METHOD 8032A

ACRYLAMIDE BY GAS CHROMATOGRAPHY

1.0   SCOPE AND APPLICATION

    1.1   Method 8032 is used to determine trace amounts of acrylamide monomer (CAS No. 79-06-1) in aqueous matrices. This method may be applicable to other matrices and to other similar analytes.

    1.2   The method detection limit (MDL) in an aqueous matrix is 0.032 µg/L.

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

2.0   SUMMARY OF METHOD

    2.1   Method 8032 is based on bromination of the acrylamide double bond. The reaction product (2,3-dibromopropionamide) is extracted from the reaction mixture with ethyl acetate, after salting out with sodium sulfate. The extract is cleaned up using a Florisil column, and analyzed by gas chromatography with electron capture detection (GC/ECD).

    2.2   Compound identification should be supported by at least one additional qualitative technique. Analysis using a second gas chromatographic column or gas chromatography/mass spectrometry may be used for compound confirmation.

3.0   INTERFERENCES

    3.1   No interference is observed from seawater or in the presence of 8.0% of ammonium ions derived from ammonium bromide.

    3.2   Impurities from potassium bromide are removed by the Florisil cleanup procedure.

4.0   APPARATUS AND MATERIALS

    4.1   Gas chromatographic system

        4.1.1   Gas chromatograph suitable for on-column injections with all required accessories, including detector, analytical columns, recorder, gases, and syringes. A data system for measuring peak heights and/or peak areas is recommended.

        4.1.2   GC Column - 2 m x 3 mm glass column, 5% FFAP (free fatty acid polyester) on 60-80 mesh acid washed Chromosorb W, or equivalent.

        4.1.3   Detector - electron capture detector.

    4.2   Separatory funnel - 150-mL.

4.3   Volumetric flask (Class A) - 100-mL, with ground-glass stopper; 25-mL, amber, with ground-glass stopper.

4.4   Syringe - 5-mL.

4.5   Microsyringes - 5-µL, 100-µL.

4.6   Pipets (Class A).

4.7   Glass chromatography column (30 cm x 2 cm).

4.8   Mechanical shaker.

5.0   REAGENTS

5.1   Reagent grade 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   Organic-free reagent water. All references to water in this method refer to organic-free reagent water, as defined in Chapter One.

5.3   Solvents - All solvent must be pesticide quality, or equivalent.

5.3.1   Ethyl acetate, $C_2H_5CO_2C_2H_5$

5.3.2   Diethyl ether, $C_2H_5OC_2H_5$. Must be free of peroxides as indicated by test strips (EM Quant, or equivalent). Procedures for removal of peroxides are provided with the test strips. After cleanup, 20 mL of ethyl alcohol preservative must be added to each liter of ether.

5.3.3   Methanol, CH_3OH

5.3.4   Benzene, C_6H_6

5.3.5   Acetone, CH_3COCH_3

5.4   Saturated bromine water - Prepare by shaking organic-free reagent water with bromine and allowing to stand for 1 hour, in the dark, at 4°C. Use the aqueous phase.

5.5   Sodium sulfate (anhydrous, granular), Na_2SO_4. Purify this reagent by heating at 400°C for 4 hours in a shallow tray, or by precleaning the sodium sulfate with methylene chloride. If the sodium sulfate is precleaned with methylene chloride, a method blank must be analyzed, demonstrating that there is no interference from the sodium sulfate.

5.6   Sodium thiosulfate, $Na_2S_2O_3$, 1 M aqueous solution.

5.7   Potassium bromide, KBr, prepared for infrared analysis.

5.8   Concentrated hydrobromic acid, HBr, specific gravity 1.48.

5.9   Acrylamide monomer, $H_2C{:}CHCONH_2$, electrophoresis reagent grade, minimum 95% purity.

5.10   Dimethyl phthalate, $C_6H_4(COOCH_3)_2$, 99.0% purity.

5.11   Florisil (60/100 mesh): Prepare Florisil by activating at 130°C for at least 16 hours. Alternatively, store Florisil in an oven at 130°C. Before use, cool the Florisil in a desiccator. Pack 5 g of the Florisil, suspended in benzene, in a glass column (Sec. 4.8).

