Control No. AM339

METHOD WRITEUP FOR

WATER QUALITY BRANCH

ANALYTICAL METHODS MANUAL

by

Yvonne D. Stokker

Analytical Methods Division
National Water Research Institute
Canada Centre for Inland Waters
Burlington, Ontario, Canada L7R 4A6

November 1986
NWRI Contribution # 87-61

METHOD FOR THE ANALYSIS OF DINOSEB IN NATURAL WATERS BY *in situ* ACETYLATION (gas chromatographic)

1. SCOPE AND APPLICATION

1.1 This method is applicable to the qualitative and quantitative gas chromatographic determination of 2-sec-butyl-4,6-dinitrophenol (Dinoseb) in natural waters.

1.2 The practical limits of measurement using electron capture detection range from 0.1 to 10 µg/L. These concentration levels have been validated and are based on using a 1-L water sample.

2. PRINCIPLE AND THEORY

2.1 The Dinoseb in a 1-L water sample undergoes *in situ* acetylation with acetic anhydride in the presence of 0.5% potassium bicarbonate, while simultaneously being extracted into petroleum ether.

2.2 The organic extract, containing dinoseb acetate, is dried through anhydrous sodium sulphate and, with iso-octane added as a keeper, concentrated by rotary evaporation to 1 mL.

2.3 The extract is made up to its final volume with toluene and analyzed by EC-GLC.

2.4 The method presented here can readily be modified for the simultaneous analysis of other phenols.

3. INTERFERENCES

3.1 All reagents must be thoroughly checked and any interferences from this source eliminated.

3.2 Because the acetylation reaction is specific to phenols, the potential interference from other acidic compounds is greatly reduced.

3.3 Extraneous matter, especially in highly coloured water samples, is a potential interference. The optional cleanup procedures discussed in the Remarks section will usually eliminate this source of interference.

4. SAMPLING PROCEDURE AND STORAGE

4.1 Water samples should be collected and stored in an all-glass system and acidified immediately to pH 1 or less with dilute sulphuric acid (1+1). This method would then require neutralization of the sample immediately before it undergoes extractive acetylation.

4.2 Teflon-lined bottle caps are recommended to prevent contamination of the water sample from contact with the cap. If Teflon-lined caps are unavailable, the use of solvent-washed aluminum foil beneath the cap is acceptable.

4.3 Samples should be stored in the dark at 4°C and extracted as soon as possible.

5. SAMPLE PREPARATION

5.1 If the water sample has been acidified, it should first be neutralized with sodium or potassium hydroxide before being subjected to the extractive acetylation procedure.

6. APPARATUS

6.1 Capillary GLC analysis of Dinoseb acetate

6.1.1 A gas chromatograph with good sensitivity equipped with a split/splitless capillary column injection port and an electron capture detector (^{63}Ni) such as Hewlett-Packard Model 5880A or equivalent.

6.1.2 Automatic Liquid Sampler such as Hewlett-Packard Model 7671A or equivalent. If this is not available, use a 10-µL Hamilton micro-syringe and inject 2 µL.

6.1.3 Fused Silica Capillary Column (FSCC): 12 m x 0.2 mm i.d. column, coated with cross-linked dimethyl silicone gum (0.33 µm thickness) and surface deactivated by siloxane, such as Hewlett-Packard OV-1 (Part No. 19091-60312) or equivalent.

6.1.4 Chromatographic conditions:

Injection Port: splitless mode, splitless valve on for 30 s

Injection Port Temperature: 250°C

Detector: Ni-63 ECD

Detector Temperature: 300°C

Detector Make-Up Gas: Argon/Methane (95+5) at 25 mL/min

Carrier Gas: Helium

Linear Velocity: 25 cm/s

Column Temperature Initial: 70°C; hold for 0.5 min

Programming Rate 1: 25°C/min (70° to 140°C);

Programming Rate 2: 2°C/min (140° to 180°C); hold for 5 min at 180°C

Column Post-Run Final Temperature: 220°C for 15 min

Chart Speed: 0.75 cm/min

6.2 Magnetic stirrers with 51 mm x 9.5 mm o.d. Teflon-coated spinbars.

6.3 Solvent Evaporator with thermostatted bath such as Büchi Rotavapor Model RE-120 or equivalent, available from Brinkman Instruments.

