METHOD 643: ANALYSIS OF BENTAZON IN WASTEWATER BY LIQUID CHROMATOGRAPHY

1. SCOPE AND APPLICATION

1.1 This method covers the determination of bentazon in municipal and industrial wastewater.

Parameter	CAS No.
Bentazon (Basagran)	25057-89-0

1.2 The estimated detection limit (EDL) for bentazon is listed in Table 1. The EDL was calculated from the minimum detectable response being equal to 5 times the background noise using a 5-mL final extract volume of a 1-liter sample and an injection volume of 100 µL. The EDL for a specific wastewater may be different depending on the nature of interferences in the sample matrix.

1.3 This is a high performance liquid chromatographic (HPLC) method applicable to the determination of bentazon in municipal and industrial discharges. When this method is used to analyze unfamiliar samples for bentazon, compound identification should be supported by at least one additional qualitative technique. This method describes analytical conditions for a second HPLC column that can be used to confirm measurements made with the primary column.

1.4 This method is restricted to use by or under the supervision of analysts experienced in the operation of liquid chromatographs and in the interpretation of liquid chromatograms.

2. SUMMARY OF METHOD

2.1 Bentazon is removed from an acidified sample matrix by extraction with methylene chloride. The extract is discarded after back extraction with aqueous base. HPLC conditions are described which permit the separation and measurement of bentazon in the aqueous extract.

3. INTERFERENCES

3.1 Solvent, reagents, glassware, and other sample processing hardware may yield discrete artifacts and/or elevated baselines causing misinterpretation in liquid chromatograms. All of these materials must be demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 9.1

3.1.1 The use of high-purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

3.1.2 Glassware must be scrupulously cleaned (1). Clean all glassware as soon as possible after use by rinsing with the last solvent used in it. This should be followed by detergent washing with hot water and rinses with tap water and reagent water. It should then be drained dry and heated in a muffle furnace at 400°C for 15 to 30 minutes. Solvent rinses with acetone and pesticide-quality hexane may be substituted for the muffle furnace heating. Volumetric ware should not be heated in a muffle furnace. After drying and cooling, glassware should be sealed and stored in a clean environment to prevent any accumulation of dust or other contaminants. Store the glassware inverted or capped with aluminum foil.

3.2 Matrix interferences may be caused by UV-active contaminants that are co-extracted from the samples. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality being sampled. Unique samples may require additional cleanup approaches to achieve the detection limit listed in Table 1.

4. SAFETY

4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. 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 data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified (2-4) for the information of the analyst.

5. APPARATUS AND EQUIPMENT

5.1 SAMPLE CONTAINERS - Narrow-mouth glass bottles, 1-liter or 1-quart volume, equipped with polytetrafluoroethylene (PTFE)-lined screw caps. Wide-mouth glass bottles, 1-quart volume, equipped with PTFE-lined screw caps may also be used. Prior to use, wash bottles and cap liners with detergent and rinse with tap and reagent water. Allow the bottles and cap liners to air dry, then muffle the bottles at 400°C for 1 hour. After cooling, rinse the bottle and cap liners with hexane, seal the bottles, and store in a dust-free environment.

5.1.1 Automatic sampler (optional)--Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler

uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing should be thoroughly rinsed with methanol, followed by repeated rinsings with reagent water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow-proportional composites.

5.2 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) APPARATUS — Analytical system complete with liquid chromatograph and all required accessories including syringes, analytical columns, and mobile phases. The system must be compatible with the specified detectors and strip-chart recorder. A data system is recommended for measuring peak areas.