NOTE: Benzene is toxic, and should only be used in a ventilated laboratory hood.

5.12   Stock standard solution

Prepare a stock standard solution of acrylamide monomer as described below. 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. Commercially-prepared standards can be used at any concentration if they are certified by the manufacturer or by an independent source.

Dissolve 105.3 mg of acrylamide monomer in organic-free reagent water in a 100-mL volumetric flask, and dilute to the mark with organic-free reagent water. Dilute the solution of acrylamide monomer so as to obtain standard solutions containing 0.1 - 10 mg/L of acrylamide monomer.

5.13   Calibration standards

Dilute the acrylamide stock solution with organic-free reagent water to produce standard solutions containing 0.1 - 5 mg/L of acrylamide. Prior to injection the calibration standards are reacted and extracted in the same manner as environmental samples (Sec. 7.0).

5.14   Internal standards

The suggested internal standard is dimethyl phthalate. Prepare a solution containing 100 mg/L of dimethyl phthalate in ethyl acetate. The concentration of dimethyl phthalate in the sample extracts and calibration standards should be 4 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   Bromination

7.1.1   Pipet 50 mL of sample into a 100-mL glass-stoppered flask. Dissolve 7.5 g of potassium bromide into the sample, with stirring.

7.1.2   Adjust the pH of the solution with concentrated hydrobromic acid until the pH is between 1 and 3.

7.1.3   Wrap the flask with aluminum foil in order to exclude light. Add 2.5 mL of saturated bromine water, with stirring. Store the flask and contents in the dark, at 0°C, for at least 1 hour.

7.1.4   After reacting the solution for at least 1 hour, decompose the excess of bromine by adding 1 M sodium thiosulfate solution, drop by drop, until the solution becomes colorless.

7.1.5   Add 15 g of sodium sulfate and stir vigorously using a magnetic stirrer.

7.2   Extraction

7.2.1   Transfer the solution into a 150-mL separatory funnel. Rinse the reaction flask three times with 1-mL aliquots of organic-free reagent water. Transfer the rinsings into the separatory funnel.

7.2.2   Extract the aqueous solution twice with 10-mL portions of ethyl acetate for 2 min each extraction, using a mechanical shaker at approximately 240 strokes per minute. Dry the organic phase with 1 g of sodium sulfate.

7.2.3   Transfer the organic phase into a 25-mL amber volumetric flask. Rinse the sodium sulfate with three 1.5-mL portions of ethyl acetate and combine the rinsings with the organic phase.

7.2.4   Add exactly 100 µg of dimethyl phthalate to the flask and make the solution up to the 25 mL mark with ethyl acetate. Inject a 5-µL aliquot of this solution into the gas chromatograph.

7.3   Florisil cleanup - Whenever interferences are observed, the samples should be cleaned up as follows.

7.3.1   Transfer the dried extract into an evaporation vessel with   15 mL of benzene. Evaporate the solvent at 70°C under reduced pressure, and concentrate the solution to about 3 mL.

7.3.2   Add 50 mL of benzene and subject the solution to Florisil column chromatography at a flow rate of 3 mL/min. Elute the column first with 50 mL of diethyl ether/benzene (1:4) at a flow rate of 5 mL/min, and then with 25 mL of acetone/benzene (2:1) at a flow rate of 2 mL/min. Discard all of the first eluate and the initial 9 mL portion of the second eluate, and use the remainder for the determination, using dimethyl phthalate (4 mg/L) as an internal standard.

NOTE: Benzene is toxic, and should be only be used in a ventilated laboratory hood.

