ETHYLENE DIBROMIDE

Contents

I. DETERMINATIVE METHOD

A. INTRODUCTION

Theory

Ethylene dibromide (EDB) has very low solubility in water and has a high vapor pressure, making codistillation over water with another solvent (hexane) a practical separation technique. The hexane-EDB condensate is trapped in a Barrett distilling receiver that is cooled in an ice bath. The water is drained from the receiver, the volume of hexane read, and the hexane layer transferred to a 20 mL scintillation vial containing 2-3 g Na_2SO_4. The dried condensate is injected directly on a gas chromatograph equipped with an electron capture detector.

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B. EQUIPMENT

1. Apparatus

a. Scintillation vial, 20 mL, Wheaton Scientific No. 98654, or equivalent.

NOTE: Adhesive in the foil-lined cap of the vial may produce interfering peaks on GC. This can be prevented by adding a Teflon liner to the cap.

b. Volumetric flasks, 100 mL and 10 mL.

c. Syringes: 10 μL, 100 μL, and 1000 μL.

d. Condenser water jacket, Kimax No. 18190 with 24/40 ground glass fitting, or equivalent.

e. Barrett distillation receiver, 20 mL, Pyrex No. 3622, or equivalent.

f. 1000 mL round-bottom flask with 24/40 ground glass fitting single neck. (Refer to note following item 1.g.)

g. Heating mantle, 1 L, Glas-Cal Apparatus Company, 711 Hulman Street, Terre Haute, IN, 47803, catalog No. 0-408, or equivalent.

NOTE: Flask size can be reduced to 500 mL and a hot water bath can be substituted for the heating mantle if these items are not available.

2. Instrumentation

Gas chromatograph, Hewlett-Packard 5880 (or equivalent) equipped with Electron Capture detector (^{63}Ni)

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C. REAGENTS AND SOLUTIONS

Reagent and Solution List

a. Hexane, UV grade, Burdick and Jackson, or equivalent.

b. Distilled water, Waters Milli Q treated, or equivalent.

c. Sodium sulfate, reagent grade (tested for interfering peaks), anhydrous crystals.

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D. STANDARDS

1. Source	1,2-dibromoethane (> 99%), Aldrich Gold Label No. 24, 0065-6, b.p. 131-132° C.
2. Preparation of Standards	Prepare fresh working external and recovery studies once per month. Each fresh standard shall be prepared as stated in the methodology and compared to current standard. a. Stock standard (0.5 μg/mL): Add 23 μL of 2.179 specific gravity EDB to 100 mL volumetric flask and dilute to volume with hexane. b. Working standard (0.5 μg/mL): Add 100 μL of stock standard to a 100 mL volumetric flask and dilute to volume with hexane. c. External standard (1.0 ng/mL): Add 200 μL working standard to 100 mL volumetric flask and dilute to volume with hexane. d. Recovery standard: i. 1 ppb: Add 1 mL working standard to 500 mL hexane. ii. Add 10 mL recovery standard solution to 10 g blank tissue.
3. Storage Conditions	Store all standards in a freezer at 0° C.
4. Shelf Life Stability	Only long enough to conduct all necessary analyses—volatile compound.

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E. EXTRACTION PROCEDURE

1. Sample Preparation

To prevent cross-contamination, sample is finely chopped on filter paper just before analysis.

2. Sample Extraction

a. Weigh 10 g of finely chopped sample in 1000 mL round-bottom flask and add 300 mL distilled water, 10 mL hexane, and boiling chips.

b. Connect to distillation apparatus and place in heating mantle.

c. Increase heat until water just boils and collect distillate until 2-3 mL water layer appears in Barrett trap.

d. Drain water layer and discard.

e. Add contents of trap (8.8 to 10.0 mL hexane) to 20 mL scintillation vial containing 2-3 g of anhydrous Na_2SO_4, shake vial, and let stand. When hexane is no longer cloudy, inject 5 μL on GC.

f. Prepare a reagent blank by distilling 10 mL hexane with 300 mL water and collect in the same manner as a sample.

g. Prepare a tissue blank by distilling 10 g of the tissue of interest with 10 mL hexane and 300 mL water, and collect in the same manner as a sample.

h. Perform recoveries at 1 ppb equivalent EDB by repeating steps a-e above. Repeat with 100 ppb EDB equivalent, if samples are found in this range. Use standards in section D.2.d.i, ii.

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F. ANALYTICAL QUANTITATION

Instrumental Settings and Conditions

a. 6 ft × 2 mm glass column packed with 15% OV-17 on 80/100 mesh Chromosorb WAW or 20% OV-225/20% OV-17 (2 + 1) on 80/100 mesh Chromosorb W-HP.

b. Argon/methane carrier gas 95/5: 20 mL/min for 15% OV-17 column, 37 mL/min for 20% OV-225/20% OV-17 column.

c. Oven temperature programmed from 100° C for 8 min at 16° C/min to 220° C, hold for 8 to 10 min, time delay 15 min for equilibration. Injection temperature 160° C, detector temperature 350° C.

NOTE: Detector temperature can be maintained at 260° C for greater sensitivity during the analysis, but must be elevated to 350° C periodically to prevent contamination.

NOTE: Instrument should be adjusted to give approximately 50% full-scale response and a retention time of about 5-6 min for a 1 ppb external standard.

