

Determination of Carbonyl Compounds by Reversed-Phase High-Performance Liquid Chromatography

INTRODUCTION

Large quantities of carbonyl compounds are used worldwide as raw materials and intermediates in the chemical and plastics manufacturing industries. These compounds are eye, skin, and respiratory system irritants and many have also been identified as animal teratogens, mutagens, and carcinogens. Because of their widespread, high-volume industrial usage they are present in workplace air spaces and industrial waste materials. They are also present in dump site leachates, which can contaminate ground water that may eventually be used for drinking water.

This application note describes a method wherein carbonyl compounds in aqueous samples or extracts are first derivatized with dinitrophenylhydrazine (DNPH). After derivatization, the carbonyl compounds are extracted and injected onto a C18, reversed-phase high-performance liquid chromatography (RP-HPLC) column and separated using gradient elution. The separated compounds are then quantified by UV detection at 360 nm.

These analytical procedures are patterned after and consistent with U.S. EPA Method 554, which specifically addresses drinking water samples. It is also consistent with U.S. EPA Method 8315, which addresses aqueous and solid waste, soil, stack gas, and indoor air samples.

EQUIPMENT

Dionex DX-500 HPLC system consisting of:

- GP40 Gradient Pump (SST)
- LC5 High-Pressure Injection Valve (SST)
- AD20 Absorbance Detector
- Eluent Organizer

PeakNet® Chromatography Workstation

REAGENTS AND STANDARDS

Reagents

Reagent grade chemicals should be used for all tests. Water should be free from substances that interfere with the test. Such substances include the analytes of interest that should be below the limits of detection as well as residual chlorine and phenolic compounds. Because formaldehyde is ubiquitous, care must be taken to prevent it from contaminating the water.

- Acetonitrile (HPLC grade or equivalent)
- Acetic acid
- Sodium acetate
- Citric acid
- Sodium citrate (trisodium dihydrate)
- 2,4-dinitrophenylhydrazine (DNPH), recrystallized with acetonitrile
- Hydrochloric acid
- Methylene chloride (HPLC grade or equivalent)
- Perchloric acid
- Sodium chloride
- Sodium hydroxide (pellets)
- Sodium sulfate (granular, anhydrous)
- Sodium sulfite (anhydrous)

Standards

Analytical grade aldehydes and ketones should be purchased for the preparation of the DNPH derivative standards. Alternatively, prederivatized DNPH stock solutions can be obtained as 100 µg/mL concentrates from AccuStandard (New Haven, Connecticut, USA).

CONDITIONS

Option 1

Column: Zorbax® ODS column,
4.6 mm x 250 mm i.d.,
5- μ m particle size

Eluent 1: Acetonitrile

Eluent 2: Water

Gradient:

Time (min)	A (%)	B (%)
0	60	40
20	60	40
35	100	0
50	100	0

Flow Rate: 1.2 mL/min

Inj. Volume: 20 μ L

Detection: UV, 360 nm

Option 2

Column: Two Zorbax ODS columns,
4.6 mm x 250 mm i.d.,
5- μ m particle size

Eluent 1: Acetonitrile

Eluent 2: Water

Gradient:

Time (min)	A (%)	B (%)
0	60	40
30	75	25
50	100	0
55	100	0
56	60	40

Flow Rate: 1.0 mL/min

Inj. Volume: 25 μ L

Detection: UV, 360 nm

PREPARATION OF SOLUTIONS

Stock Solutions

Acetate Buffer (for formaldehyde analysis only)

Add 40 mL of 5 M acetic acid to 60 mL of 5 M sodium acetate. Mix thoroughly and adjust to pH 5 with NaOH (or HCl) as needed.

1 M Citric Acid

Place 192.1 g of citric acid in a 1-L volumetric flask. Add 500 mL of water and mix until dissolved. Mix and dilute to volume with water.

1 M Sodium Citrate

Place 258.1 g of sodium citrate in a 1-L volumetric flask. Add 500 mL of water and mix until dissolved. Mix and dilute to volume with water.

1 M Citrate Buffer, pH 3

Add 80 mL of 1 M citric acid to 20 mL of 1 M sodium citrate. Mix thoroughly and adjust the pH with NaOH (or HCl) as needed.

2,4-Dinitrophenylhydrazine, 3.00 mg/mL

Dissolve 428.7 mg of DNPH (solids containing 30% water) into 100 mL of acetonitrile. (See Appendix A for DNPH purification procedure.)