5.2.1 Gradient pumping system, constant flow.

5.2.2 Injector valve (Rheodyne 7125 or equivalent) with 100-µL loop.

5.2.3 Column 1 — 250 mm by 4.6 mm ID, stainless steel, packed with reverse-phase Ultrasphere ODS, 10 µ, or equivalent.

5.2.4 Column 2 — 300 mm by 4.0 mm ID, packed with reverse phase µ Bondapak C18, 10µ, (Waters Associates), or equivalent.

5.2.5 Ultraviolet detector, variable wavelength, capable of monitoring at 340 nm.

5.2.6 Strip-chart recorder compatible with detector, 250-mm. (A data system for measuring peak areas is recommended.)

5.3 MISCELLANEOUS

5.3.1 Balance—analytical, capable of accurately weighing to the nearest 0.0001 g.

5.3.2 Separatory funnels—2-liter, and 250 mL, equipped with PTFE stopcocks.

5.3.3 Standard solution storage containers—15-mL bottles with PTFE-lined screw caps.

5.3.4 Pasteur pipets with bulbs.

6. REAGENTS AND CONSUMABLE MATERIALS

6.1 REAGENTS

6.1.1 Acetone, hexane, methanol, and methylene chloride—Demonstrated to be free of analytes and interferences.

6.1.2 Reagent water—Reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest.

6.1.3 Sodium hydroxide solution (0.1N)—Dissolve 0.4 gram of NaOH in reagent water and dilute to 100 mL.

6.1.4 Sodium chloride—(ACS) Crystals.

6.1.5 Sodium thiosulfate—(ACS) Granular.

6.1.6 Sulfuric acid solution (1+1)—Slowly add 50 mL of H_2SO_4 (specific gravity 1.84) to 50 mL of reagent water.

6.1.7 Sodium hydroxide solution (6N)—Dissolve 24 g of NaOH in reagent water and dilute to 100 mL.

6.1.8 Acetate buffer solution—Dissolve 0.41 g of anhydrous sodium acetate (ACS) and 1.5 mL of glacial acetic acid (ACS) in 100 mL of reagent water.

6.1.9 Glacial acetic acid—(ACS).

6.1.10 HPLC mobile phase buffer (pH 4.7, 0.062 M acetate)—Dissolve 0.87 g of anhydrous sodium acetate (ACS) and 3.0 mL of glacial acetic acid (ACS) in 1 L of reagent water.

6.2 STANDARD STOCK SOLUTION (1.00 µg/µL)—This solution may be purchased as a certified solution or prepared from a pure standard material using the following procedures.

6.2.1 Prepare the stock standard solution by accurately weighing about 0.0100 gram of pure material. Dissolve the material in pesticide quality methanol, dilute to volume in a 10-mL volumetric flask. Larger volumes can be used at the convenience of the analyst. When compound purity is certified at 96 percent or greater, the weight can be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards can be used at any concentration if they are certified by the manufacturer or by an independent source.

6.2.2 Transfer the stock standards in Teflon^R-sealed screw-cap bottles. Store at 4°C and protect from light. Stock standards should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.

6.2.3 Stock standards must be replaced after 6 months, or when comparison with quality control check samples indicates a problem.

7. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

7.1 Collect all samples in duplicate. Grab samples must be collected in glass containers. Conventional sampling practices (5) should be followed, except that the bottle must not be prewashed with sample before collection.

7.2 The samples must be iced or refrigerated at 4°C from the time of collection until extraction. If the samples will not be extracted within 48 hours of collection, the sample should be adjusted to a pH range of 6.0 to 8.0 with sodium hydroxide or sulfuric acid and 35 mg of sodium thiosulfate per ppm of free chlorine per liter must be added.

7.3 All samples must be extracted within 7 days and completely analyzed within 30 days of extraction. (6)

8. CALIBRATION

8.1 Establish liquid chromatographic operating parameters equivalent to those indicated in Table 1.

8.2. Prepare calibration standards at a minimum of three concentration levels of bentazon by adding volumes of the stock standard to a volumetric flask and diluting to volume with HPLC mobile phase (35-percent methanol in HPLC mobile phase buffer or 40-percentmethanol in HPLC mobile phase buffer). One of the external standards should be at a concentration near, but greater than, the EDL, and the other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.