7.4   Gas chromatographic conditions

Nitrogen carrier gas flow rate: 40 mL/min
Column temperature: 165°C.
Injector temperature: 180°C
Detector temperature: 185°C.
Injection volume: 5 µL

7.5   Calibration

7.5.1   Inject 5 µL of a method blank (organic-free reagent water carried through all sample storage, handling, bromination and extraction procedures) into the GC.

7.5.2   Prepare a minimum of five standard solutions of acrylamide as described in Sec. 5.13.1. One standard should be near the detection limit of the method. The remaining four standards should bracket the expected sample concentrations and cover the linear working range of the instrument. Brominate and extract each standard solution as described in Secs. 7.1 and 7.2.

7.5.3   Inject 5 µL of each of the brominated and extracted standards, and record the instrument response.

7.5.4   Calculate the response factor relative to the internal standard for each calibration standard according to the guidance in Sec. 7.0 of Method 8000.

7.5.5   Calculate the mean, standard deviation, and relative standard deviation of the response factors from the five calibration standards, using the equations found in Sec. 7.0 of Method 8000.

7.5.6   If the RSD of the response factors is less than or equal to 20%, then the calibration can be assumed to be linear, and an average response factor may be used to calculate sample results. If the RSD is greater than 20%, see Method 8000 for alternative approaches to calibration.

7.6   Sample analysis

7.6.1   Inject 5-µL portions of each sample extract (containing 4 mg/L internal standard) into the gas chromatograph. An example GC/ECD chromatogram is shown in Figure 1.

7.6.2   The concentration of acrylamide monomer in the sample is calculated according to the following equation.

$$\text{Concentration } (\mu g/L) = \frac{(A_x)(C_{is})(D)(V_i)}{(A_{is})(\overline{RF})(V_s)(1000)}$$

where:

A_x = Area (or height) of the peak for the analyte in the sample.
A_{is} = Area (or height) of the peak for the internal standard.
C_{is} = Concentration of the internal standard in the concentrated sample extract (µg/L).
D = Dilution factor, if the sample or extract was diluted prior to analysis. If no dilution was made, $D = 1$. The dilution factor is always dimensionless.
V_i = Volume of the extract injected (µL). The injection volume for samples and calibration standards must be the same.
$\overline{RF}$ = Mean response factor from the initial calibration.
V_s = Volume of the aqueous sample extracted or purged (mL). If units of liters are used for this term, multiple the results by 1000.

The 1000 in the denominator represents the number of µL in 1 mL. If the injection (V_i) is expressed in mL, then the 1000 may be omitted.

Using the units specified here for these terms will result in a concentration in units of ng/mL, which is equivalent to µg/L.

8.0   QUALITY CONTROL

8.1   Refer to Chapter One and Method 8000 for specific quality control (QC) procedures. Quality control procedures to ensure the proper operation of the various sample preparation and/or sample introduction techniques can be found in Methods 3500 and 5000.   Each laboratory should maintain a formal quality assurance program. The laboratory should also maintain records to document the quality of the data generated.

8.2   Quality control procedures necessary to evaluate the GC system operation are found in Method 8000, Sec. 7.0 and includes evaluation of retention time windows, calibration verification and chromatographic analysis of samples.

8.3   Initial Demonstration of Proficiency - Each laboratory must demonstrate initial proficiency with each sample preparation and determinative method combination it utilizes, by generating data of acceptable accuracy and precision for target analytes in a clean matrix. The laboratory must also repeat the following operations whenever new staff are trained or significant changes in instrumentation are made. See Method 8000, Sec. 8.0 for information on how to accomplish this demonstration.

8.4   Sample Quality Control for Preparation and Analysis - The laboratory must also have procedures for documenting the effect of the matrix on method performance (precision, accuracy, and detection limit). At a minimum, this includes the analysis of QC samples including a method blank, a matrix spike, a duplicate, and a laboratory control sample (LCS) in each analytical batch.