Sodium Chloride, Saturated Solution

Dissolve an excess of sodium chloride (greater than 38 g/100 mL) in water.

5.0 N Sodium Hydroxide

Place 100 mL water in a 200-mL volumetric flask. Add 40.0 g of sodium hydroxide, and mix and cool the solution to 25 °C. Bring to volume with water.

0.1 M Sodium Sulfite Solution (freshly prepared)

Place 150 mL of water in a 250-mL volumetric flask. Add 3.15 g of anhydrous sodium sulfite, dissolve, and dilute to volume with water.

Stock Standard Solutions

Prederivatized DNPH stock solutions can be obtained as 100 μ g/mL concentrates, in acetonitrile, from AccuStandard (New Haven, Connecticut, USA). Alternatively, underivatized stock standard solutions may be made from the pure standards and derivatized, as needed, according to the directions listed below.¹

Preparation of Stock Standard Solutions (1000 mg/L)

Add 100 mg of the pure material other than formaldehyde to 90 mL of water and dilute to 100 mL to give a final volume concentration of 1000 mg/L. Store all standard solutions in the dark at 4 °C in a glass vial with a Teflon®-lined cap with minimum headspace. They should be stable for about six weeks.

Preparation of Stock Formaldehyde Solution (1000 mg/mL)

Prepare by diluting an appropriate amount of the certified formaldehyde solution. If a certified formaldehyde solution is not available, dilute approximately 2.67 mL of commercially available 37% formaldehyde solution to 1 L with water. Standardize by using the procedure discussed in Appendix B. The solution should be stored at room temperature in the dark and it should be standardized every 6 months. The solution appears to be stable indefinitely.

Preparation of Calibration Standards

Calibration Standards

Prepare calibration standards at a minimum of five concentrations for each analyte of interest in water from the stock standard solution. The lowest concentration of each standard should meet or slightly exceed the MDLs listed in Table 1. The other concentrations of the calibration curve should correspond to the expected range of concentrations found in the samples of interest.

Formaldehyde Solution (10.0 mg/L)

Dilute 1.00 mL of the formaldehyde stock solution to 100 mL with water. Prepare fresh daily.

SAMPLE PREPARATION AND PRESERVATION

Aqueous Samples

Aqueous samples should be stored at 4 °C and derivatized within three days of being taken. Derivatized compounds are extracted using methylene chloride (liquid/liquid) extraction or C18 cartridge (liquid/solid) adsorption, followed by ethanol extraction. The methylene chloride extracts are exchanged into either acetonitrile or ethanol and analyzed by HPLC. The ethanol extracts are analyzed directly by HPLC.

Table 1 Method Detection Limits

Analyte	MDL ^a Option 1	MDL ^a Option 2
Acetaldehyde	12 (µg/L)	0.6 (µg/L)
Acetone	N/A	2
Acrolein	N/A	0.4
Benzaldehyde	N/A	2
Butanal	9	9
Crotonaldehyde	1	2
Cyclohexanone	0.5	N/A
Decanal	6	N/A
2,5-Dimethylbenzaldehyde	N/A	10
Formaldehyde	0.8	0.4
Heptanal	3	N/A
Hexanal	3	3
Isovaleraldehyde	N/A	5
Nonanal	9	N/A
Octanal	3	N/A
Pentanal	2	5
Propanal	8	2
<i>m</i> -Tolualdehyde	N/A	5
<i>o</i> -Tolualdehyde	N/A	4
<i>p</i> -Tolualdehyde	N/A	5

^aM.D.L. = (s.d.) × (t)_{99.5%}, where (t)_{99.5%} is for a 99.5% single-sided student's *t* test distribution.
N/A = Not applicable

Solid Samples

Solid samples should be stored at 4 °C and extracted within three days. Samples are extracted with an acetate buffer solution and this buffered extract is derivatized, extracted and analyzed by HPLC as described for aqueous samples.

Stack Gas Samples

Stack gas samples are drawn through a sampling train where the carbonyl compounds are derivatized *in situ*. These samples should be stored at 4 °C and analyzed within 30 days of being taken. The derivatized samples are extracted with methylene chloride and the extracted compounds exchanged into acetonitrile, followed by HPLC analysis.

Indoor Air Samples

Indoor air samples are drawn through a sampling train where the carbonyl compounds are derivatized *in situ*. These samples should be stored at 4 °C and analyzed within 30 days of being taken. The derivatized compounds are extracted with acetonitrile and analyzed by HPLC.