8.3 Using injections of 100 µL of each calibration standard, tabulate peak height or area responses against the mass injected. The results are used to prepare a calibration curve for the analytes. Alternatively, if the ratio of response to amount injected (calibration factor) is a constant over the working range (<10 percent relative standard deviation, RSD), linearity of the calibration curve can be assumed and the average ratio or calibration factor can be used in place of a calibration curve.

8.4 The working calibration curve or calibration factor must be verified on each working day by the measurement of one or more calibration standards. If the response for bentazon varies from the predicted response by ±10 percent, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or factor must be prepared.

8.5 Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution pattern and the absence of interferences from the reagents.

9. QUALITY CONTROL

9.1 MONITORING FOR INTERFERENCES

Analyze a laboratory reagent blank each time a set of samples is extracted. A laboratory reagent blank is an aliquot of reagent water. If the reagent blank contains a reportable level of bentazon, immediately check the entire analytical system to locate and correct for possible interferences and repeat the test.

9.2 ASSESSING ACCURACY

9.2.1 After every 10 samples, and preferably in the middle of each day, analyze a laboratory control standard. Calibration standards may not be used for accuracy assessments and the laboratory control standrd may not be used for calibration of the analytical system.

9.2.1.1 Laboratory Control Standard Concentrate (100 µg/L)—From stock standards prepared as described in Section 6.2, prepare a laboratory control standard concentrate that contains bentazon at a concentration of 2 µg/µL in methanol.(7)

9.2.1.2 Laboratory Control Standard—Using a pipet or microliter syringe, add 50.0 µL of the laboratory control standard concentrate to a 1.0 L aliquot of reagent water contained in a 1-L volumetric flask.

9.2.1.3 Analyze the laboratory control standard as described in Section 10. Calculate the percent recovery (P_i) with the equation:

$$P_i = \frac{100\ S_i}{T_i}$$

where S_i = the analytical results from the laboratory control standard, in µg/L; and
T_i = the known concentration of the spike, in µg/L.

9.2.2 At least annually, the laboratory should participate in formal performance evaluation studies, where solutions of unknown concentrations are analyzed and the performance of all participants is compared.(7)

9.3 ASSESSING PRECISION

9.3.1 Precision assessments for this method are based upon the analysis of field duplicates (Sect. 7.1). Analyze both samples for at least 10% of all samples. To the extent

practical, the samples for duplication should contain reportable levels of bentazon.

9.3.2 Calculate the relative range (7) (RR_i) with the equation:

$$RR_i = \frac{100\ R_i}{X_i}$$

where R_i = the absolute difference between the duplicate measurements X_1 and X_2, in µg/L; and

X_i = the average concentration found ($[X_1 + X_2]/2$), in µg/L.

9.3.3 Individual relative range measurements are pooled to determine average relative range or to develop an expression of relative range as a function of concentration.

10. PROCEDURE

10.1 SAMPLE EXTRACTION

10.1.1 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample (approximately one liter) into a 2-liter separatory funnel. Add 35 mg/L of sodium thiosulfate per ppm of free chlorine. Check the pH of the sample with wide-range pH paper and adjust to within the range of 2.5 to 3.5 with sulfuric acid. Add 200 grams of sodium chloride and mix to dissolve.

10.1.2 Add 60 mL of methylene chloride to the sample bottle and shake for 30 seconds to rinse the walls. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for 2 minutes with periodic venting to release vapor pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends on the sample, but may include stirring, filtration of the emulsion through glass wool, or centrifugation. Collect the extract in a 250-mL separatory funnel.

10.1.3 Add an additional 60-mL volume of methylene chloride to the sample bottle and complete the extraction procedure a second time, combining the extracts in the 250-mL separatory funnel.

10.1.4 Perform a third extraction in the same manner. Add 2 mL of 0.1 M NaOH in reagent water to the 250-mL separatory funnel, and extract by shaking the funnel for 2 minutes with periodic venting to release vapor pressure. Allow the

organic layer to separate from the water phase for a minimum of 10 minutes. Drain the methylene chloride into a 250-mL Erlenmeyer flask. Transfer the aqueous layer with a Pasteur pipet to a 5-mL volumetric flask.