8.4.1   Documenting the effect of the matrix should include the analysis of at least one matrix spike and one duplicate unspiked sample or one matrix spike/matrix spike duplicate pair. The decision on whether to prepare and analyze duplicate samples or a matrix spike/matrix spike duplicate must be based on a knowledge of the samples in the sample batch. If samples are expected to contain target analytes, then laboratories may use one matrix spike and a duplicate analysis of an unspiked field sample. If samples are not expected to contain target analytes, laboratories should use a matrix spike and matrix spike duplicate pair.

8.4.2   A Laboratory Control Sample (LCS) should be included with each analytical batch. The LCS consists of an aliquot of a clean (control) matrix similar to the sample matrix and of the same weight or volume.   The LCS is spiked with the same analytes at the same concentrations as the matrix spike. When the results of the matrix spike analysis indicate a potential problem due to the sample matrix itself, the LCS results are used to verify that the laboratory can perform the analysis in a clean matrix.

8.4.3   See Method 8000, Sec. 8.0 for the details on carrying out sample quality control procedures for preparation and analysis.

8.5   It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the

laboratory and the nature of the samples. Whenever possible, the laboratory should analyze standard reference materials and participate in relevant performance evaluation studies.

9.0 METHOD PERFORMANCE

9.1 The following performance data have been generated under the conditions described in this method:

9.1.1 The calibration curve for Method 8032 has been found to be linear over the range 0-5 µg/L of acrylamide monomer.

9.1.2 In previous analyses, the limit of detection for an aqueous matrix has been found to be 0.032 µg/L.

9.1.3 The yields of the brominated compound have been found to be 85.2 ± 3.3% and 83.3 ± 0.9%, at fortification concentrations of 1.0 and 5.0 µg/L, respectively.

9.2 Table 1 provides the recoveries of acrylamide monomer from river water, sewage effluent, and sea water.

9.3 The recovery of the bromination product as a function of the amount of potassium bromide and hydrobromic acid added to the sample is shown in Figure 2.

9.4 The effect of the reaction time on the recovery of the bromination product is shown in Figure 3. The yield was constant when the reaction time was more than 1 hour.

9.5 Figure 4 shows the recovery of the bromination product as a function of the initial pH from 1 to 7.35. The yield was constant within this pH range. The use of conventional buffer solutions, such as sodium acetate - acetic acid solution or phosphate solution, caused a significant decrease in yield.

10.0 REFERENCES

1. Hashimoto, A., "Improved Method for the Determination of Acrylamide Monomer in Water by Means of Gas-Liquid Chromatography with an Electron-Capture Detector", Analyst, 101:932-938, 1976.

TABLE 1

RECOVERY OF ACRYLAMIDE FROM WATER SAMPLES AS 2,3-DIBROMOPROPIONAMIDE

Sample Matrix	Acrylamide Monomer Spiked/μg	Amount of 2,3-DBPA[a]/μg Calculated	Found[b]	Bromination Recovery %[b]	Recovery of Acrylamide Monomer (%)[b]	RSD (%)
Standard	0.05	0.162	0.138	85.2	-	3.3
	0.20	0.649	0.535	82.4	-	1.0
	0.25	0.812	0.677	83.3	-	0.9
River Water	0.20	0.649	0.531	81.8	99.4	2.5
Sewage Effluent	0.20	0.649	0.542	83.5	101.3	3.0
Seawater	0.20	0.649	0.524	80.7	98.8	3.5

