METHOD 8032

ACRYLAMIDE BY GAS CHROMATOGRAPHY

1.0 SCOPE AND APPLICATION

1.1 Method 8032 is used to determine trace amounts of acrylamide monomer in aqueous matrices. This method may be applicable to other matrices. The following compound can be determined by this method:

Compound Name	CAS No.[a]
Acrylamide	79-06-01

[a] Chemical Abstract Services Registry Number.

1.2 The method detection limit (MDL) in clean water is 0.032 µg/L.

1.3 This method is restricted to 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 sea water or in the presence of 8.0% of ammonium ions derived from ammonium bromide. Impurities from potassium bromide are removed by the Florisil clean up 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 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 Kuderna-Danish (K-D) apparatus.

4.2.1 Concentrator tube - 10 mL graduated (Kontes K-570050-1025 or equivalent). A ground glass stopper is used to prevent evaporation of extracts.

4.2.2 Evaporation flask - 500 mL (Kontes K-570001-500 or equivalent). Attach to concentrator tube with springs, clamps, or equivalent.

4.2.3 Snyder column - Three ball macro (Kontes K-503000-0121 or equivalent).

4.2.4 Snyder column - Two ball micro (Kontes K-569001-0219 or equivalent).

4.2.5 Springs - 1/2 inch (Kontes K-662750 or equivalent).

4.3 Separatory funnel - 150 mL.

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

4.5 Syringe - 5 mL.

4.6 Microsyringes - 5 µL, 100 µL.

4.7 Pipets (Class A).

4.8 Glass column (30 cm x 2 cm).

4.9 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

5.3.1 Ethyl acetate, $C_2H_5CO_2C_2H_5$. Pesticide quality, or equivalent.

5.3.2 Diethyl ether, $C_2H_5OC_2H_5$. Pesticide quality, or equivalent. 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. Pesticide quality, or equivalent.

5.3.4 Benzene, C_6H_6. Pesticide quality, or equivalent.

5.3.5 Acetone, CH_3COCH_3. Pesticide quality, or equivalent.

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 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).

5.12 Stock standard solutions

5.12.1 Prepare a stock standard solution of acrylamide monomer as specified in Sec. 5.12.1.1. 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.

5.12.1.1 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

5.13.1 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).

5.14 Internal standards

5.14.1 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 an hour, decompose the excess of bromine by adding 1 M sodium thiosulfate solution, dropwise, until the color of the solution is discharged.

7.1.5 Add 15 g of sodium sulfate, using a magnetic stirrer to effect vigorous stirring.

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 with two 10 mL portions of ethyl acetate for 2 min each, using a mechanical shaker (240 strokes per min). 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 5 µL portions 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 a Kuderna-Danish evaporator 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 under 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 sample blank (organic-free reagent water carried through all sample storage, handling, bromination and extraction procedures).

7.5.2 Prepare standard solutions of acrylamide as described in Sec. 5.13.1. Brominate and extract each standard solution as described in Secs. 7.1 and 7.2.

7.5.2.1 Inject 5 µL of each of a minimum of five standard solutions: one should be near the detection limit; one should be near, but below, the expected concentrations of the analyte; one should be near, but above, the expected concentrations of the analyte.

7.5.2.2 Prepare a calibration curve using the peak areas of the standards. If the calibration curve deviates significantly from a straight line, prepare a new calibration curve with the existing standards, or, prepare new standards and a new calibration curve. See Method 8000, Sec. 7.4.3, for additional guidance on calibration by the internal standard method.

