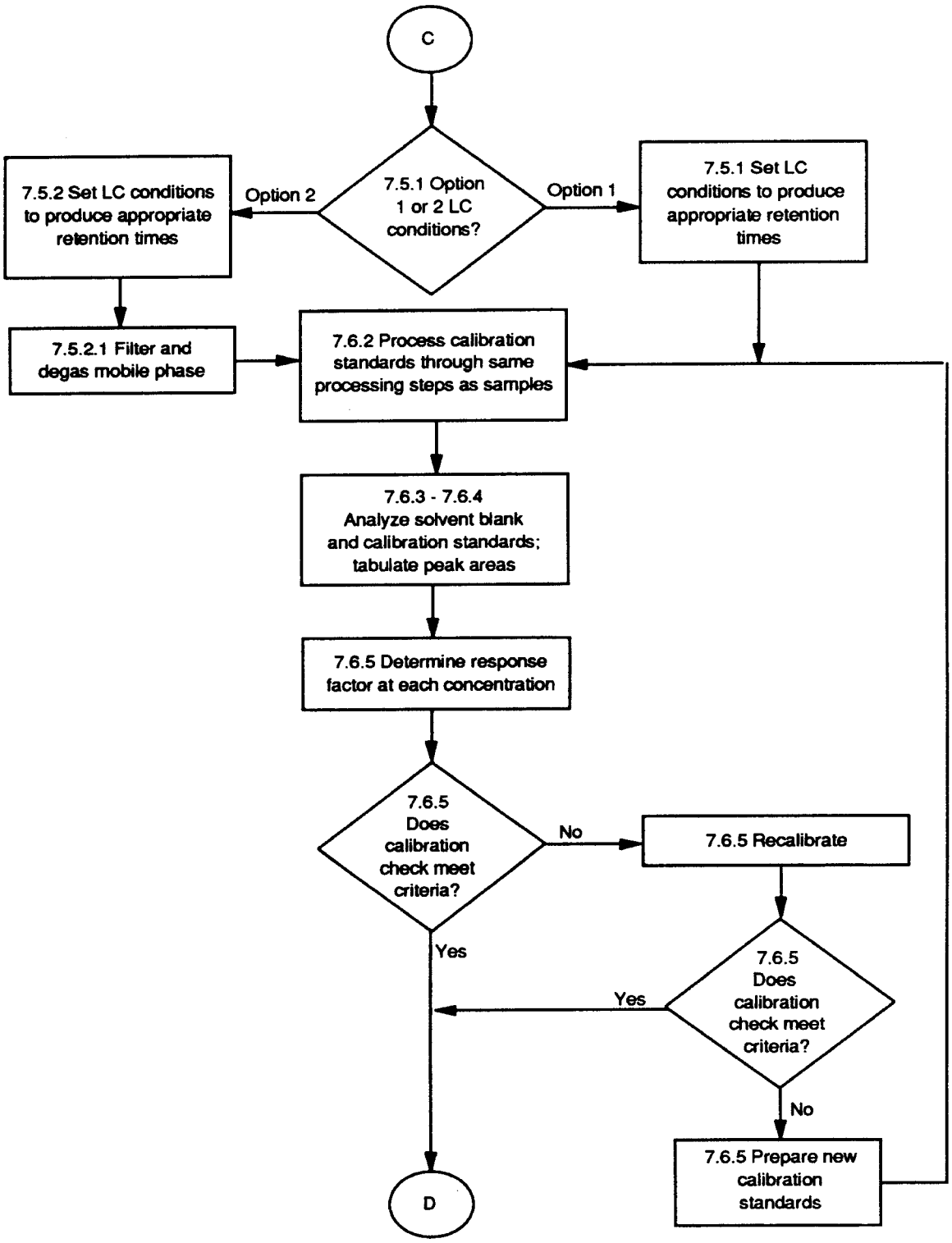
Number	Compound	Concentration (ng/µL)
1	Formaldehyde	1.140
2	Acetaldehyde	1.000
3	Acrolein	1.000
4	Acetone	1.000
5	Propanal	1.000
6	Crotonaldehyde	1.000
7	Butanal	0.905
8	Benzaldehyde	1.000
9	Isovaleraldehyde	0.450
10	Pentanal	0.485
11	o-Tolualdehyde	0.515
12	m-Tolualdehyde	0.505
13	p-Tolualdehyde	0.510
14	Hexanal	1.000
15	2,4-Dimethylbenzaldehyde	0.510