[a] 2,3-Dibromopropionamide

[b] Mean of five replicate determinations

FIGURE 1
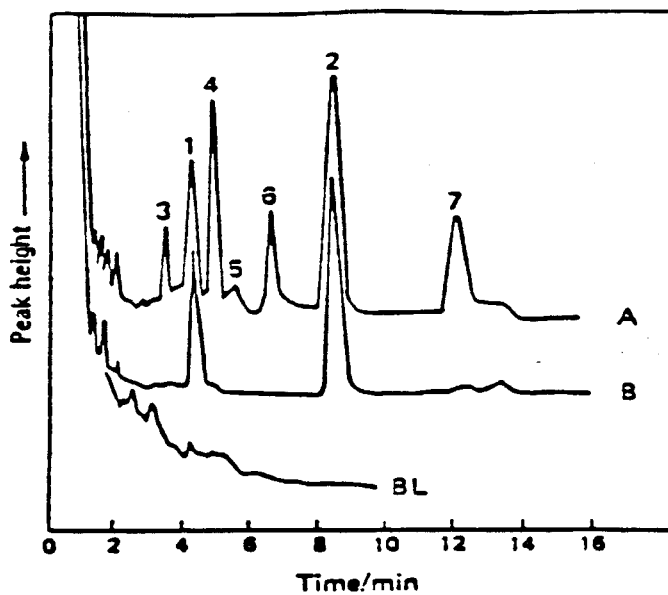
Typical gas chromatograms of the bromination product obtained from aqueous acrylamide monomer solution:

A. Untreated
B. With Florisil cleanup
BL. Chromatogram of blank, concentrated five-fold before gas chromatographic analysis.

Peaks:

1. 2,3-Dibromopropionamide
2. Dimethyl phthalate
4-7. Impurities from potassium bromide

Sample size = 100 mL; acrylamide monomer = 0.1 µg
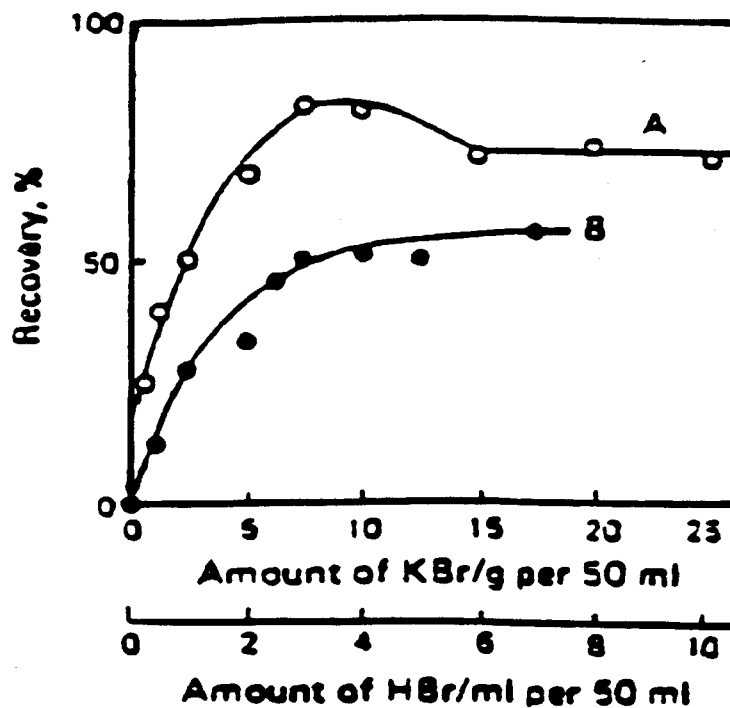
FIGURE 2

Effect of (A) potassium bromide and (B) hydrobromic acid on the yield of bromination.

