

SOP# METH-65

Revision #3 Date 08/93

DETERMINATION OF SUBSTITUTED UREA HERBICIDES, LINURON, DIURON
AND THEIR METABOLITES IN SOIL AND AGRICULTURAL PRODUCTS

Reason for Revision: To incorporate higher distillation volume for collection
for some matrices as determined during MTO phase.

INTRODUCTION:

This SOP was written in conjunction with the use of a new distillation still (see figure 1)
developed by DuPont to improve recovery, precision and to reduce analysis time.

Method Reference:

1. Morse Laboratories, Inc. SOP# Meth-42, Rev. #3.
2. Morse Laboratories, Inc. SOP# Meth-61.
3. Morse Laboratories, Inc. SOP# Meth-25, Rev. #2.

Principle:

Linuron, Diuron and their metabolites are hydrolyzed by alkaline reflux to 3,4-
dichloroaniline (3,4-DCA), followed by a distillation of the amine into an acid solution.
The acidic distillate is then made alkaline with concentrated base and subsequently extracted
into an organic solvent (in this case, hexane) and analyzed by gas chromatography.

Note: During all analyses, equivalent apparatus, solvents, glassware, or techniques (such as
sample concentration) may be substituted for those specified in the method.

Sample Preparation:

Samples are prepared for analysis according to specifications in the protocol.

Reagents:

Sodium hydroxide: 50% solution (VWR Scientific)

Deionized water

Antifoam: 30% silicone defoamer (DOW Corning)

BEST AVAILABLE COPY

SOP# METH-65, Page 2

Granular zinc: 20 mesh, "Baker Analyzed" (J.T. Baker)

Titanium Trichloride solution: 15% (Riedel-de Haën-14010): (Crescent Chemical
516/348-0333, catalogue no.
UN1760)

Concentrated hydrochloric acid: "Baker Analyzed" (J.T. Baker)

Hexane: "Baker Resi-Analyzed", nanograde (J.T. Baker)

Phenolphthalein: "Baker Analyzed" (J.T. Baker)

Acetone: "Baker Analyzed", reagent grade (J.T. Baker)

Sodium sulfate: Anhydrous, granular (Mallinckrodt)

Si Bond Elut: Test each box for suitability prior to use (500 mg. Bond Elut LRC,
Varian, Part #1211-3036)

Benzene: Omni Solv (EM Science)

Prepares:

1. Spiking Standards: Prepare stock standard solutions at 1000 $\mu\text{g/mL}$ or 100 $\mu\text{g/mL}$ in acetone, methanol or acetonitrile (depending upon protocol requirements). Prepare diluted standard solutions in acetone or acetonitrile.
2. 3,4 dichloroaniline GC standard: Prepare stock solution of 3,4-dichloroaniline at 1000 $\mu\text{g/mL}$ or 100 $\mu\text{g/mL}$ in methanol or acetonitrile (depending upon protocol requirements). A stock standard solution at 100 $\mu\text{g/mL}$ may be prepared in benzene. Prepare appropriate GC standard solutions by diluting the stock standard in hexane or benzene depending on the solvent used in the final sample extract for instrumentation.
3. 40% sodium hydroxide solution: 50 mL deionized water plus 200 mL 50% NaOH (sodium hydroxide).
4. Dilute hydrochloric acid solution: 10 mL concentrated hydrochloric acid plus 20 mL of deionized water.
5. Phenolphthalein indicator solution: 1.0 g in 200 mL of 95% ethyl alcohol.

BEST AVAILABLE COPY

APPARATUS:

One liter reaction flasks with 29/42 ground-glass fittings

Suitable size Teflon sleeves (for reflux condenser 29/42 ground glass joint)

Heating mantles - one liter capacity

Variable transformers for heating mantles.

Cork rings - 500 mL size

Glass beads (Glassballs #300, VWR catalogue #26396-596)

Erlenmeyer flasks - 300 mL, graduated, with 29/40 ground fittings (receiving flasks)

Filtering funnels - top I.d. 3.0 inches

Filter paper - Whatman #4, 15.0 cm diameter

Separatory funnels - Kimax, 250 mL and 500 mL sizes

DuPont Distillation Still (see figure 1)

Lab-Line orbit shaker

Zymark TurboVap evaporator

Zymark tubes

Reacti-Vap - Pierce Model 18780

Assorted graduated cylinders, pipets, flasks and beakers

Vac Elut SPS 24 (Varian #1223-4022) attached to a suitable pump and waste collection vessel

VWR Scientific heat block

BEST AVAILABLE COPY

Procedure:

Hydrolysis -

1. Prepare 30 mL dilute hydrochloric acid (10 mL concentrated HCl + 20 mL DI water) and place in a 300 mL graduated distillation receiving flask; set aside.
2. Weigh the sample into a 1 liter reaction flask (no flaws or cracks) with 29/42 ground-glass fitting. Fortify at this point.

Note: All reaction flasks must be checked for cracks, flaws and thinning of glass prior to use. Flawed glassware may cause breakage due to rupture.