DERIVATIZATION

Measure an aliquot of the sample which is appropriate to the anticipated concentration range (nominally 100 mL). Quantitatively transfer the sample aliquot to a 250-mL Florence flask. 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 water. Record the original sample volume prior to dilution.

For analytes other than formaldehyde, add 4 mL of citrate buffer and adjust the pH to 3.0 ± 0.1 with 6 N HCl or 6 N NaOH. If formaldehyde is the only analyte of interest, add 4 mL of acetate buffer and adjust the pH to 5.0 ± 0.1 with 6 N HCl or 6 N NaOH. In either case, following pH adjustment add 6 mL of DNPH reagent, seal the container, and place in a heated orbital shaker at 40 °C for 1 hour. Adjust the agitation to produce a gentle swirling of the reaction solution.

EXTRACTION PROCEDURE

Liquid-Solid Extraction

This procedure requires the use of a vacuum manifold, which is capable of simultaneous extraction of up to 12 samples. In addition, solid sorbent cartridges packed with 2 g of C18 resin will be required.

Attach the assembled vacuum manifold to a water aspirator or vacuum pump. Attach the 2 g sorbent cartridges to the vacuum manifold and condition them by passing 10 mL of 40 mM citrate buffer through each one. Remove the Florence flask from the shaker at the end of the 1-hour period and add to it 10 mL of saturated sodium chloride solution.

Pour the reaction solution into the sorbent cartridges and apply a vacuum so that the solution is drawn through 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. While maintaining the vacuum, elute each cartridge into a 10-mL volumetric flask, with approximately 9 mL of acetonitrile. Dilute the solution to volume with acetonitrile, mix thoroughly, and

place in a tightly sealed vial until analyzed. Due to the excess of DNPH used in the derivatization process, the sorbent cartridges will retain the yellow color after elution. This discoloration does not indicate non-recovery of the analyte derivatives.^{1,2}

Liquid-Liquid Extraction

After the 1-hour derivatization period, 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 g of anhydrous sodium sulfate. Swirl the contents to complete the drying process.

Assemble a Kuderna-Danish (K-D) concentrator by attaching a 10-mL concentrator tube to a 500-mL evaporator flask. Pour the methylene chloride extract into the evaporator flask, taking care to minimize transfer of the sodium sulfate granules. Wash the Erlenmeyer flask with 30 mL of methylene chloride and add the wash to the evaporator flask.

Add one or two boiling chips to the flask and attach a three-ball Snyder column. Pre-wet the Snyder column by adding about 1 mL of methylene chloride to the top. Place the K-D apparatus in a hot water bath (80–90 °C) so that the concentrator tube is partially immersed in the hot water and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 10–15 minutes. At the proper rate of distillation the balls of the column will actively chatter, but the chambers will not flood with the condensed solvent. When the apparent volume of the liquid reaches 5 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes.

Prior to liquid chromatographic analysis, the extract solvent must be exchanged to acetonitrile. Remove the three-ball Snyder column and evaporator flask. Add 5 mL of acetonitrile and a new boiling chip and attach a micro-Snyder column to the concentrator tube. Concentrate the extract using 1 mL of acetonitrile to pre-wet the Snyder column. Place the K-D apparatus in the water bath so that the concentrator tube is partially immersed in the hot water and proceed as described above. 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.

Remove the Snyder column and rinse the flask and its lower joint with 1–2 mL of acetonitrile and add to the concentrator tube. Quantitatively transfer the sample to a 10-mL volumetric flask using a 5-mL syringe with an attached Acrodisk® 0.45-mm 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 for longer than two 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.¹

PREPARATION FOR HPLC ANALYSIS

Filter the mobile phase through a 0.22-mm polyester membrane filter in an all glass and Teflon suction apparatus. Degas each filtered solution by purging with helium for 10 to 15 minutes (100 mL/ min) or by heating to 60 °C for 5 to 10 minutes in an Erlenmeyer flask covered with a watch glass. A constant backpressure restrictor 500 kPa (75 psi) or 15–30 cm of 0.25-mm i.d. Teflon tubing should be placed after the detector to eliminate further mobile phase outgassing.

Allow the system to pump the initial solvent mixture ratio (60% CH₃CN/40% H₂O) for 20 to 30 minutes at a flow rate of 1 mL/min and monitor the detector output to establish a stable baseline.