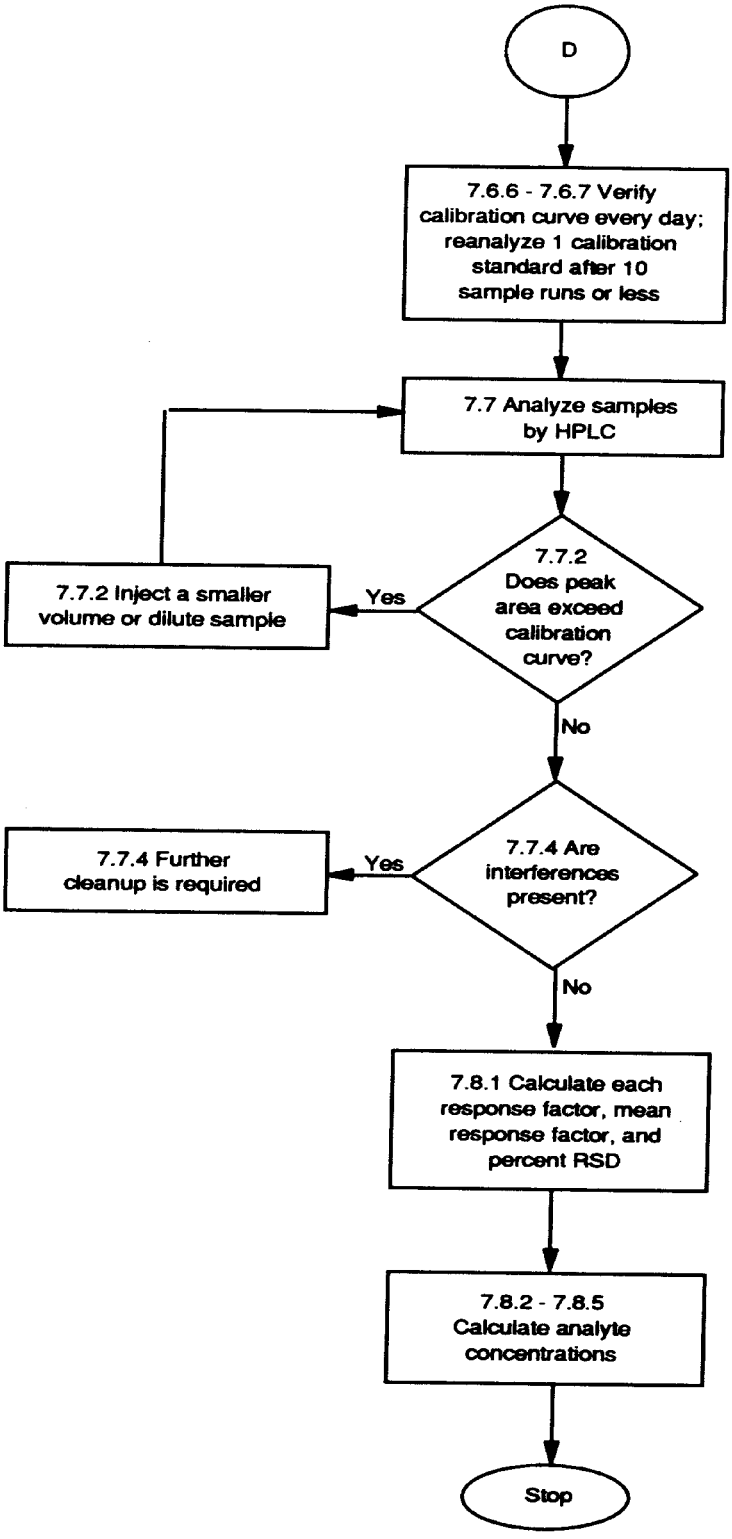
DETERMINATION OF CARBONYL COMPOUNDS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)




A
7.4.2.2 - 7.4.2.3 Connect sample cartridge to clean syringe and place in syringe rack
7.4.2.4 Backflush cartridge with acetonitrile
7.4.2.4 Does eluate flow become blocked?
Yes
7.4.2.4 Displace trapped air with acetonitrile in syringe using a long-tip disposable Pasteur pipet
No
7.4.2.5 Dilute to 5 mL with acetonitrile; label flask; pipet 2 aliquots into sample vials
7.4.2.6 Store sample at 4C
C


