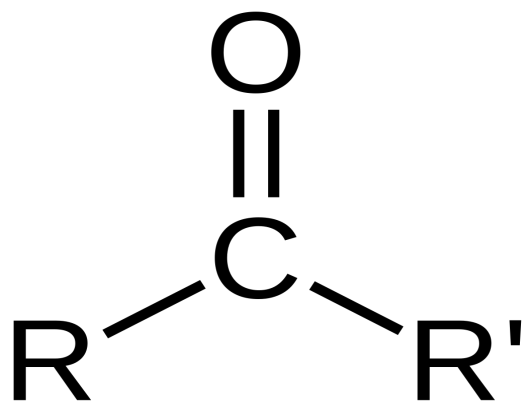


Determination Of Carbonyl Compounds In Water By Dinitrophenylhydrazine Derivatization and HPLC/UV*



UCT Part Numbers

EUC1812M15Unendcapped C18 -
2000 mg/15 mL cartridge

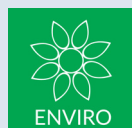
Method Summary:

This method provides procedures for the determination of free carbonyl compounds and aldehydes in water. A measured volume of sample is buffered to pH 3 and the analytes derivatized at 40°C for one hour using 2,4-dinitrophenylhydrazine (DNPH) then extracted through SPE cartridges containing 2000 mg of C18. The cartridge(s) are eluted with 10 mL of ethanol and the derivatives are determined by absorbance at 360 nm after separation by HPLC.

The following compounds can be determined using this method:

Analyte	CASRN
2,5-Dimethylbenzaldehyde	5779-94-2
Acetaldehyde	75-07-0
Acetone	67-64-1
Acrolein	107-02-8
Benzaldehyde	100-52-7
Butanal	123-72-8
Crotonaldehyde	123-73-9
Cyclohexanone	108-94-1
Decanal	112-31-2
Formaldehyde	50-00-0
Heptanal	111-71-7
Hexanal (hexaldehyde)	66-25-1
Hexanal	66-25-1
Isovaleraldehyde	590-86-3
m-Tolualdehyde	620-23-5
Nonanal	124-19-6
Octanal	124-13-0
o-Tolualdehyde	529-20-4
Pentanal (propionaldehyde)	110-62-3
Propanal	123-38-6
p-Tolualdehyde	104-87-0

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



Stock Standard Solutions:

Stock standard formaldehyde solution approximately 1 mg/mL

- Prepare by diluting 265 µL of formalin to 100 mL with reagent water

Standardization of formaldehyde stock solution

- Transfer 25 mL of a 0.1M Na₂SO₃ solution to a beaker
- Record the pH
- Add a 25.0 mL aliquot of the formaldehyde stock solution and record the pH
- Titrate this mixture back to the original pH using 0.1 N HCl
- Calculate the formaldehyde concentration using the following equation:

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

where: N HCl = Normality of HCl solution used

mL HCl = mL of standardized HCl solution used

30.03 = MW of formaldehyde

Note: The pH value of the 0.1 Na₂SO₃ should be 10.5 ±0.2 when the stock formaldehyde solution and the 0.1 M Na₂SO₃ solution are mixed together. The pH should be 11.5 ±0.2. It is recommended that new solutions be prepared if the pH deviates from this value.

Stock aldehyde(s) and ketone(s)

- Prepare by adding an appropriate amount of the analyte to 90 mL of methanol
- Dilute to 100 mL to give a final concentration of 1.0 mg/mL. Replace after six weeks, or sooner, if comparison with check standards indicates a problem

Reaction Solutions:

2,4-Dinitrophenylhydrazine (DNPH [2,4-(O₂N)₂ C₆ H₃]NHNH₂) (3.00 g/L)

- Dissolve 428.7 mg of 70% (w/w) reagent in 100 mL absolute ethanol. Sonication may be needed

Note: If the DNPH does not completely dissolve, filter the solution

Citrate buffer pH 3 (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 6N NaOH or 6N HCl

Sodium Chloride Solution (saturated)

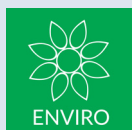
- Prepare by mixing an excess of the reagent grade solid with reagent water