DISCUSSION AND RESULTS

This application note describes two options for this HPLC analysis of carbonyl compounds. Option 1 uses a single C18 column and separates a limited set of compounds. This option has been found suitable for the analysis of drinking water, waste water, soil and stack gas samples. Option 2 separates a somewhat different and broader range of compounds. This option has been found suitable for the analysis of indoor air samples. Table 2 lists the compounds determined by each option. See Figures 1 and 2 on page 6 for example chromatograms.

Method detection limits for both options were obtained using fortified, organic-free reagent water. MDLs are based upon analyses of eight replicate spiked blank injections. Method performance is illustrated in U.S. EPA Method 8315.

Table 2 Carbonyl Compounds that Can Be Determined by This Method

Compound Name	Option
Acetaldehyde	1, 2
Acetone	2
Acrolein	2
Benzaldehyde	2
Butanal	1, 2
Crotonaldehyde	1, 2
Cyclohexanone	1
Decanal	1
2,5-Dimethylbenzaldehyde	2
Formaldehyde	1
Heptanal	1
Hexanal	1, 2
Isovaleraldehyde	2
Nonanal	1
Octanal	1
Pentanal	1, 2
Propanal	1, 2
<i>m</i> -Tolualdehyde	2
<i>o</i> -Tolualdehyde	2

PRECAUTIONS

Hazards

The toxicity or carcinogenicity of each reagent in this method has not been precisely defined; however, each compound should be treated as a potential health hazard. For example, glacial acetic acid can produce skin burns and severely injure the eyes. Formaldehyde is a severe skin, eye, and mucous membrane irritant and can cause acute hypersensitivity. Formaldehyde may also be a potential carcinogen. DNPH is flammable. The laboratory is responsible for maintaining a current awareness of OSHA regulations regarding the safe handling of the chemicals specified in this method.

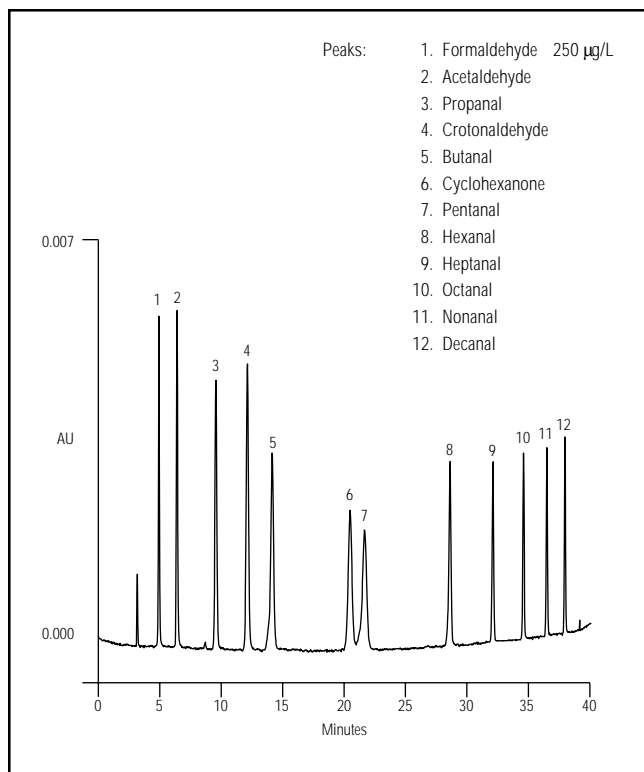


Figure 1. Separation of carbonyl compounds using Option 1.

Interferences

Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in the chromatogram. All of these materials must be routinely demonstrated to be free from interferences by running blanks. Formaldehyde contamination of the DNPH is a frequently encountered problem because formaldehyde is prevalent in the environment; thus, DNPH should be recrystallized to prevent this type of contamination using the procedure Appendix A.

Matrix interference may be caused by contaminants that are co-extracted from the sample. If this is a problem, additional sample cleanup may be necessary.

Acetaldehyde is generated during derivatization if ethanol is present in the sample. This background will impair the measurement of acetaldehyde at levels below 500 µg/L. Phenols, residual chlorines, sulfites, and other reactive substances combine with some free carbonyls to form other compounds. Such bound carbonyls are not meant to be measured by this test method.