B
7.4.1.1 Measure volume of aqueous phase of sample; pour sample into separatory funnel and drain methylene chloride (from Method 0011) into volumetric flask.
7.4.1.2 Extract aqueous solution with methylene chloride; add methylene chloride extracts to volumetric flask.
7.4.1.3 Dilute to volume with methylene chloride; mix well; remove aliquot.
7.4.1.4 Does sample have a high concentration of formal-dehyde?
No
Yes
7.4.1.4 Exchange solvent with methanol as in 7.3.5.5.
7.4.1.4 Dilute extract with mobile phase.
7.4.1.4 Does sample have a low concentration of formalde-hyde?
No
Yes
7.4.1.5 Store sample at 4°C.
7.4.1.4 Concentrate extract during solvent exchange process.
C


C
7.5.1 Option 1 or 2 LC conditions?
Option 2
Option 1
7.5.2 Set LC conditions to produce appropriate retention times
7.5.1 Set LC conditions to produce appropriate retention times
7.5.2.1 Filter and degas mobile phase
7.6.2 Process calibration standards through same processing steps as samples
7.6.3 - 7.6.4 Analyze solvent blank and calibration standards; tabulate peak areas
7.6.5 Determine response factor at each concentration
7.6.5 Does calibration check meet criteria?
No
7.6.5 Recalibrate
Yes
7.6.5 Does calibration check meet criteria?
Yes
No
7.6.5 Prepare new calibration standards
D


D
7.6.6 - 7.6.7 Verify calibration curve every day; reanalyze 1 calibration standard after 10 sample runs or less
7.7 Analyze samples by HPLC
7.7.2 Does peak area exceed calibration curve?
Yes
7.7.2 Inject a smaller volume or dilute sample
No
7.7.4 Are interferences present?
Yes
7.7.4 Further cleanup is required
No
7.8.1 Calculate each response factor, mean response factor, and percent RSD
7.8.2 - 7.8.5 Calculate analyte concentrations
Stop

APPENDIX A

RECRYSTALLIZATION OF 2,4-DINITROPHENYLHYDRAZINE (DNPH)

NOTE: This procedure should be performed in a properly ventilated hood. Inhalation of acetonitrile can result in nose and throat irritation (brief exposure at 500 ppm) or more serious effects at higher concentration and/or longer exposures.

A.1 Prepare a saturated solution of DNPH by boiling excess DNPH in 200 mL of acetonitrile for approximately 1 hour.

A.2 After 1 hour, remove and transfer the supernatant to a covered beaker on a hot plate and allow gradual cooling to 40 to 60°C. Maintain this temperature range until 95% of the solvent has evaporated, leaving crystals.

A.3 Decant the solution to waste and rinse the remaining crystals twice with three times their apparent volume of acetonitrile.

A.4 Transfer the crystals to a clean beaker, add 200 mL of acetonitrile, heat to boiling, and again let the crystals grow slowly at 40 to 60°C until 95% of the solvent has evaporated. Repeat the rinsing process as in Sec. A.3.

A.5 Take an aliquot of the second rinse, dilute 10 times with acetonitrile, acidify with 1 mL of 3.8 M perchloric acid per 100 mL of DNPH solution, and analyze with HPLC as in Sec. 7.0 for Procedure 2. An acceptable impurity level is less than 0.025 ng/µL of formaldehyde in recrystallized DNPH reagent or below the sensitivity (ppb, v/v) level indicated in Table 3 for the anticipated sample volume.

A.6 If the impurity level is not satisfactory, pipet off the solution to waste, repeat the recrystallization as in Sec. A.4 but rinse with two 25 mL portions of acetonitrile. Prep and analyze the second rinse as in Sec. A.5.

A.7 When the impurity level is satisfactory, place the crystals in an all-glass reagent bottle, add another 25 mL of acetonitrile, stopper, and shake the bottle. Use clean pipets when removing the saturated DNPH stock solution to reduce the possibility of contamination of the solution. Maintain only a minimum volume of the saturated solution adequate for day to day operation to minimize waste of the purified reagent.