7.5.2.3 Calculate the response factor for each standard according to Equation 1.

$$RF = \frac{(P_s)\ (M_{is})}{(P_{is})\ (M_A)} \qquad \text{Equation 1}$$

RF = Response factor
P_s = Peak height of acrylamide
M_{is} = Amount of internal standard injected (ng)
P_{is} = Peak height of internal standard
M_A = Amount of acrylamide injected (ng)

7.5.3 Calculate the mean response factor according to Equation 2.

$$\overline{RF} = \frac{\sum_{i=1}^{n} RF}{n} \qquad \text{Equation 2}$$

$\overline{RF}$ = Mean response factor
RF = Response factors from standard analyses (calculated in Equation 1)
n = Number of analyses

7.6 Gas chromatographic analysis:

7.6.1 Inject 5 µL portions of each sample (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 given by Equation 3.

$$[A] = \frac{(P_A)\ (M_{is})}{(P_{is})\ (\overline{RF})\ (V_i)\ (V_s)} \qquad \text{Equation 3}$$

[A] = Concentration of acrylamide monomer in sample (mg/L)

P_A = Peak height of acrylamide monomer
M_{is} = Amount of internal standard injected (ng)
V_s = Total volume of sample (mL)
$\underline{P_{is}}$ = Peak height of internal standard
RF = Mean response factor from Equation 2
V_i = Injection volume (µL)

8.0 QUALITY CONTROL

8.1 Refer to Chapter One and Method 8000 for specific quality control procedures.

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 is linear over the range 0-5 µg/L of acrylamide monomer.

9.1.2 The limit of detection for an aqueous solution is 0.032 µg/L.

9.1.3 The yields of the brominated compound are 85.2 $\pm$ 3.3% and 83.3 $\pm$ 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

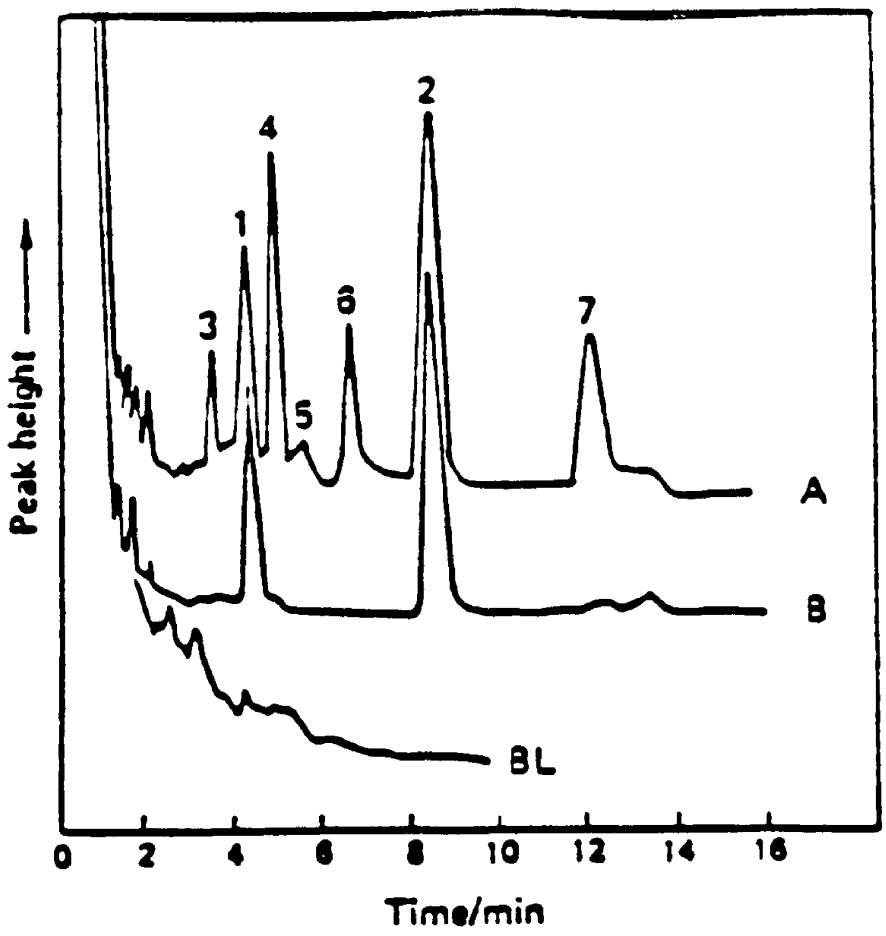
RECOVERY OF ACRYLAMIDE FROM WATER SAMPLES AS
2,3-DIBROMOPROPIONAMIDE

Sample Matrix	Acrylamide Monomer Spiked/µg	Amount of 2,3-DBPA[a]/µg		Overall Bromination Recovery %[b]	Recovery of Acrylamide Monomer, %[b]	Coefficient of Variation
		Calculated	Found[b]			
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
Sea Water	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:

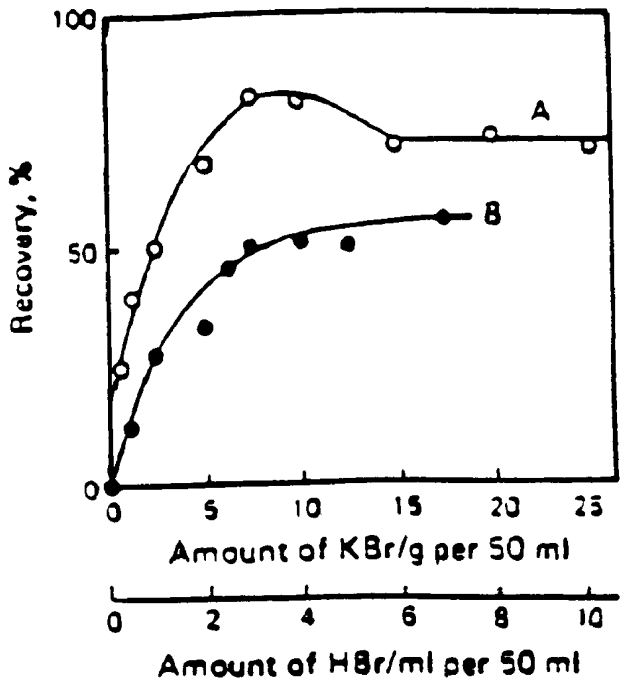
A. Untreated
B. With Florisil cleanup
BL. Chromatogram of blank, concentrated five-fold before gas chromatographic analysis.

Peaks:

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

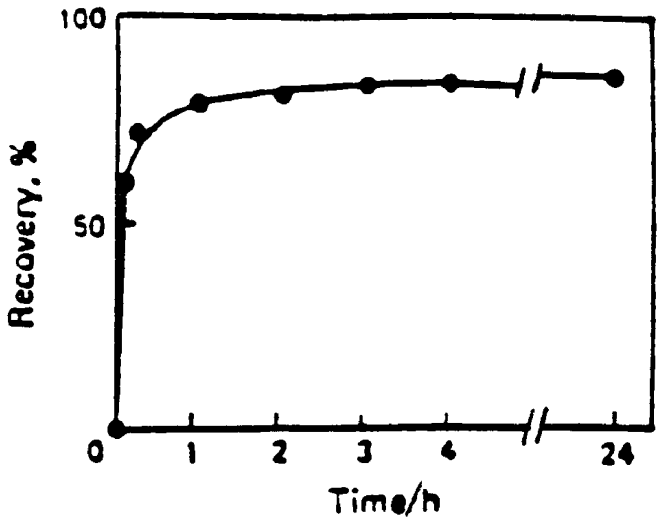
Sample size = 100 mL; acrylamide monomer = 0.1 µg

