

METHOD 8316

ACRYLAMIDE, ACRYLONITRILE AND ACHOLEIN BY HIGH PERFORMANCE
LIQUID CHROMATOGRAPHY (HPLC)

1.0 SCOPE AND APPLICATION

1.1 The following compounds can be determined by this method:

Compound Name	CAS No. ^a
Acrylamide	79-06-1
Acrylonitrile	107-13-1
Acrolein (Propenal)	107-02-8

^a Chemical Abstract Services Registry Number.

1.2 The method detection limits (MDLs) for the target analytes in organic-free reagent water are listed in Table 1. The method may be applicable to other matrices.

1.3 This method is restricted to use by or under the supervision of analysts experienced in the use of high performance liquid chromatographs and skilled in the interpretation of high performance liquid chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method.

2.0 SUMMARY OF METHOD

2.1 Water samples are analyzed by high performance liquid chromatography (HPLC). A 200 µL aliquot is injected onto a C-18 reverse-phase column, and compounds in the effluent are detected with an ultraviolet (UV) detector.

3.0 INTERFERENCES

3.1 Contamination by carryover can occur whenever high-concentration and low-concentration samples are sequentially analyzed. To reduce carryover, the sample syringe must be rinsed out between samples with solvent. Whenever an unusually concentrated sample is encountered, it should be followed by the analysis of solvent to check for cross contamination.

4.0 APPARATUS AND MATERIALS

4.1 HPLC system

4.1.1 One high pressure pump.

4.1.2 Octadecyl Silane (ODS, C-18) reverse phase HPLC column, 25 cm x 4.6 mm, 10 μ m, (Zorbax, or equivalent).

4.1.3 Variable wavelength UV detector.

4.1.4 Data system.

4.2 Other apparatus

4.2.1 Water degassing unit - 1 liter filter flask with stopper and pressure tubing.

4.2.2 Analytical balance - \pm 0.0001 g.

4.2.3 Magnetic stirrer and magnetic stirring bar.

4.2.4 Sample filtration unit - syringe filter with 0.45 μ m filter membrane, or equivalent disposable filter unit.

4.3 Materials

4.3.1 Syringes - 10, 25, 50 and 250 μ L and 10 mL.

4.3.2 Volumetric pipettes, Class A, glass - 1, 5 and 10 mL.

4.3.3 Volumetric flasks - 5, 10, 50 and 100 mL.

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

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 Acrylamide, $\text{CH}_2\text{:CHCONH}_2$, 99+% purity, electrophoresis reagent grade.

5.3 Acrylonitrile, $\text{H}_2\text{C:CHCN}$, 99+% purity.

5.4 Acrolein, $\text{CH}_2\text{:CHCHO}$, 99+% purity.

5.5 Organic-free reagent water. All references to water in this method refer to organic-free reagent water, as defined in Chapter One. Sparge with He to eliminate O₂ to prevent significant absorption interference from O₂ at the 195 nm wavelength.

5.6 Stock standard solutions - Can be prepared from pure standard materials or can be purchased as certified solutions. Commercially prepared stock standards can be used if they are certified by the manufacturer and verified against a standard made from pure material.

5.6.1 Acrylamide

5.6.1.1 Weigh 0.0100 g of acrylamide neat standard into a 100 mL volumetric flask, and dilute to the mark with organic-free reagent water. Calculate the concentration of the standard solution from the actual weight used. When compound purity is assayed to be 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard.

5.6.1.2 Transfer the stock solution into vials with Teflon lined screw caps or crimp tops. Store at 4°C, protected from light.

5.6.1.3 Stock solutions must be replaced after six months, or sooner if comparison with the check standards indicates a problem.

5.6.2 Acrylonitrile and Acrolein - Prepare separate stock solutions for acrylonitrile and acrolein.

5.6.2.1 Place about 9.8 mL of organic-free reagent water into a 10 mL volumetric flask before weighing the flask and stopper. Weigh the flask and record the weight to the nearest 0.0001 g. Add two drops of neat standard, using a 50 µL syringe, to the flask. The liquid must fall directly into the water, without contacting the inside wall of the flask.

CAUTION: Acrylonitrile and acrolein are toxic. Standard preparation should be performed in an laboratory fume hood.

5.6.2.2 Stopper the flask and then reweigh. Dilute to volume with organic-free reagent water. Calculate the concentration from the net gain in weight. When compound purity is assayed to be 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard.

5.6.2.3 Stock solutions must be replaced after six months, or sooner if comparison with the check standards indicates a problem.

5.7 Calibration standards

5.7.1 Prepare calibration standards at a minimum of five concentrations by diluting the stock solutions with organic-free reagent water.

5.7.2 One calibration standard should be prepared at a concentration near, but above, the method detection limit; the remaining standards should correspond to the range of concentrations found in real samples, but should not exceed the working range of the HPLC system (1 mg/L to 10 mg/L).

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

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

7.0 PROCEDURE

7.1 HPLC Conditions

Mobile Phase:	Degassed organic-free reagent water
Injection Volume:	200 μ L
Flow Rate:	2.0 mL/min
Pressure:	38 atm
Temperature:	25°C
Detector UV wavelength:	195 nm

7.2 Calibration:

7.2.1 Prepare standard solutions of acrylamide as described in Sec. 5.7.1. Inject 200 μ L aliquots of each solution into the chromatograph. See Method 8000 for additional guidance on calibration by the external standard method.

7.3 Chromatographic analysis:

7.3.1 Analyze the samples using the same chromatographic conditions used to prepare the standard curve. Suggested chromatographic conditions are given in Sec. 7.1. Table 1 provides the retention times that were obtained under these conditions during method development.

8.0 QUALITY CONTROL

8.1 Refer to Chapter One for specific quality control procedures.

8.2 Before processing any samples, the analyst must demonstrate, through the analysis of a method blank, that all glassware and reagents are interference free.

9.0 METHOD PERFORMANCE

9.1 Method performance data are not available.

10.0 REFERENCES

1. Hayes, Sam; "Acrylamide, Acrylonitrile, and Acrolein Determination in Water by High Pressure Liquid Chromatography," USEPA.

TABLE 1
ANALYTE RETENTION TIMES AND METHOD DETECTION LIMITS

Compound	Retention Time (min)	MDL ($\mu\text{g/L}$)
Acrylamide	3.5	10
Acrylonitrile	8.9	20
Acrolein (Propenal)	10.1	30

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