

Method 1661
*The Determination of
Bromoxynil in Municipal and
Industrial Wastewater*

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1. SCOPE AND APPLICATION

- 1.1 This method covers the determination of bromoxynil in waste by direct aqueous injection high-performance liquid chromatography (HPLC) with an ultraviolet detector (UV).
- 1.2 This method is designed to meet the monitoring requirements of the U.S. Environmental Protection Agency under the Clean Water Act at 40 *CFR* Part 455. Any modification of this method beyond those expressly permitted shall be considered a major modification subject to application and approval of alternative test procedures under 40 *CFR* 136.4 and 136.5.
- 1.3 When this method is applied to the analysis of unfamiliar samples, compound identity must be supported by at least one additional qualitative technique. This method lists a second UV wavelength that can be used to confirm measurements made with the primary wavelength.
- 1.4 The detection limit of this method is usually dependent on the level of interferences rather than instrumental limitations. The limit in Table 1 typifies the minimum quantity that can be detected with no interferences present.
- 1.5 This method is for use by or under the supervision of analysts experienced in the use of a high-performance liquid chromatograph and interpretation of liquid chromatographic data. Each laboratory that uses this method must demonstrate the ability to generate acceptable results using the procedure in Section 8.2.

2. SUMMARY OF METHOD

- 2.1 A 40- μ L aliquot of sample is injected into the HPLC. Chromatographic conditions are described that permit the separation and measurement of bromoxynil by reverse-phase C18 column HPLC with a multiple-wavelength UV detector.
- 2.2 Identification of bromoxynil is performed by comparing the retention time of the chromatograph peak with that of an authentic standard. Compound identity is confirmed when the retention times agree, and when the response at a second wavelength agrees with the response at the primary wavelength.
- 2.3 Quantitative analysis is performed using an authentic standard of bromoxynil to produce a calibration factor or calibration curve, and using the calibration data to determine the concentration of bromoxynil in the sample.
- 2.4 Quality is assured through reproducible calibration and testing of the HPLC system.

3. CONTAMINATION AND INTERFERENCES

- 3.1 Solvents, reagents, glassware, and other sample-processing hardware may yield artifacts and/or elevated baselines causing misinterpretation of chromatograms. All materials used in the analysis shall be demonstrated to be free from interferences under the conditions of analysis by running method blanks as described in Section 8.4.
- 3.2 Glassware and, where possible, reagents are cleaned by rinsing with solvent and baking at 450°C for a minimum of 1 hour in a muffle furnace or kiln. Some thermally stable materials may not be eliminated by this treatment and thorough rinsing with acetone and pesticide-quality acetonitrile may be required.
- 3.3 Specific selection of reagents and purification of solvents by distillation in all-glass systems may be required.
- 3.4 Interferences coextracted from samples will vary considerably from source to source, depending on the diversity of the site being sampled.

4. SAFETY

- 4.1 The toxicity or carcinogenicity of each compound or reagent used in this method has not been precisely determined; however, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. 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 handling sheets should also be made available to all personnel involved in these analyses. Additional information on laboratory safety can be found in References 1 to 3.
- 4.2 Unknown samples may contain high concentrations of volatile toxic compounds. Sample containers should be opened in a hood and handled with gloves that will prevent exposure.

5. APPARATUS AND MATERIALS

NOTE: Brand names, suppliers, and part numbers are for illustrative purposes only. No endorsement is implied. Equivalent performance may be achieved using apparatus and materials other than those specified here, but demonstration of equivalent performance meeting requirements of this method is the responsibility of the laboratory.

- 5.1 Sampling equipment for discrete or composite sampling.
 - 5.1.1 Sample bottle, amber glass, 40-mL minimum, with screw-cap. If amber bottles are not available, samples shall be protected from light.
 - 5.1.2 Bottle caps: Threaded to fit sample bottles. Caps shall be lined with PTFE.
 - 5.1.3 Cleaning.

