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METHOD 8315

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 (UV) detection to identify and quantitate the target analytes using two (2) different sets of conditions: Option 1 and Option 2. Option 1 has been shown to perform well for one set of target analytes for the following matrices: aqueous samples, soil or waste samples, and stack samples collected by Method 0011. Option 2, and its set of target analytes, has been shown to work well for indoor air samples collected by Method 00YY. The two sets of target analytes overlap for some compounds. Refer to the Analysis Option number listed in the following table to determine which analytes may be analyzed by which option. The following analytes may be determined by this method:

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

[a] Chemical Abstract Services Registry Number.
[b] This list of target analytes contains an overlapping list of compounds that have been evaluated using different analysis techniques.

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 Gas - for degassing mobile phase solvents. (Option 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 Teflon and use a 0.22 μm polyester membrane filter. (Option 2)

4.2 Apparatus and Materials for Option 1

4.2.1 Reaction vessel - 250 mL Florence flask.

4.2.2 Separatory funnel - 250 mL, with Teflon stopcock.

4.2.3 Kuderna-Danish (K-D) apparatus.

4.2.3.1 Concentrator tube - 10 mL graduated (Kontes K-570050-1025 or equivalent). A ground glass stopper is used to prevent evaporation of extracts.

4.2.3.2 Evaporation flask - 500 mL (Kontes K-570001-500 or equivalent). Attach to concentrator tube with springs, clamps, or equivalent.

4.2.3.3 Snyder column - Three ball macro (Kontes K-503000-0121 or equivalent).

4.2.3.4 Snyder column - Two ball micro (Kontes K-569001-0219 or equivalent).

4.2.3.5 Springs - 1/2 inch (Kontes K-662750 or equivalent).

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

4.2.7 pH meter - Capable of measuring to the nearest 0.01 units.

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

4.2.9 Solid sorbent cartridges - Packed with 500 mg C18 (Baker or equivalent).

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

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

4.2.12 Pipet - Capable of accurately delivering 0.10 mL solution (Pipetman or equivalent).

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

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

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

4.2.16 Syringe Filters - 0.45 µm filtration disks (Gelman Acrodisc 4438 or equivalent).

4.3 Volumetric Flasks - 5 mL, 10 mL, and 250 or 500 mL.

4.4 Vials - 10, 25 mL, glass with Teflon lined screw caps or crimp tops.

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

4.6 Glass Funnels

4.7 Polyethylene Gloves - used to handle silica gel cartridges.

5.0 REAGENTS

5.1 Reagent grade 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 - All references to water in this method refer to organic-free reagent water, as defined in Chapter One.

5.2 Option 1 Reagents

5.3.1 Methylene chloride, CH_2Cl_2 - HPLC grade or equivalent.

5.3.2 Methanol, CH_3OH - HPLC grade or equivalent.

5.3.3 Ethanol (absolute), CH_3CH_2OH - HPLC grade or equivalent.

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

5.3.5 Sodium chloride, NaCl.

5.3.6 Sodium sulfite solution, Na_2SO_3, 0.1 M.

5.3.7 Sodium sulfate, Na_2SO_4 - granular, anhydrous.

5.3.8 Citric Acid, $C_8H_8O_7$, 1.0 M - solution.

5.3.9 Sodium Citrate, $C_6H_5Na_3O_7.2H_2O$, 1.0 M - trisodium salt dihydrate solution.

5.3.10 Acetic acid (glacial), CH_3CO_2H.

5.3.11 Hydrochloric Acid, HCl, 0.1 N.

5.3.12 2,4-Dinitrophenylhydrazine (DNPH) (70% (W/W)), $[2,4-(O_2N)_2C_6H_3]NHNH_2$, in organic-free reagent water.

5.3.13 DNPH (3.00 μg/L) - Dissolve 428.7 mg of 70% (w/w) reagent in 100 mL absolute ethanol. Slight heating or sonication may be necessary to effect dissolution.

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

5.3.15 Sodium chloride solution (saturated) - Prepare by mixing an excess of the reagent grade solid with organic-free reagent water.

5.3.16 Extraction fluid for Option 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 $\pm$ 0.02.

5.3 Option 2 Reagents

Note: Sampling reagents and procedures for the preparation of stock standard solutions and DNPH derivative standards for Option 2 are given in Method OOYY.

5.3.1 Acetonitrile, CH_3CN - UV grade (for option 2).

5.3.2 2,4-Dinitrophenylhydrazine (DNPH), $C_6H_6N_4O_4$ - recrystallize at least twice with UV grade acetonitrile using the procedure in Appendix A.

5.4 Formaldehyde, CH_2O - ACS reagent, 37% solution (W/W).

5.5 Aldehydes and Ketones - analytical grade, used for preparation of DNPH derivative standards of target analytes other than formaldehyde. See list on page 1 for possible target analytes.

5.6 Stock Standard Solutions for Option 1

5.6.1 Stock formaldehyde (approximately 1.00 mg/mL) - Prepare by diluting 265 μL formaldehyde to 100 mL with organic-free reagent water.

5.6.1.1 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 (Section 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:

$$\text{Concentration (mg/mL)} = \frac{(30.03)(\text{N HCl})(\text{mL HCl})}{25.0 \text{ mL}}$$

where:

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

5.6.2 Stock aldehyde(s) and ketone(s) - Prepare by adding an appropriate amount of the analyte to 90 mL of methanol and dilute to 100 mL to give a final concentration of 1.00 mg/mL.

5.6.3 Stock standard solutions must be replaced after six weeks, or sooner, if comparison with check standards indicates a problem.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1 See the introductory material to this Chapter, Organic Analytes, Section 4.1.