Sample size = 50 mL;
Acrylamide monomer = 0.25 µg

FIGURE 3
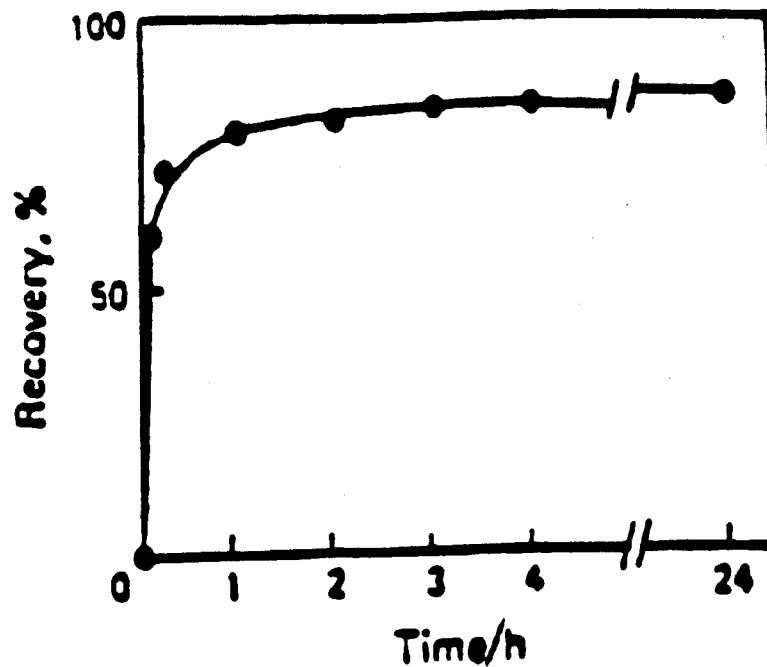
Effect of reaction time on the bromination. Reaction conditions:

    50 mL of sample;
    0.25 µg of acrylamide monomer;
    7.5 g of potassium bromide;
    2.5 mL of saturated bromine water

Extraction conditions:

    15 g of sodium sulfate;
    extraction at pH 2;
    solvent = 10 mL of ethyl acetate (X2)

FIGURE 4
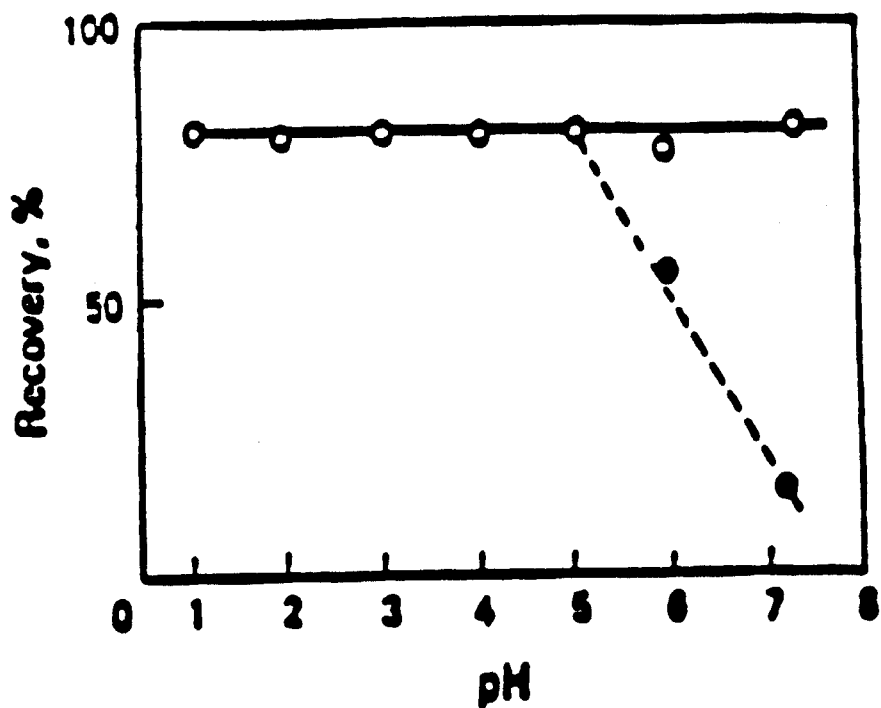
Effect of initial pH on the bromination. Reaction and extraction conditions as in Figure 3. The pH was adjusted to below 3 with concentrated hydrobromic acid, and to 4 - 5 with dilute hydrobromic acid. Reaction at pH 6 was in distilled water. A pH of 7.35 was achieved by careful addition of dilute sodium hydroxide solution. The broken line shows the result obtained by the use of sodium acetate - acetic acid buffer solution.