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- 5.1.3.1** Bottles are detergent water washed, then rinsed with solvent rinsed or baked at 450°C for a minimum of 1 hour before use.
- 5.1.3.2** Liners are detergent-water washed, then rinsed with reagent water and solvent, and baked at approximately 200°C for a minimum of 1 hour prior to use.
- 5.1.4** Compositing equipment: Automatic or manual compositing system incorporating glass containers cleaned per bottle cleaning procedure above. Sample containers are kept at 0 to 4°C during sampling. Glass or PTFE tubing only shall be used. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used in the pump only. Before use, the tubing shall be thoroughly rinsed with methanol, followed by repeated rinsings with reagent water to minimize sample contamination. An integrating flow meter is used to collect proportional composite samples.
- 5.2** Equipment for glassware cleaning.
- 5.2.1** Laboratory sink with overhead fume hood.
- 5.2.2** Kiln: Capable of reaching 450°C within 2 hours and holding 450°C within $\pm 10^\circ\text{C}$, with temperature controller and safety switch (Cress Manufacturing Co, Sante Fe Springs, CA, B31H or X31TS, or equivalent).
- 5.3** pH meter, with combination glass electrode.
- 5.4** Sample vials: Amber glass, 10- to 15-mL with PTFE-lined screw- or crimp-cap, to fit HPLC autosampler.
- 5.5** Balance: analytical, capable of weighing 0.1 mg.
- 5.6** Miscellaneous glassware.
- 5.6.1** Pipettes, glass, volumetric, 1.00-, 5.00-, and 10.0-mL.
- 5.6.2** Pipettes, glass, Pasteur, 150 mm long x 5 mm ID (Fisher Scientific 13-678-6A, or equivalent).
- 5.6.3** Volumetric flasks, 10.0-, 25.0-, and 50.0-mL.
- 5.7** High-performance liquid chromatograph (HPLC): Analytical system complete with pumps, sample injector, column oven, and multiple-wavelength ultra-violet (UV) detector.
- 5.7.1** Pumping system: Capable of isocratic operation (Waters 600E, or equivalent).
- 5.7.2** Sample injector: Capable of automated injection of up to 30 samples (Waters 700, or equivalent).

- 5.7.3 Column oven: Capable of operation at room ambient to 50°C (Waters TCM, or equivalent).
- 5.7.4 Column: 150 mm long x 4.6 mm ID 300 Angstrom C18 column (Vydac 201 TP5415, or equivalent), operated at the conditions shown in Table 1.
- 5.7.5 Detector: UV operated at 255 nm and 280 nm (Waters 490E, or equivalent).
- 5.8 Data system
 - 5.8.1 Data acquisition: The data system shall collect and record LC peak areas and retention times on magnetic media.
 - 5.8.2 Calibration: The data system shall be used to calculate and maintain lists of calibration factors (response divided by concentration) and multi-point calibration curves. Computations of relative standard deviation (coefficient of variation) are used to test calibration linearity.
 - 5.8.3 Data processing: The data system shall be used to search, locate, identify, and quantify the compounds of interest in each analysis. Displays of chromatograms are required to verify results.
 - 5.8.4 Statistics on initial (Section 8.2) and ongoing (Section 12.5) performance shall be computed and maintained.
- 6. **REAGENTS AND STANDARDS**
 - 6.1 Sample preservation: Sodium thiosulfate (ACS), granular.
 - 6.2 pH Adjustment.
 - 6.2.1 Sodium hydroxide (10N): Dissolve 40 g NaOH in 100 mL reagent water.
 - 6.2.2 Sulfuric acid (1+1): Reagent grade, 6N in reagent water. Slowly add 50 mL H₂SO₄ (specific gravity 1.84) to 50 mL reagent water.
 - 6.3 Solvents: Methanol; pesticide-quality, lot-certified to be free of interferences.
 - 6.4 Reagent water: HPLC grade water in which the compounds of interest and interfering compounds are not detected by this method.
 - 6.5 Standard: Purchased as a solution with certification as to purity, concentration, and authenticity, or prepared from materials of known purity and composition. If compound purity is 96% or greater, the weight may be used without correction to compute the concentration of the standard. When not being used, standards are stored in the dark at -20°C to -10°C in screw-capped vials with PTFE-lined lids. A mark is placed on the vial at the level of the solution so that solvent evaporation loss can be detected. The vials are brought to room temperature prior to use.