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G. CALCULATIONS

1. Procedure	Each chromatogram should contain injections of hexane, reagent blank, tissue blank, external standard (1 ng/mL), and 1 ppb recovery standard. Calculation is made against the external standard without correction for recovery.
2. References	Rains, D. and Holder, J., J. Assoc. Off. Anal. Chem., Vol. 64, pp 1252-1254, (1981)

I. DETERMINATIVE METHOD

H. HAZARD ANALYSIS

1. Method Title

Analysis and Confirmation for Ethylene Dibromide in Animal Tissue by Codistillation.

2. Required Protective Equipment

Safety glasses, plastic gloves, lab coat, face mask.

3. Procedure Steps

	Hazards	*Recommended Safe Procedures*
D. Standards		
1, 2-dibromoethane	This compound is flammable and extremely corrosive to the skin, eyes, and respiratory system. It is also considered to be a carcinogen.	Pipetting and diluting must be performed in an efficient fume hood. Electric hot plates or open flames should not be present.
E. Extraction	Vapor leaks, explosions	The procedure should be followed using ice baths and checking that the distillation components are sealed securely. It would be desirable for this procedure to be performed in a fume hood.

4. Disposal Procedures

Ethylene dibromide solutions	See above.	The solutions should be combined in a separate storage container until picked up by the contractor or in-house specialist.

I. DETERMINATIVE METHOD

J. QUALITY ASSURANCE PLAN

1. Performance Standards

Compound	*Analytical Range (ppb)*	*Acceptable Recovery (%)*	*Repeatability CV (%)*
Ethylene dibromide	0.5-2.0	65%-100%†	15

†With average of 10 last recoveries not less than 75%.

2. Critical Control Points and Specifications

Record	*Acceptable Control*
Lot no, source, date checked, analyst.	No deflection > 0.05 ppb as EDB within ± 0.5 min of EDB R.T.

3. Readiness to Perform

a. Familiarization.

i. Phase I: Standards—3 levels, 3 replicates each.

(a) 0.5 ppb.

(b) 1.0 ppb.

(c) 2.0 ppb.

ii. Phase II: Fortified samples—at least 3 levels, 3 replicates each.

(a) 0.5 ppb.

(b) 1.0 ppb.

(c) 2.0 ppb.

Blanks: Reagent; Tissue: 1 of each with each set.

NOTE: Phases I and II may be performed concurrently.

iii. Phase III: Check samples for analyst accreditation.

(a) Three samples from FSIS Science Western Laboratory (or supervisor if only one laboratory is performing this test).

(b) Submit analytical findings to Chemistry Division, QSB.

Notification from Chemistry Division required to commence official analysis.

b. Acceptability criteria.

Refer to section J. 1 above.

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J. QUALITY ASSURANCE PLAN (Continued)

4. Intralaboratory Check Samples

a. System, minimum contents.

i. Frequency: Initially, 1 per set, gradually reduced to a minimum of 1 per week per analyst, or 20% of official samples analyzed (whichever is smaller). Blind samples or random duplicates chosen by the supervisor or LSO.

ii. Records to be maintained by analyst and reviewed by supervisor and LSO.

(a) Running average difference between replicates.

(b) All % recoveries recorded.

(d) For all recoveries the running average, standard deviation, and coefficient of variation.

(e) Appropriate CUSUM charts.

b. Acceptability criteria.

If unacceptable values are obtained, then:

i. Stop all official analyses for that analyst.

ii Investigate and identify probable cause.

iii. Take corrective action.

iv. Repeat Phase III of section J.3 above if cause was analyst-related.

5. Sample Acceptability and Stability

a. Matrix: Fat.

b. Sample receipt size: Varied; enough to obtain matrix required for all quantitative tests and reserve sample.

c. Condition upon receipt: Cold.

d. Sample storage:

i. Time: Maximum unknown—analyte dissipates.

ii. Condition: Frozen.

6. Sensitivity

a. Lowest detectable limit (LDL): 0.5 ppb.

b. Lowest reliable quantitation (LRQ): 1.0 ppb.

c. Minimum proficiency level (MPL): 1.0 ppb.

II. CONFIRMATORY METHOD

Confirmation of EDB Residue

a. Prior to injecting the samples, inject 5 μL of hexane to determine if there are any interfering peaks.

b. For EDB residue between 1 and 5 ppb, inject 5 μL of the 10 mL hexane distillate into the mass spectrometer, utilizing the following equipment and conditions:

   i. Gas chromatograph equipped with a 25 M OV-1 column. Injection port 150° C , column temperature 50° C. Use a splitless injection system for the analysis.

   ii. Operate mass spectrometer in the negative chemical ionization mode using methane as reagent gas. Tune the instrument on ion 633. Adjust the electronics for maximum response while just attaining separation between 633 and 634. Monitor ions 79 and 81. Interface temperature 200° C. Source temperature 200° C.

   iii. Minimum detectable amount should be between 1 and 5 pg of EDB when monitoring ions 79 and 81, with a retention time of 4.5 min.

c. For EDB residues between 5 and 20 ppb, concentrate the 10 mL hexane distillate to 1 mL at room temperature under a stream of nitrogen. Inject 5 μL of this concentrate into the mass spectrometer using the same GC conditions shown in procedure b above and the following MS conditions:

   i. Operate mass spectrometer in the electron impact mode. Perform a normal tune procedure using PFTBA as standard. Source and interface remain at 200° C. Monitor ions 107 and 109.

   ii. Minimum detectable amount should be less than the 250 pg injected.

d. A positive confirmation is reported if in procedure b both ions, 79 and 81, are present at the correct retention time and the ratio of 79 and 81 is approximately 1.00 $\pm$ 10%, and in procedure c if both ions, 102 and 109 are present at the correct retention time and the ratio of 107 and 109 is approximately 1.10 $\pm$ 10%.