Reducing agent, ammonium chloride (100 mg/L)

- Add to all samples containing residual chlorine. The ammonium chloride may be added as a solid with stirring until dissolved, to each volume of water
- Sodium thiosulfate is not recommended because it may produce a residue of elemental sulfur that can interfere with some analytes

Elution Fluid

- Dilute 64.3 mL of 1.0N NaOH and 5.7 mL of glacial acetic acid to 900 mL with reagent water. The pH should be 4.93 ±0.02
- Dilute to 1 L with water



Procedure:

1. Derivatization Procedure (formaldehyde)

- a) Quantitatively transfer 100 mL of sample into a 250 mL Florence or Erlenmeyer flask

Note: Other volumes may be used depending on the expected concentration of the analytes. If less than 100 mL is used adjust volume to 100 mL using reagent water

- b) Add 4 mL acetate buffer and adjust pH to 5.0 ± 0.1 with 6M HCl or 6M NaOH
- c) Add 6 mL of DNPH reagent, seal the flask and place in a heated orbital shaker at 40 °C for 1 hour
- d) Adjust the agitation to produce a gentle swirling of the reaction solution
- e) Proceed to Step 2. **Cartridge Conditioning for analysis of other aldehydes**
- f) Add 4 mL of citrate buffer and adjust the pH to 3.0 ± 0.1 with 6M HCl or 6M NaOH
- g) Add 6 mL of DNPH reagent, seal and place the flask in a heated orbital shaker 40 °C for 1 hour

2. Cartridge Conditioning

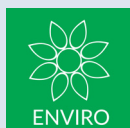
- a) Assemble a vacuum manifold
- b) Place **EUC1812M15** cartridge(s) on manifold
- c) Condition the cartridge by adding 15 mL of acetonitrile (ACN)
- d) Draw through then rinse using a 15 mL portion of reagent water
- e) While cartridge is still wet add 10 mL of dilute citrate buffer (10 mL of 1M citrate buffer dissolved in 250 mL of water)
- f) Remove the reaction vessel from the shaker after 1 hour and add 10 mL of saturated NaCl solution to the flask
- g) Quantitatively transfer the reaction solution to the SPE cartridge and apply a vacuum to draw the solution through at 3-5 mL/min
- h) Continue to apply vacuum for about 1 minute after the liquid sample has passed through the cartridges

3. Cartridge Elution

- a) Turn off vacuum and place 10 mL volumetric flasks under cartridges
- b) Turn on vacuum and elute the cartridges with approximately 9 mL of acetonitrile directly into flasks
- c) Bring the eluate to 10 mL volume with ACN, mix thoroughly, then transfer aliquot to analysis vial and seal

Note: Because this method uses an excess of DNPH, the cartridges will retain a yellow color after this step. The color does not indicate incomplete recovery of the analyte derivatives

HPLC Analysis	
HPLC Column	C18, 250 mm x 4.6 mm i.d., 5 µm particle size
Mobile Phase	70/30 methanol/water (v/v)
Injection Vol	20 µL
Flow Rate	1.2 mL/min
UV Detector	360 nm
Flow Program	70/30 methanol/water (v/v) for 20 min, to 100% acetonitrile in 15 minutes hold at 100% acetonitrile for 10 minutes



Representative Retention Times in Reagent Water Using Specified Analysis Conditions

Analyte	Retention Time (min)
Formaldehyde	5.3
Acetaldehyde	7.4
Propanal	11.7
Crotonaldehyde	16.1
Butanal	18.1
Cyclohexanone	27.6
Pentanal	28.4
Hexanal	34.1
Heptanal	35.0
Octanal	40.1
Nonanal	40.4
Decanal	44.1

References:

- [1] Adapted from Method 8315a, Determination Of Carbonyl Compounds By High Performance Liquid Chromatography (HPLC), and EPA Method 554, Revision 1.0, James W. Eichelberger, W.J. Bashe (Technology Applications, Incorporated), Environmental Monitoring Systems Laboratory, Office Of Research And Development, U. S. Environmental Protection Agency, Cincinnati, Ohio 45268

DCN-316140-261

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