- 6.6** Preparation of stock solutions: Prepare in water per the steps below. Observe the safety precautions in Section 4.
- 6.6.1** Dissolve an appropriate amount of assayed reference material in solvent. For example, weigh 10 mg bromoxynil in a 100-mL ground-glass stoppered volumetric flask and fill to the mark with water. After the bromoxynil is completely dissolved, transfer the solution to a 150-mL vial with PTFE-lined cap.
 - 6.6.2** Stock solutions should be checked for signs of degradation prior to the preparation of calibration or performance test standards.
 - 6.6.3** Stock solutions shall be replaced after 6 months, or sooner if comparison with quality control check standards indicates a change in concentration.
- 6.7** Secondary mixtures: Using stock solutions (Section 6.6), prepare mixtures for calibration and calibration verification (Sections 7.3 and 12.4), for initial and ongoing precision and recovery (Sections 8.2 and 12.5), and for spiking into the sample matrix (Section 8.3).
- 6.7.1** Calibration solutions: Prepare in water at the concentrations given in Table 2. The low level solution is used for calibration verification (Section 12.4)
 - 6.7.2** Precision and recovery standard and matrix spike solution: Prepare in water at a concentration of 10 µg/mL.
- 6.8** Stability of solutions: All standard solutions (Sections 6.6 through 6.7) shall be analyzed within 48 hours of preparation and on a monthly basis thereafter for signs of degradation. Standards will remain acceptable if the peak area remains within ±15% of the area obtained in the initial analysis of the standard.

7. *SETUP AND CALIBRATION*

- 7.1** Configure the HPLC system described in Sections 5.7 through 5.8 and establish the operating conditions in Table 1.
- 7.2** Attainment of minimum level: Determine that the minimum level in Table 1 is met at each wavelength.
- 7.3** Calibration.
- 7.3.1** Inject 40 µL of each calibration solution (Table 2) into the HPLC system, beginning with the lowest concentration and proceeding to the highest. Compute and store, as a function of the concentration injected, the retention time and the peak area of Bromoxynil each wavelength (primary and confirmatory).
 - 7.3.2** Calibration factor (ratio of area to amount injected).
 - 7.3.2.1** Compute the coefficient of variation (relative standard deviation) of the calibration factor over the calibration range at each wavelength.

7.3.2.2 Linearity: If the calibration factor is constant ($C_v < 15\%$) over the calibration range, an average calibration factor may be used; otherwise, the complete calibration curve (area vs. amount) shall be used.

8. QUALITY CONTROL

8.1 Each laboratory that uses this method is required to operate a formal quality control program.⁴ The minimum requirements of this program consist of an initial demonstration of laboratory capability, an ongoing analysis of standards and blanks as tests of continued performance, and analysis of spiked samples to assess accuracy. Laboratory performance is compared to established performance criteria to determine if the results of analyses meet the performance characteristics of the method.

8.1.1 The analyst shall make an initial demonstration of the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

8.1.2 The analyst is permitted to modify this method to improve separations or lower the costs of measurements, provided all performance requirements are met. Each time a modification is made to the method or a cleanup procedure is added, the analyst is required to repeat the procedure in Section 8.2 to demonstrate method performance. If minimum level will be affected by the modification, the analyst is required to repeat demonstration of the minimum level (Section 7.2).

8.1.3 The laboratory shall, on an ongoing basis, demonstrate through calibration verification and the analysis of the precision and recovery standard (Section 6.7.2) that the analysis system is in control. These procedures are described in Sections 12.1, 12.4, and 12.5.

8.1.4 The laboratory shall maintain records to define the quality of data that is generated. Development of accuracy statements is described in Section 8.3.

8.1.5 Analyses of blanks are required to demonstrate freedom from contamination. The procedures and criteria for analysis of a blank are described in Section 8.4.

8.2 Initial precision and recovery: To establish the ability to generate acceptable precision and accuracy, the analyst shall perform the following operations.

8.2.1 Analyze one set of four 10-mL aliquots of reagent water spiked with 100 μL of the precision and recovery standard (Section 6.7.2) according to the procedure in Sections 10 and 11.

8.2.2 Using the results of the set of four analyses, compute the average recovery (X) and the standard deviation of recovery (s) for bromoxynil.