10.1.5 Add the methylene chloride back to the 250-mL separatory funnel, and extract with an additional 2 mL of 0.1 M NaOH. Combine the extracts in the 5-mL volumetric flask.

10.1.6 Add two drops of glacial acetic acid to the volumetric flask, and dilute to volume with acetate buffer solution (Section 6.1.7).

10.1.7 Determine the original sample volume by refilling the sample bottle to the mark and transferring the liquid to a 1,000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

10.2 CLEANUP AND SEPARATION

10.2.1 The cleanup procedure recommended in this method involves the back extraction of a methylene chloride extract with aqueous base, and has been used for the analysis of various clean waters and industrial effluents. If additional cleanup is required, a one liter sample is adjusted to pH 12 with 6N NaOH and extracted with three, 60 mL aliquots of methylene chloride in a 2L seporatary funnel. The methylene chloride extracts are discarded and the aqueous sample adjusted to pH range of 2.5 to 3.5 with 1:1 sulphuric acid solution for re-extraction as per sect. 10.1.1. If additional cleanup is required, or if particular circumstances demand the use of an alternate cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery for each compound of interest is no less than 85 percent.

10.3 LIQUID CHROMATOGRAPHY ANALYSIS

10.3.1 Table 1 summarizes the recommended operating conditions for the liquid chromatograph. Included in this table are the estimated retention times and estimated detection limit that can be achieved by this method. An example of the separation achieved by Column 2 is shown in Figure 1. Other columns, chromatographic conditions, or detectors may be used if data quality comparable to table 2 is achieved.

10.3.2 Calibrate the system daily as described in Section 8.

10.4 Inject 100 µL of the sample extract. Monitor the column eluent at 340 nm. Record the resulting peak size in area or peak height units.

10.5 The retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time for a compound can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

10.6 If the response for the peak exceeds the working range of the system, dilute the sample with mobile phase and reanalyze.

10.7 If the measurement of the peak response is prevented by the presence of interferences, additional cleanup is required.

11. CALCULATIONS

11.1 Determine the concentration of bentazon in the sample.

11.1.1 Calculate the amount of bentazon injected from the peak response from the calibration curve. The concentration in the sample can be calculated from the equation:

$$\text{Concentration, } \mu g/L = \frac{(A)\ (V_t)}{(V_i)\ (V_s)}$$

where:
A = Amount of bentazon injected (nanograms),
V_i = Volume of extract injected (µL),
V_t = Volume of total extract (µL), and
V_s = Volume of water extracted (mL).

11.2 Report results in µg/L without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.

12. METHOD PERFORMANCE

12.1 The EDL and associated chromatographic conditions for bentazon are listed in Table 1(8). The EDL is defined as the minimum response being equal to 5 times the background noise, assuming a 5-mL final extract volume of a 1-liter sample and an HPLC injection volume of 100 µL.

12.2 Single operator accuracy and precision studies were conducted by Environmental Science and Engineering, Incorporated (6), in the designated matrices. The results of these studies are presented in Table 2.

REFERENCES

1. ASTM Annual Book of Standards, Part 31, D3694, "Standard Practice for Preparation of Sample Containers and for Preservation," American Society for Testing and Materials, Philadelphia, PA, p. 679, 1980.

2. "Carcinogens - Working with Carcinogens," Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, Publication No. 77-206, Aug. 1977.

3. "OSHA Safety and Health Standards, General Industry" (29 CFR 1910), Occupational Safety and Health Administration, OSHA 2206 (Revised, January 1976).

4. "Safety in Academic Chemistry Laboratories," American Chemical Society Publication, Committee on Chemical Safety, 3rd Edition, 1979.

5. ASTM Annual Book of Standards, Part 31, D3370, "Standard Practice for Sampling Water," American Society for Testing and Materials, Philadelphia, PA, p. 76, 1980.

6. Test procedures for Pesticides in Wastewaters, EPA Contract Report No. 68-03-2897 (In Preparation). Unpublished report available from U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268.