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



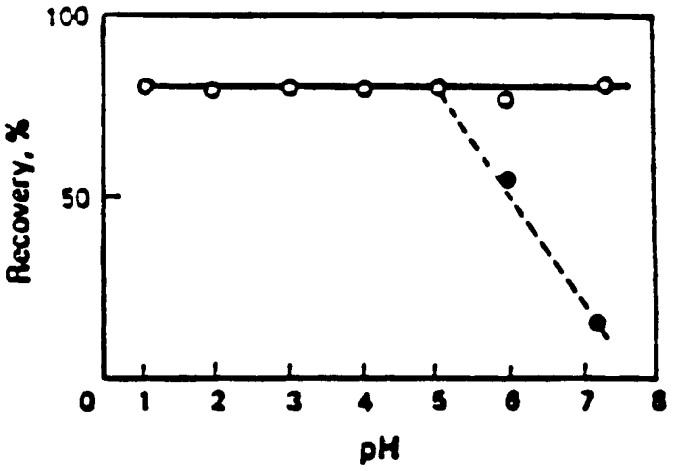
Effect of reaction time on the bromination. Reaction conditions:

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

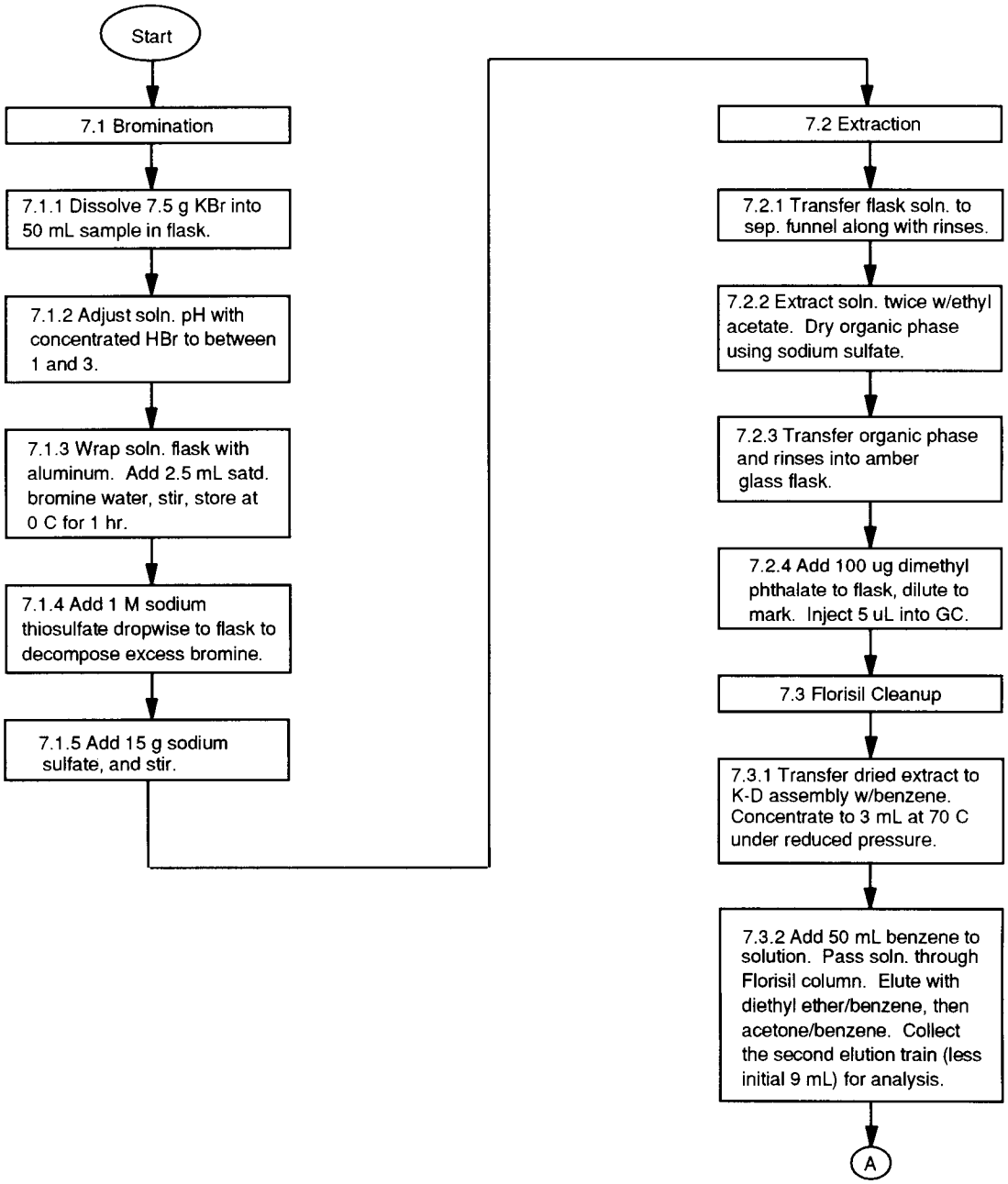
Extraction conditions:

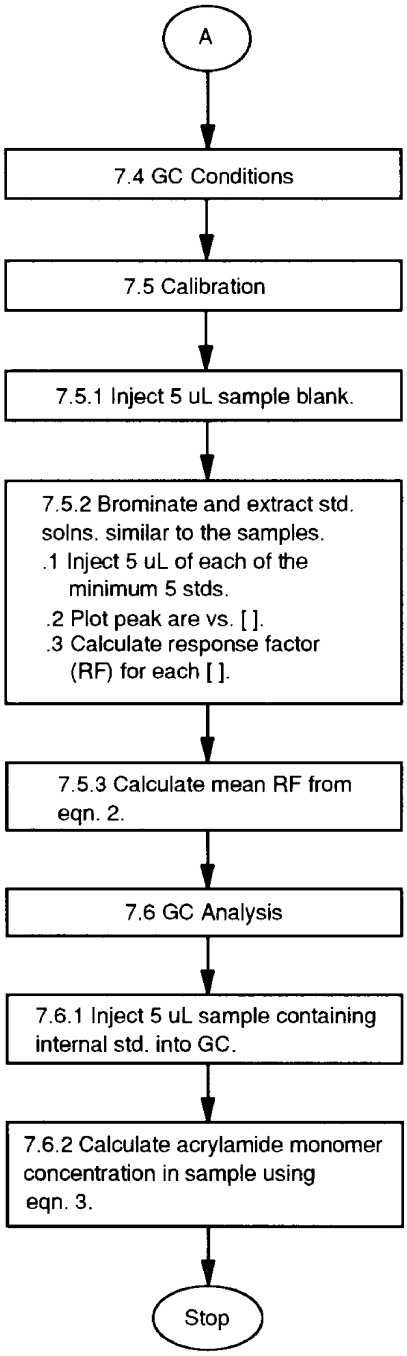
15 g of sodium sulfate;
extraction at pH 2;
solvent = 10 mL of ethyl acetate (X2)

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. pH 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.


Start
7.1 Bromination
7.1.1 Dissolve 7.5 g KBr into 50 mL sample in flask.
7.1.2 Adjust soln. pH with concentrated HBr to between 1 and 3.
7.1.3 Wrap soln. flask with aluminum. Add 2.5 mL satd. bromine water, stir, store at 0 C for 1 hr.
7.1.4 Add 1 M sodium thiosulfate dropwise to flask to decompose excess bromine.
7.1.5 Add 15 g sodium sulfate, and stir.
7.2 Extraction
7.2.1 Transfer flask soln. to sep. funnel along with rinses.
7.2.2 Extract soln. twice w/ethyl acetate. Dry organic phase using sodium sulfate.
7.2.3 Transfer organic phase and rinses into amber glass flask.
7.2.4 Add 100 ug dimethyl phthalate to flask, dilute to mark. Inject 5 uL into GC.
7.3 Florisil Cleanup
7.3.1 Transfer dried 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.
A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7.4 GC Conditions
7.5 Calibration
7.5.1 Inject 5 uL sample blank.
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 [ ].
7.5.3 Calculate mean RF from eqn. 2.
7.6 GC Analysis
7.6.1 Inject 5 uL sample containing internal std. into GC.
7.6.2 Calculate acrylamide monomer concentration in sample using eqn. 3.
Stop