6.2 Samples must be refrigerated at 4°C. Aqueous samples must be derivatized and extracted within 3 days of sample collection. Higher molecular weight aldehydes (heptanal to decanal) evidenced losses greater than 50% in a liquid matrix over a 3 day holding time. Accordingly, liquid samples should be derivatized and extracted within 24 hours of sample collection. Likewise, the holding times of extracts 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 00YY 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 (Option 1)

7.3.1 For aqueous samples, measure a 100 mL aliquot of sample. Other aqueous sample volumes, from 50 to 200 mL, may be employed to accommodate the anticipated analyte concentration range. Quantitatively transfer the sample aliquot to the reaction vessel (Section 4.2).

7.3.2 For solid samples, 1 to 10 mL of extract (Section 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.

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

7.3.4 Liquid-Solid Derivatization and Extraction

7.3.4.1 Add 4 mL of citrate buffer and adjust the pH to 3.0 ± 0.1 with 6 M HCl or 6 M NaOH. Add 6 mL of DNPH reagent, seal the container, and place in a heated, orbital shaker (Section 4.2.14) set at 40°C for 1 hour. Adjust the agitation to produce a gentle swirling of the reaction solution.

7.3.4.2 Assemble the vacuum manifold and connect to a water aspirator or vacuum pump. Entrain three (3) solid sorbent cartridges, using connectors supplied by the manufacturer, and attach the sorbent train 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 the sorbent cartridge train.

7.3.4.3 Remove the reaction vessel from the shaker and add 10 mL saturated NaCl solution to the vessel.

7.3.4.4 Add the reaction solution to the sorbent train and apply a vacuum so that the solution is drawn through the cartridges at a rate of 3 to 5 mL/min. Continue applying the vacuum for about 10 minutes after the liquid sample has passed through the cartridges.

7.3.4.5 Maintain vacuum conditions described in Section 7.3.4.4, elute each cartridge train with approximately 9 mL of absolute ethanol directly into a 10 mL volumetric flask. Dilute the solution to volume with absolute ethanol, mixed 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 this step (7.3.4.5). Its presence is not indicative of non-recovery of the analyte derivatives.

complete concentration. At the proper rate of distillation the balls of the column will actively chatter, but the chambers will not flood. When the apparent volume of liquid reaches less than 5 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes.

7.3.5.5.2 Remove the Snyder column and rinse the flask and its lower joint with 1-2 mL of methanol and add to concentrator tube. Quantitatively transfer the sample to a 10 mL volumetric flask using a 5 mL syringe with an attached Acrodisk 0.45 μm filter cassette. Adjust the extract volume to 10 mL. Stopper the flask and store refrigerated at 4°C if further processing will not be performed immediately. If the extract will be stored longer than two (2) days, it should be transferred to a vial with a Teflon lined screw cap or crimp top. Proceed with HPLC chromatographic analysis if further cleanup is not required.

7.4 Extraction of Samples from Methods 0011 and 00~~YY~~ 0100 (Options 1 and 2)

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

7.4.1.1 Measure the aqueous volume of the sample prior to extraction (for moisture determination in case 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 levels of formaldehyde are present, the extract can be diluted with mobile phase, otherwise the extract must be solvent exchanged as described in Section 7.3.5.5. If low levels 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 (2) days, it should be transferred to a vial with a Teflon lined screw cap or crimp top with Teflon-lined septa. Proceed with HPLC chromatographic analysis if further cleanup is not required.

7.4.2 Indoor air samples collected by Method 00~~YY~~ 0100 (Option 2)

7.4.2.1 Refer to Section 7.1 of Method ~~00YY~~ 0100 for information regarding desorption of the sample analytes from the sample cartridge.

Add actual Elution Instructions for 0100

7.4.2.2 Store the sample at 4°C. Proceed with HPLC chromatographic analysis if further cleanup is not required.

7.5 Chromatographic Conditions (Recommended):

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

Column:	C18, 250 mm x 4.6 mm ID, 5 µm particle size
Mobile Phase Gradient:	70%/30% methanol/water (v/v) for 20 min. 70%/30% methanol/water to 100% methanol in 15 min. 100% methanol for 15 min.
Flow Rate:	1.5 mL/min
Detector:	Ultraviolet, operated at 360 nm
Injection Volume:	20 µL
Run Time:	About 50 minutes.

7.5.2 Option 2 - For indoor air samples collected by Method ~~00YY~~ 0100.

Column:	Two Zorbax ODS columns in series (4.6 mm i.d. x 25 cm or equivalent)
Mobile Phase Gradient:	Linear gradient program of acetonitrile (ACN) and water beginning after sample injection: From 60%/40% to 75%/25% ACN/Water in 30 minutes. From 75%/25% to 100%/0% ACN/Water in 20 minutes. Maintain 100% ACN for 5 minutes. From 100%/0% to 60%/40% ACN/Water in 1 minute. Maintain 60%/40% ACN/Water ratio for 15 minutes.
Detector:	Ultraviolet, operated at 360 nm
Flow Rate:	1.0 mL/min
Sample Injection volume:	25 µL (suggested)
Run Time:	About 1 hour and 11 minutes for a complete cycle.

Note: For Option 2 analysts are advised to experiment with their HPLC systems to optimize chromatographic conditions for their particular analytical needs. The separation of acrolein, acetone, and propionaldehyde should be a minimum goal of the optimization.

7.5.2.1 Option 2 suggests that filtering and degassing of the mobile phase be done to remove dissolved gasses using the following procedure:

7.5.2.1.1 Filter each of the liquids (water and acetonitrile) through a 0.22 µm polyester membrane filter in an all glass and Teflon suction filtration apparatus.