6.4 Oven, capable of maintaining 200°C.

6.5 Hamilton micro-syringes (500 µL, 100 µL, 10 µL).

6.6 Disposable Pasteur Pipettes (23 cm x 5 mm i.d.).

6.7 Volumetric flasks, "low-actinic" (100 mL).

6.8 Separatory funnels with Teflon stop-cocks (1000 mL).

6.9 Coarse (70-100 μm) sintered-glass filter funnels (100 mm x 40 mm i.d.) with a tapered ground-glass joint and suction side-arm, available from Pegasus Industrial Specialties Ltd.

6.10 Round-bottom flasks (500 mL).

6.11 Graduated centrifuge tubes (15 mL) with ground-glass stoppers.

NOTE: All glassware must be thoroughly washed with a hot solution of laboratory detergent followed by rinses with hot tap water, 2-3 rinses of distilled water and a final acetone rinse to remove the water. The glassware (except for volumetric pipettes and syringes) should be dried at 130°C for at least two hours. Thorough rinsing with organic solvent immediately prior to using the glassware is recommended.

7. REAGENTS

7.1 All solvents must be Distilled-in-Glass, Pesticide Residue grade and must be checked before use for low blank values.

7.1.1 Petroleum Ether (30°-60°C).

7.1.2 Iso-octane

7.1.3 Toluene

7.1.4 Acetone

7.1.5 Methanol

7.2 All chemicals must be of highest purity and should be washed with solvent and preheated where necessary.

7.2.1 Purified (organic-free) water. Pass distilled water through Milli-Q Water System (Millipore Corp.). Alternatively, extract 1L distilled water three times by stirring with 50 mL dichloromethane for 30 minutes. Discard organic layers.

7.2.2 Anhydrous Potassium Bicarbonate. Heat $KHCO_3$ overnight at 130°C.

7.2.3 Acetic Anhydride. Distill AnalaR grade $(CH_3CO)_2O$ reagent, available from BDH Chemicals, three times and collect the 138°-140°C fraction for acetylation reactions.

CAUTION: Acetic Anhydride is corrosive and moisture-sensitive.

7.2.4 Sodium sulphate (anhydrous, Reagent grade), available from BDH Chemicals. Heat 18 hours at 650°C and store in a clean glass bottle in a dessicator.

7.3 Analytical Standards. Obtain analytical grade Dinoseb (97+% purity from the manufacturer or U.S. Environmental Protection Agency and use without further purification.

7.3.1 Prepare 1000 ppm Stock Solution by dissolving 100 mg of Dinoseb in toluene and diluting to 100.0 mL in a "low-actinic" volumetric flask. Store at 4°C in the dark.

7.3.2 Prepare a Dinoseb Spiking Solution by diluting an appropriate aliquot of the Stock Solution with acetone. Use this Spiking Solution to fortify 1-L purified (organic free) water samples for the preparation of GLC calibration standards as outlined in Procedure Step 8.13.

8. PROCEDURE

8.1 Stir a 1-L water sample in a 1.14 L glass bottle or other suitable container, on a magnetic stirrer using a Teflon-coated stirring bar so that the vortex formed at the surface almost reaches the bottom of the bottle. Add 5 g potassium bicarbonate and stir until completely dissolved.

8.2 Carefully add 20 mL acetic anhydride and 50 mL petroleum ether. Tightly cover the bottle with an aluminum-lined cap. After stirring for 5 minutes, transfer the contents of the bottle to a 1-L separatory funnel and allow the layers to separate.

8.3 Return the aqueous (lower) layer to the original sample bottle.

8.4 Drain the organic layer through a (vacuum) sintered-glass filter funnel containing 50 mm of anhydrous sodium sulphate. Collect the dried extract in a clean 500-mL round-bottom flask.

8.5 Rinse the 1-L separatory funnel with two 25-mL aliquots of petroleum ether and transfer to the original sample bottle containing the aqueous layer. After stirring for 5 min, again transfer the contents of the bottle to the 1-L separatory funnel.

8.6 Repeat the layer separation in Steps 8.3 and 8.4.

8.7 Repeat Steps 8.5 and 8.6 with a third aliquot of 50 mL petroleum ether. Discard the water sample after this final extractive acetylation.

8.8 Rinse the 1-L separatory funnel twice with 10 mL petroleum ether and drain the rinsings through the sodium sulphate column into the 500-mL round-bottom flask.