8.2.3 Compare s and X with the corresponding limit for initial precision and recovery in Table 1. If s and X meet the acceptance criteria, system performance is acceptable and analysis of blanks and samples may begin. If, however, s exceeds the precision limit or X falls outside the range for accuracy, system performance is unacceptable. In this case, correct the problem and repeat the test.

8.3 Method accuracy: The laboratory shall spike (matrix spike) at least 10% of the samples from a given site type (e.g., influent to treatment, treated effluent, produced water). If only one sample from a given site type is analyzed, a separate aliquot of that sample shall be spiked.

8.3.1 The concentration of the matrix spike shall be determined as follows.

8.3.1.1 If, as in compliance monitoring, the concentration of bromoxynil in the sample is being checked against a regulatory concentration limit, the matrix spike shall be at that limit or at 1 to 5 times higher than the background concentration determined in Section 8.3.2, whichever concentration is larger.

8.3.1.2 If the concentration is not being checked against a regulatory limit, the matrix spike shall be at the level of the precision and recovery standard (Section 6.7.2) or at 1 to 5 times higher than the background concentration, whichever concentration is larger.

8.3.1.3 If it is impractical to determine the background concentration before spiking (e.g., maximum holding times will be exceeded), the matrix spike concentration shall be the regulatory concentration limit, if any; otherwise, at the level of the precision and recovery standard (Section 6.7.2) or at 1 to 5 times the expected background concentration, whichever is larger.

8.3.2 Analyze one sample aliquot to determine the background concentration (B) of bromoxynil. If necessary, prepare a standard solution appropriate to produce a level in the sample 1 to 5 times the background concentration. Spike a second sample aliquot with the standard solution and analyze it to determine the concentration after spiking (A) of each analyte. Calculate the percent recovery (P):

Equation 1

$$P = \frac{100 (A-B)}{T}$$

where

T = True value of the spike

8.3.3 Compare the percent recovery of bromoxynil with the corresponding QC acceptance criteria in Table 1. If it fails the acceptance criteria for recovery, the sample may be complex and must be diluted and reanalyzed per Section 15.

8.3.4 As part of the QC program for the laboratory, method accuracy for samples shall be assessed and records shall be maintained. After the analysis of five spiked samples of a given matrix type (e.g., influent to treatment, treated effluent,

produced water) in which the the recovery test (Section 8.3.3) is passed, compute the average percent recovery (P) and the standard deviation of the percent recovery (s_p). Express the accuracy assessment as a percent recovery interval from $P - 2s_p$ to $P + 2s_p$ for each matrix. For example, if $P = 90\%$ and $s_p = 10\%$ for five analyses of wastewater, the accuracy interval is expressed as 70 to 110%. Update the accuracy assessment in each matrix on a regular basis (e.g., after each 5 to 10 new accuracy measurements).

8.4 Blanks: reagent water blanks are analyzed to demonstrate freedom from contamination.

8.4.1 Analyze a reagent water blank with each sample batch (samples started through the extraction process on the same 8-hour shift, to a maximum of 20 samples). Analyze the blank immediately after analysis of the precision and recovery standard (Section 12.5) to demonstrate freedom from contamination.

8.4.2 If any compound or any potentially interfering compound is found in an aqueous blank at greater than 100 $\mu\text{g}/\text{L}$ (assuming the same calibration factor as bromoxynil for interfering compounds), analysis of samples is halted until the source of contamination is eliminated and a blank shows no evidence of contamination at this level.

8.5 The specifications contained in this method can be met if the apparatus used is calibrated properly, then maintained in a calibrated state. The standards used for calibration (Section 7), calibration verification (Section 12.4), and for initial (Section 8.2) and ongoing (Section 12.5) precision and recovery should be identical, so that the most precise results will be obtained. The HPLC instrument will provide the most reproducible results if dedicated to the settings and conditions required for the analyses of the analytes given in this method.

8.6 Depending on specific program requirements, field replicates and field spikes may be required to assess the precision and accuracy of the sampling and sample transporting techniques.

9. *SAMPLE COLLECTION, PRESERVATION, AND HANDLING*

9.1 Collect samples in glass containers following conventional sampling practices,⁵ except that the bottle shall not be prerinsed with sample before collection. Aqueous samples which flow freely are collected in refrigerated bottles using automatic sampling equipment.

