DETERMINATION OF 2,4-DB, 2,4-D AND 2,4-DICHLOROPHENOL IN SOIL

1. Introduction

The method of analysis for 2,4-DB, 2,4-D and 2,4-Dichlorophenol has been condensed from EPA Method 8150 for Chlorinated Herbicides and EPA Method 8040 for Phenols and combined to simplify the analysis. Both methods are found in the EPA Test Methods for Evaluating Solid Waste (SW-846) and are approved by the Office of Solid Waste.

2. Summary

Fifty grams of soil is acidified and extracted with 20:80 Acetone/Diethyl ether. After filtration, the extract is partitioned with additional Diethyl ether and split into two fractions. Fraction 1 is concentrated and analyzed by GC/ECD for 2,4-Dichlorophenol. Fraction 2 is hydrolyzed with KOH. The base extract is washed with Diethyl ether, acidified and partitioned into Diethyl ether. After concentration Fraction 2 is derivatized with diazomethane for analysis of 2,4-D and 2,4-DB, by GC/ECD.

3. Reagents

Organic-free water
11N sulfuric acid
12N potassium hydroxide
Acetone, residue grade
Diethyl ether, residue grade
Sodium sulfate, anhydrous
Diazomethane from diazald, ACS grade

4. Apparatus

Aldrich diazald kit
Filter flasks, 500ml
Beakers, 400ml
Graduated cylinders, 250ml, 500ml, 1000ml
Separatory funnel, 250ml, 500ml
Graduated centrifuge tubes, 15ml
Round bottom flask, 250ml, 500ml
Centrifuge bottle, 250ml
Buchner funnel, 110ml
Filter paper, Automatic Drip Coffee Machine type
Vials, 2ml screw cap
Vials, 20ml teflon lined screw cap
Centrifuge
Rotary evaporator
N, vap, Nitrogen evaporator

4. Apparatus (continued)

Mechanical shaker
Vacuum pump
Drying oven

5. Instrumentation

Gas chromatograph, Shimadzu GC-14A
Data processor, Shimadzu C-R4A
Detector, Electron Capture (ECD)
Column, J&W DB5 15m 0.53mm ID Film thickness 1.5 um
Balance, accurate to 0.0001g
Balance, accurate to 0.1g
Syringes 10ul/100ul/500ul

6. Standard Solutions

Stock:	Combine 10 mg 2,4-DB, 10mg 2,4-D and 10mg 2,4-Dichlorophenol in 100ml Diethyl ether. (100ng/ul)
Fortification:	Use stock.
Calibration:	Dilute stock 1:10 and derivatize with Diazomethane for 2,4-D and 2,4 DB only, (prepared according to Diazald Method, Aldrich) (10ng/ul). Make serial dilution of 1600ng/ml, 800ng/ml, 400ng/ml, 200ng/ml and 100 ng/ml for calibration curve.

7. Extraction

Acid rinse and silanize all glassware.

Weight 50g of well mixed soil into a 250ml centrifuge bottle. Add 30ml reagent water to moisten. While stirring, adjust pH to <2 with concentrated H_2SO_4. Add 100ml 20:80 Acetone:Diethyl ether and shake for 20 minutes. Decant organic phase into filter flask through a Buchner funnel lined with a pre-rinsed automatic drip coffee filter. Adjust pH of aqueous phase to <2 with conc. H_2SO_4 and repeat extraction two times with 50ml 20:80 Acetone:Diethyl ether. Combine all three extracts into a 500ml separatory funnel. Add 100ml reagent water and adjust pH to <2 with conc. H_2SO_4.

Gently shake separatory funnel for one minute and allow layers to separate. Collect aqueous phase (bottom layer) in a clean 400ml beaker. Transfer the organic phase into a 250ml graduated cylinder. Check pH and repeat the partition two times with 25ml Diethyl ether. Combine all three extracts into the 250ml graduated cylinder. Adjust volume to exactly 200ml and split extract 50:50 into two 500ml round bottoms,

7. Extraction (continued)

labeled Fraction 1 and Fraction 2. Concentrate Fraction 1 by roto-evaporation at 30°C to <5ml. Transfer Fraction 1 to a 15ml graduated centrifuge tube, rinsing the 500ml round bottom two times with 2ml Diethyl ether. Continue concentration under a gentle flow of purified nitrogen and adjust final volume to exactly 5.0ml. Fraction 1 is ready for analysis by GC/ECD.

8. Hydrolysis

Add 35ml of 5% KOH to the 500ml round bottom labeled Fraction 2 and rotovap at 35°C until ether is evaporated off. Place flask in water bath at 90°C for one hour. Remove flask and allow to cool. Transfer the basic solution to a 500ml separatory funnel and wash with 40ml Diethyl either three times. Discard the ether layers. The phenoxy acid herbicides remain soluble in the aqueous phase as potassium salts.

9. Solvent Cleanup

Adjust the pH to <2 with cold sulfuric acid. Be sure to check pH. Extract with 50ml diethyl ether three times. Combine extracts and dry by passing them through a funnel containing 5g 5% acidified sodium sulfate into a 500ml round bottom flask. Discard the aqueous layer. (Extract can be stored overnight under refrigeration).

Concentrate Fraction 2 to approximately 2ml by roto-evaporation at 35°C. Do not let extract go dry! Transfer Fraction 2 to a 15ml graduated centrifuge tube rinsing the flask two times with 2ml Diethyl ether. Continue concentration under a gentle flow of nitrogen to approximately 1ml.

10. Esterification

Add 0.1ml of diazomethane, prepared by the Diazald Kit Method, and let stand for 10 minutes. Wash wall of vial with 2ml of dimethyl ether and adjust to a final volume of exactly 5.0ml for analysis by GC/ECD.

11.1 Gas Chromatographic Ana. - For 2,4-DB

Column: J&W DB-5
15m 0.53mm ID
1.5um film thickness
Detector: Electron capture
Conditions

11.1 Gas Chromatographic Analysis For 2,4-DB (continued)

Temperatures: Injection 250°C
Detector 300°C
Oven 160°C for 6 minutes to
200°C @ 20°C/min hold for 1 min.
Gas Flows: Carrier Helium 12ml/min
Make up Nitrogen 60ml/min

11.2 Gas Chromatographic Analysis For 2,4-D

Column: J&W DB-5
15m 0.53mm ID
1.5um film thickness
Detector: Electron capture
Conditions
Temperatures: Injection 250°C
Detector 300°C
Oven 140°C for 6 minutes to
200°C @ 20°C/min hold for 1 min.
Gas Flows: Carrier Helium 12ml/min
Make up Nitrogen 60ml/min

11.3 Gas Chromatographic Analysis For 2,4-Dichlorophenol

Column: J&W DB-5
15m 0.53mm ID
1.5um film thickness
Detector: Electron capture
Conditions
Temperatures: Injection 250°C
Detector 300°C
Oven 80°C for 6 minutes to
200°C @ 25°C/min hold for 1 min.
Gas Flows: Carrier Helium 12ml/min
Make up Nitrogen 60ml/min

12. Quantitiation

The method of quantitation employs external standard linear regression analysis. Nanograms injected are plotted against peak heights to obtain the linear regression curve. (ng found) is taken from the regression curve. ug/kg are calculated by the following equation:

ug/Kg = ng/g = ppb

$$ng/g = \frac{(ng\ found/ul\ injected)\ \ (5,000ul)}{50g}$$