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        ┌───────────────────────────┐          ┌───────────────────────────┐
        │      7.1 Bromination.     │          │      7.2 Extraction.      │
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                        │                                   │
                        ▼                                   ▼
        ┌───────────────────────────┐          ┌───────────────────────────┐
        │ 7.1.1 Dissolve 7.5 g KBr   │          │ 7.2.1 Transfer flask soln. │
        │ into 50 mL sample in flask.│          │ to sep. funnel along with  │
        │                            │          │         rinses.            │
        └───────────────────────────┘          └───────────────────────────┘
                        │                                   │
                        ▼                                   ▼
        ┌───────────────────────────┐          ┌───────────────────────────┐
        │ 7.1.2 Adjust pH with       │          │ 7.2.2 Extract soln. twice  │
        │ concentrated HBr to        │          │ w/ethyl acetate. Dry       │
        │ between 1 and 3.           │          │ organic phase using        │
        │                            │          │ sodium sulfate.            │
        └───────────────────────────┘          └───────────────────────────┘
                        │                                   │
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        ┌───────────────────────────┐          ┌───────────────────────────┐
        │ 7.1.3 Wrap soln. flask     │          │ 7.2.3 Transfer organic     │
        │ with aluminum. Add 2.5 mL  │          │ phase and rinses into      │
        │ satd. bromine water, stir, │          │ amber glass flask.         │
        │ store at 0° C for 1 hr.    │          │                            │
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                        │                                   │
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        ┌───────────────────────────┐          ┌───────────────────────────┐
        │ 7.1.4 Add 1 M sodium       │          │ 7.2.4 Add 100 µg dimethyl  │
        │ thosulfate dropwise to     │          │ phthalate to flask; dilute │
        │ flask to decompose excess  │          │ to mark. Inject 5 µL into  │
        │ bromine.                   │          │ GC.                        │
        └───────────────────────────┘          └───────────────────────────┘
                        │                                   │
                        ▼                                   ▼
        ┌───────────────────────────┐          ┌───────────────────────────┐
        │ 7.1.5 Add 15 g sodium      │          │ 7.3.1 Transfer dried       │
        │ sulfate and stir.          │──────────│ extract to K-D assembly    │
        │                            │          │ w/benzene. Concentrate to  │
        └───────────────────────────┘          │ 3 mL at 70°C under reduced │
                                                │ pressure.                  │
                                                └───────────────────────────┘
                                                             │
                                                             ▼
                                                ┌───────────────────────────┐
                                                │ 7.3.2 Add 50 mL benzene to │
                                                │ solution. Pass soln.       │
                                                │ through Florisil column.   │
                                                │ Elute with diethyl         │
                                                │ ether/benzene, then        │
                                                │ acetone/benzene. Collect   │
                                                │ the second elution train   │
                                                │ (less initial 9 mL) for    │
                                                │ analysis.                  │
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        |      7.4 GC Conditions        |
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        |       7.5 Calibration         |
        +-------------------------------+
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                      v
        +-------------------------------+
        | 7.5.1 Inject 5 uL sample blank.|
        +-------------------------------+
                      |
                      v
        +-------------------------------+
        | 7.5.2 Brominate and extract std.|
        | solns. similar to the samples.  |
        | .1 Inject 5 uL of each of the   |
        |    minimum 5 stds.              |
        | .2 Plot peak are vs. [ ].       |
        | .3 Calculate response factor    |
        |    (RF) for each [ ].           |
        +-------------------------------+
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                      v
        +-------------------------------+
        | 7.5.3 Calculate mean RF from   |
        | eqn. 2.                        |
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        |       7.6 GC Analysis          |
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        +-------------------------------+
        | 7.6.1 Inject 5 uL sample containing|
        | internal std. into GC.         |
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        | 7.6.2 Calculate acrylamide monomer|
        | concentration in sample using  |
        | eqn. 3.                        |
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                   ( Stop )
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