9.2 Maintain samples at 0 to 4°C from the time of collection until extraction. If the samples will not be extracted within 72 hours of collection, adjust the sample to a pH of 3.0 to 7.0 using sodium hydroxide or hydrochloric acid solution. Record the volume used. If residual chlorine is present in aqueous samples, add 80 mg sodium thiosulfate per liter of water. EPA Methods 330.4 and 330.5 may be used to measure residual chlorine.⁶

9.3 Begin sample extraction within 7 days of collection, and analyze all extracts within 40 days of extraction.

10. PREPARATION OF SAMPLE AND QC ALIQUOTS

- 10.1** Mix sample thoroughly.
- 10.2** Pour approximately 10 mL of sample into a clean HPLC autosampler vial. If a matrix spike is to be prepared, pour 10.0 mL into a second clean vial.
- 10.3** For each sample or sample batch (to a maximum of 20) to be analyzed in the same 8-hour shift, place two 10.0-mL aliquots of reagent water (Section 6.4) in clean auto-sampler vials. One reagent water aliquot serves as the blank.
- 10.4** Spike 100 μ L of the precision and recovery standard (Section 6.7.2) into the remaining reagent water aliquot.
- 10.5** Spike the sample designated as the matrix spike at the level directed in Section 8.3.
- 10.6** Analyze the sample and QC aliquots per Section 11.

11. HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

Table 1 summarizes the recommended operating conditions for the HPLC system. Included in this Table is the retention times for bromoxynil achieved under these conditions. An example of the separation achieved by the column system is shown in Figure 1.

- 11.1** Calibrate the system as described in Section 7.
- 11.2** Set the injection volume on the auto-sampler to inject 40 μ L of all standards, blanks, and samples.
- 11.3** Set the data system or HPLC control to begin data collection upon injection and to stop data collection after bromoxynil is expected to elute.

12. SYSTEM AND LABORATORY PERFORMANCE

- 12.1** At the beginning of each 8-hour shift during which analyses are performed, HPLC system performance and calibration are verified at both wavelengths. For these tests, analysis of the calibration verification standard (Section 6.7.1) shall be used to verify all performance criteria. Adjustment and/or recalibration (per Section 7) shall be performed until all performance criteria are met. Only after all performance criteria are met may samples, blanks, and precision and recovery standards be analyzed.
- 12.2** Retention times.
 - 12.2.1** The absolute retention time of bromoxynil shall be no less than 2.5 minutes.
 - 12.2.2** The absolute retention time of the bromoxynil peak maximum shall be within ± 15 seconds of the average of the retention times in the initial calibration (Section 7.3.1).

- 12.3** GC resolution: Resolution is acceptable if the peak width at half-height of bromoxynil is less than 15 seconds.
- 12.4** Calibration verification.
- 12.4.1** Inject the calibration verification standard (Section 6.7.1).
- 12.4.2** Compute the concentration of bromoxynil based on the calibration factor or calibration curve (Section 7.3).
- 12.4.3** Compare this concentration with the limits for calibration verification in Table 1. If calibration is verified, system performance is acceptable and analysis of blanks and samples may begin. If, however, the recovery falls outside the calibration verification range, system performance is unacceptable. In this case, correct the problem and repeat the test, or recalibrate (Section 7).
- 12.5** Ongoing precision and recovery.
- 12.5.1** Analyze the precision and recovery standard prepared with each sample batch (Section 10.3).
- 12.5.2** Compute the recovery of bromoxynil.
- 12.5.3** Compare the recovery with the limit for ongoing recovery in Table 1. If the recovery meets the acceptance criteria, the analytical process is in control and analysis of blanks and samples may proceed. If, however, the recovery falls outside the acceptable range, these processes are not in control. In this event, correct the problem and repeat the ongoing precision and recovery test.
- 12.5.4** Add results which pass the specifications in Section 12.5.3 to initial and previous ongoing data. Update QC charts to form a graphic representation of continued laboratory performance. Develop a statement of laboratory data quality for each analyte by calculating the average percent recovery (R) and the standard deviation of percent recovery (s_r). Express the accuracy as a recovery interval from $R - 2s_r$ to $R + 2s_r$. For example, if $R = 95\%$ and $s_r = 5\%$, the accuracy is 85 to 105%.