8.9 Wash the sodium sulphate column with another 25 mL petroleum ether and apply a vacuum until the sodium sulphate is dry. Remove the column and add 1 mL iso-octane to the extract as keeper.

8.10 Evaporate the petroleum ether to 1 mL on a rotary evaporator (water bath temperature at 35°C).

8.11 Transfer the concentrated extract quantitatively to a 15-mL graduated centrifuge tube, using toluene rinses of the 500-mL round-bouttom flask to make the extract up to the required final volume.

8.12 Analyze the sample extracts by means of electron capture gas chromatography.

8.13 For calibration standards, acetylate a known amount of Dinoseb by fortifying a 1-L sample of purified (organic-free) water with 100 µL of an appropriate Spiking Solution in acetone. Add 5 g potassium bicarbonate and acetylate/extract as outlined above for the natural water samples.

9. CALCULATIONS

9.1 The concentration of dinoseb is determined by comparison of peak height or area of the samples with those of the standards. This can be done by using the following equation:

$$X_{sam} = \left(\frac{H_{sam}}{H_{std}}\right) x \left(\frac{V_{inj\ std}}{V_{inj\ sam}}\right) x \left(X_{std}\right) x \left(\frac{V_{ext}}{V_{sam}}\right)$$

where X_{sam} = dinoseb concentration in original water sample (µg/L);

H_{sam} = peak height (or area) of sample;

H_{std} = peak height (or area) of standard;

$V_{inj\ std}$ = volume of standard injected (µL);

$V_{inj\ sam}$ = volume of sample injected (µL);

X_{std} = dinoseb concentration in standard solution (pg/µL);

V_{ext} = final volume of sample extract (mL); and

V_{sam} = volume of original water sample extracted (mL).

9.2 Alternatively, dinoseb concentration may be determined by acetylation of, and comparison to, an internal standard such as pentachlorophenol.

10. PRECISION AND ACCURACY

10.1 Validation studies on both distilled and natural water samples provided better than 80% recovery at levels of fortification from 0.1 µg/L to 10 µg/L for a 1-L water sample.

11. CONFIRMATION OF IDENTITY

11.1 The identity of the dinoseb acetate peak in the chromatogram may be assigned by comparison with the retention time of the peak obtained when a concentrated dinoseb solution has been reacted under identical experimental conditions.

11.2 Additional confirmation of identity may also be obtained by the formation and analysis of different derivatives.

12. REMARKS

12.1 It is recommended that a Dinoseb acetate standard be prepared alongside each set of water samples to account for any minor variations in the extractive acetylation procedure from one set to another.

12.2 Special attention should be taken in the handling of acetic anhydride because it is corrosive and moisture-sensitive.

12.3 For very dirty water samples, the organic extracts may be cleaned up on a mini 5% deactivated Silica gel column using toluene/methanol (97+3) as eluant. A base-partitioning step

with 2% potassium bicarbonate can also be added to the procedure.

12.4 This method has been shown to be applicable to phenol and 22 chlorinated phenols. It is not, however, applicable to other nitro- and dinitrophenols.

12.5 To reduce tailing of the dinoseb acetate peak on the OV-1 FSCC, it is necessary, after about 100 injections, to replace the splitless insert and to cut off approximately 10-20 cm of the injection port end of the column.

13. REFERENCES

13.1 Lee, Hing-Biu, Robert L. Hong-You and Alfred S.Y. Chau. 1985. Analysis of Phenols by Chemical Derivatization. IV. Rapid and Sensitive Method for Analysis of 21 Chlorophenols by Improved Chloroacetylation Procedure. J. Assoc. Off. Anal. Chem. 68[3]:422-426.

13.2 Lee, Hing-Biu, Li-Da Weng and Alfred S.Y. Chau. 1984. Chemical Derivatization Analysis of Pesticide Residues. VIII. Analysis of 15 Chlorophenols in Natural Water by *in situ* Acetylation. J. Assoc. Off. Anal. Chem. 67[4]: 789-794.

13.3 Mathew, John and Alan W. Elzerman. 1981. Gas-Liquid Chromotographic Determination of Some Chloro- and Nitrophenols by Direct Acetylation in Aqueous Solution. Anal. Letters 14(A16):1351-1361.

13.4 Stokker, Yvonne D. Method for the Analysis of Nitrophenols in Natural Waters. (Unpublished results).