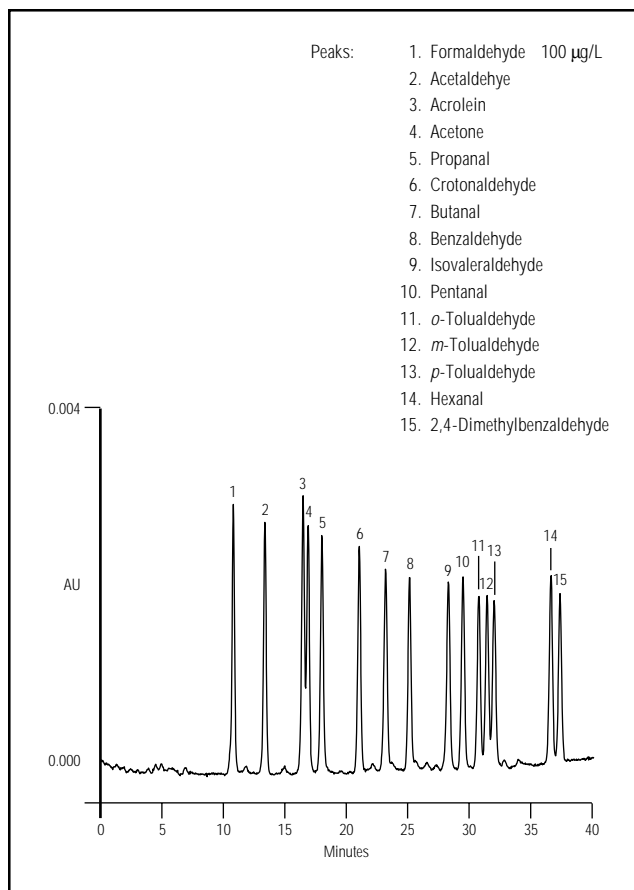


Figure 2. Separation of carbonyl compounds using Option 2.

REFERENCES

1. U.S. Environmental Protection Agency. *U.S. EPA Method 8315*, "Determination of Carbonyl Compounds by High-Performance Liquid Chromatography," November 1992.
2. U.S. Environmental Protection Agency. *U.S. EPA Method 554* "Determination of Carbonyl Compounds in Drinking Water by Dinitrophenylhydrazine Derivatization and High-Performance Liquid Chromatography (HPLC)," August 1992.

APPENDIX A: 2,4-DINITROPHENYLHYDRAZINE PURIFICATION

Formaldehyde contamination of DNPH reagent is a frequently encountered problem due to its widespread occurrence in the environment. The DNPH reagent must be purified using the following procedure.

Prepare a saturated solution of DNPH by boiling excess DNPH in 200 mL of acetonitrile for approximately 1 hour, transfer the supernatant to a covered beaker on a hot plate and allow gradual cooling to 40–60 °C. Maintain this temperature range until 95% of the solvent has evaporated, leaving crystals. Decant the solution to waste and rinse the remaining crystals twice with acetonitrile. Repeat the recrystallization process. 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 conditions for Option 2. The impurity level for formaldehyde should be less than 0.025 ng/μL. If the impurity level is not satisfactory, pipette off the solution to waste, repeat the recrystallization procedure, and rinse with two 25-mL portions of acetonitrile.

Prepare the second rinse as before and analyze. When the impurity level is satisfactory place the crystals in an all-glass reagent bottle, add another 25 mL of acetonitrile, cap, and shake the bottle. Always use clean pipettes when removing the saturated DNPH stock solution to reduce contamination of the solution.¹

Note: This procedure should be performed under a ventilated hood.

APPENDIX B: STANDARDIZATION OF FORMALDEHYDE STOCK

Calibrate a pH meter with the standard 7.0 and 10.0 pH buffers. Transfer a 25.0-mL aliquot of the 0.1 M sodium sulfite solution to an Erlenmeyer flask. Measure and record the pH while stirring. Add a 25.0-mL aliquot of the formaldehyde stock solution, mix, and allow to stand for 5 minutes. While stirring, back titrate this mixture to the original pH using 0.1 N HCl. Perform three duplications and calculate the mean concentration. Duplicates should agree within 0.3%. Calculate the concentration of the formaldehyde stock solution as follows:

$$C_F = \frac{V_A \times C_A \times MW_F}{V_F}$$

where: C_F = Concentration of formaldehyde (mg/L)

C_A = Concentration of acid used (N)


V_F = Volume of formaldehyde (mL)

V_A = Volume of acid used (mL)

MW_F = Molecular weight of formaldehyde (30.03)



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 Printed on recycled and recyclable paper.

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* Designed, developed, and manufactured under an NSAI registered ISO 9001 Quality System.



LPN 0578 500 3/01
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