13. QUALITATIVE DETERMINATION

- 13.1** Qualitative determination is accomplished by comparison of data from analysis of a sample or blank with data from analysis of the shift standard (Section 12.1), and with data stored in the retention-time and calibration libraries (Section 7.3.1). Identification is confirmed when retention-time and amounts agree per the criteria below.
- 13.2** Establish a retention-time window ± 15 seconds on either side of the mean retention-time in the calibration data (Section 7.3.1).
- 13.3** If a peak from the analysis of a sample or blank is within this window (as defined in Section 13.2) at the primary wavelength (280 nm), it is considered tentatively identified.

A tentatively identified compound is confirmed when (1) the retention time of the peak maximum at the confirmatory wavelength (255 nm) is within ± 2 seconds of the retention-time of the peak maximum at the primary wavelength, and (2) the computed amounts (Section 14) on each system (primary and confirmatory) agree within a factor of 2.

14. QUANTITATIVE DETERMINATION

- 14.1** Using the HPLC data system, compute the concentration of the analyte detected in the sample (in milligram per liter) using the calibration factor or calibration curve (Section 7.3.2).
- 14.2** If the concentration of any analyte exceeds the calibration range of the system, the sample is diluted by a factor of 10, and a 40- μ L aliquot of the diluted extract is analyzed.
- 14.3** Report results for bromoxynil found in all standards, blanks, and samples to three significant figures. Results for samples that have been diluted are reported at the least dilute level at which the concentration is in the calibration range.

15. ANALYSIS OF COMPLEX SAMPLES

- 15.1** Some samples may contain high levels ($>1000 \mu\text{g/L}$) of bromoxynil or of interfering compounds and/or polymeric materials. Some samples may overload the HPLC column and/or detector. In these instances, the sample is diluted by a factor of 10 and reanalyzed (Section 14.2).
- 15.2** Recovery of matrix spikes: in most samples, matrix spike recoveries will be similar to those from reagent water. If the matrix spike recovery is outside the range specified in Table 1, the sample is diluted by a factor of 10, respiked, and reanalyzed. If the matrix spike recovery is still outside the range, the method does not work on the sample being analyzed and the result may not be reported for regulatory compliance purposes.

16. METHOD PERFORMANCE

- 16.1** Development of this method is detailed in Reference 7.

References

1. "Carcinogens—Working with Carcinogens." Department of Health, Education, and Welfare; Public Health Service; Center for Disease Control; National Institute for Occupational Health and Safety: Publication 77-206, August 1977.
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5. "Standard Practice for Sampling Water" (ASTM Annual Book of Standards), American Society for Testing and Materials, Philadelphia, Pennsylvania: 76, 1980.
6. "Methods 330.4 and 330.5 for Total Residual Chlorine," U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, OH: EPA-600/4-70-020, March 1979.
7. "Narrative for SAS 1019," Pacific Analytical, Inc.: September 1989. Available from the U.S. Environmental Protection Agency Sample Control Center, 300 N. Lee St., Alexandria, VA 22314 (703-557-5040).

Table 1. HPLC Data and Method Acceptance Criteria for Bromoxynil*

Acceptance Criteria	Specification	Note
Minimum Level	100 µg/L	1
Method Detection Limit	20 µg/L	2
Calibration Verification (Section 12.4)	86–128 µg/L	3
Initial Precision and Recovery (Section 8.2)		3
Precision [standard deviation (s)]	28 µg/L	
Recovery [mean (X)]	74–130 µg/L	
Ongoing Precision and Recovery (Section 12.5)	72–132 µg/L	
Matrix Spike Recovery (Section 8.3.3)	68–129%	
Bromoxynil Retention Time	2.62 minutes	4

*(3,5-dibromo-4-hydroxybenzotrile; CAS 1689-84-5)

Notes:

1. This is a minimum level at which the analytical system shall give recognizable signals and acceptable calibration points.
2. Estimated; 40 *CFR* Part 136, Appendix B.
3. Test concentration 100 µg/L.
4. Column and conditions: 300 Angstrom C18 column 150 mm long x 4.6 mm ID. Column temperature 30°C. Solvent flow rate 0.5 mL/min. Isocratic at 50% methanol in water.