7. "Handbook for Analytical Quality Control in Water and Wastewater Laboratories," EPA-600/4-79-019, U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory - Cincinnati, Ohio 45268, March 1979.

8. "Evaluation of Ten Pesticide Methods," U.S. Environmental Protection Agency Contract No. 68-03-1760, Task No. 11, U.S. Environmenal Monitoring and Support Laboratory, Cincinnati, Ohio 45268.

TABLE 2. SINGLE LABORATORY ACCURACY AND PRECISION

Parameter	Matrix Type*	Range µg/L	No. of Replicates	Average Percent Recovery	Standard Deviation (%)
Bentazon	1	125	7	85.1	4.8
	2	20,400	7	88.4	8.4

*1 = 50-percent industrial effluent + 50-percent POTW effluent.
2 = 100-percent industrial effluent.

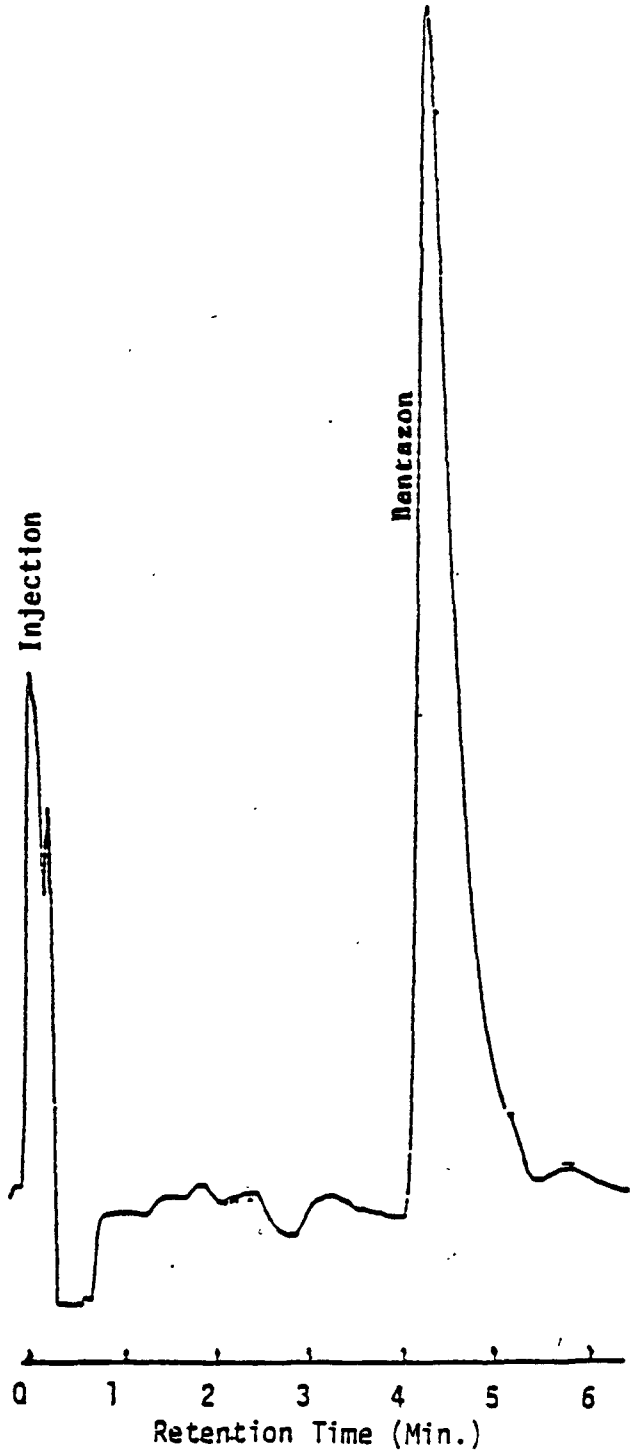

FIGURE 1. HPLC OF BENTAZON COLUMN 2