Table 2. Concentration of Bromoxynil Calibration Solutions

Level	Concentration (µg/L)
Low	100
Median	600
High	3,000

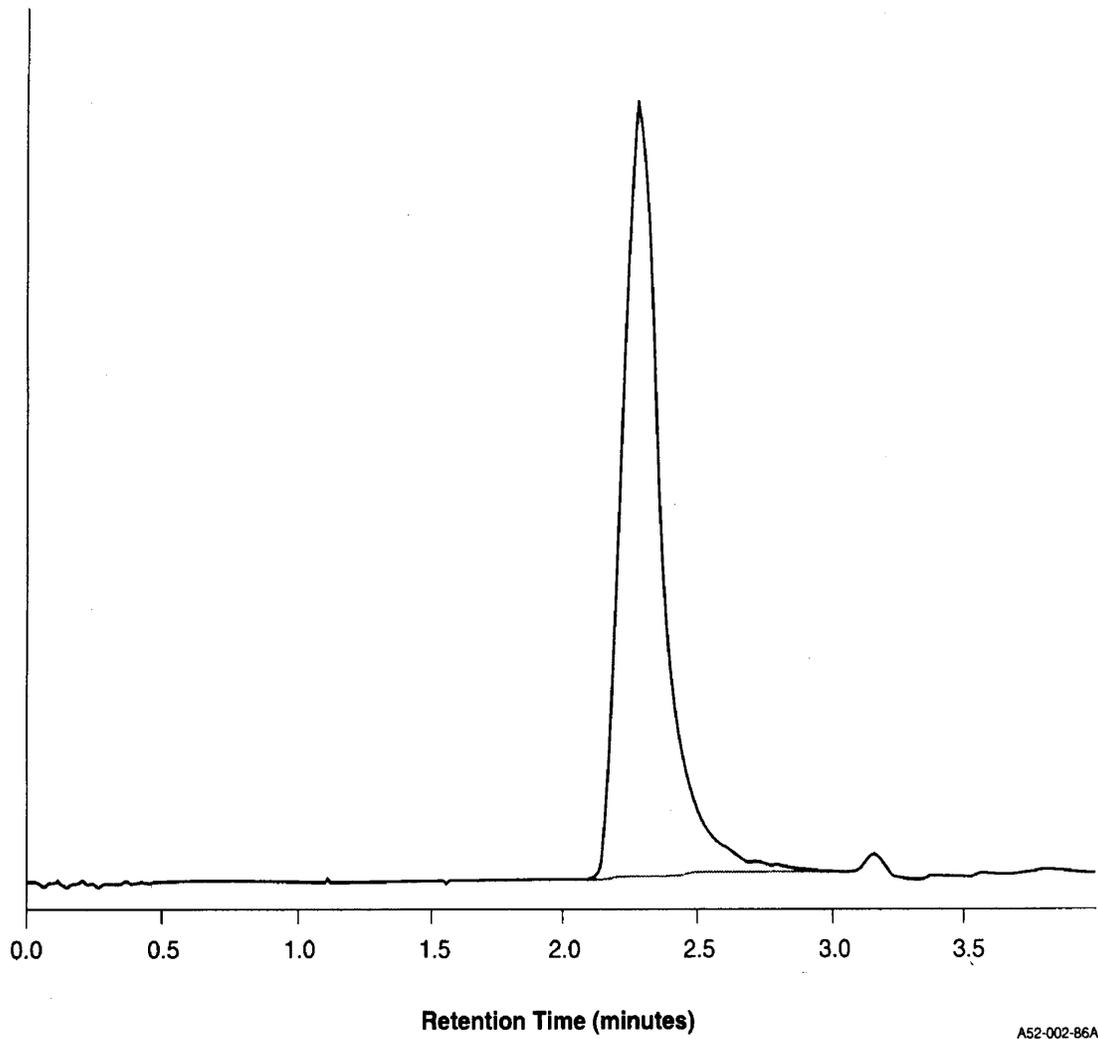


Figure 1. Chromatogram of Bromoxynil