The following are recommended sample weights for analysis:

Soils and wet crops: 25.0 grams

Oily, difficult, or dry bulky matrices: maximum of 10.0 grams

For dry matrices (except soil), hydrate the sample with a sufficient amount of DI water to completely wet the sample after fortification. Add a consistent amount of DI water for each type of matrix and record the amount of water added on the worksheet.

Note: The amount of water needed to wet the sample prior to hydrolysis may vary from matrix to matrix and should be determined at the method tryout stage.

3. Add glass beads, if necessary, to prevent bumping during reflux and distillation, or to aid in even mixing of the sample during the hydration step. Add 250 mL of 40% chilled sodium hydroxide. Swirl to mix.
For oily and difficult matrices, add 75 mL hexane to the reaction flask.
4. Add 10 mL concentrated antifoam and swirl to mix. For matrices that tend to foam excessively (such as hay, straw or stover), add 20 mL antifoam (instead of 10 mL).
5. Add 2 grams granular zinc (20 mesh) and 5 mL titanium trichloride. Immediately attach the reaction flask to the reflux condenser of the distillation still. Close the stopcock on the reflux-distillation channel. Attach the corresponding receiving flask (prepared in step 1) to the distillation condenser.

SOP# METH-65, Page 5

Note: Insure proper circulation of "ice cold" water throughout the condensers from the start of the reaction to the completion of the distillation step. This is accomplished by using dry ice (or wet ice) in the water bath.

6. For reaction mixture without hexane, set the heat transformer settings to the highest setting until the reaction mixture comes to a boil. At this point, reduce the transformer setting until the reflux point (point at which the vapor condenses to a liquid) is at the top of the first ball of the condenser (relative to the reaction vessel).

For reaction mixture containing hexane, set the heat transformer setting for each individual flask at a low setting (generally between 40 to 50 percent of the maximum setting) to effect a gentle hexane boil. The reflux point for hexane (point at which the vapor condenses to a liquid) should be maintained at the first ball of the condenser (relative to the reaction vessel).

Note: Each sample must be treated individually with reference to transformer heat settings in order to maintain equivalent reflux points for all samples in the analytical set. The reflux point, not the power setting on the transformer, is the critical aspect to be maintained here.

All samples must be refluxed for two hours starting at the time the reflux points have been set for each sample. In order to maintain a consistent reflux point, it is very important to keep "ice cold" water circulating throughout the condenser at all times.

7. At the end of the two hour reflux, open the stopcock on the reflux-distillation channel and start distillation as follows:

For reaction mixture without hexane, increase the transformer setting (85 to 90% of the maximum setting) to produce an efficient distillation.

For reaction mixture containing hexane, start distillation at the same transformer setting used for refluxing until most of the hexane has distilled off, then change the heat transformer to a high setting to produce an efficient distillation of the aqueous distillate.

In both cases, distill 120 mL aqueous distillate. The total volume of the aqueous solution in the receiving flask should be approximately 150 mL: 120 mL aqueous distillate + 30 mL acid solution. Rinse both condensers carefully with 25 mL hexane and add rinsings to the distillate in the receiving flask.

Note: For some difficult matrices (as orange dried pulp) improved, consistent recoveries may be obtained when 160 mL of aqueous distillate is collected. Determine distillation volume (either 120 or 160 mL) for collection per matrix at the Method Tryout (MTO) phase.

BEST AVAILABLE COPY

SOP# METH-65, Page 6

8. Chill the distillate before extracting using a cold water bath. This is a stopping point in the procedure and the distillate may be kept overnight or longer.

Note: All condensers (reflux and distillation) must be rinsed with acetone and deionized water prior to use. Acetone removes the antifoam residue and the water removes the acetone. Distillation heads and condenser delivery tubes should be washed the same day as the analysis takes place. See ML SOP No. Pest-82, 2-93 for cleaning and maintenance of the distillation still.

Generation of Basic Waste Requiring Neutralization -

1. Place carboy jug (1 gal. size Nalgene square-sided, wide-mouth plastic bottles labeled with appropriate caustic hazardous waste labels) under a fume hood.
2. Add 500 mL tap water to each one-gallon nalgene square bottle. Use one bottle for each sample to be neutralized.
3. Add 100 mL tap water to the cooled concentrated basic waste (which is approximately 100 mL of 100% NaOH) in the reaction flask. Pour the resulting basic mixture through a plastic strainer (to trap zinc granules) into the nalgene container while stirring or swirling. Rinse flask four times with additional 100 mL portions of tap water. This produces a waste solution of approximately 1100 mL of 9% NaOH.
4. Cap collection bottle and swirl to mix.
5. Transfer zinc granules to an appropriate container for appropriate discard or recycling.
6. Waste is ready for neutralization. See SOP# GL-67 for neutralization procedure.

Extraction of 3,4-Dichloroaniline

1. Transfer the cooled distillate into a suitable separatory funnel. Check pH; it should be at pH 1-2 at this point. Rinse the receiving flask containing the distillate with 25 mL n-hexane and add the rinsing to the separatory funnel. Shake for two minutes using a Lab-Line Orbit shaker adjusted to a suitable manufacturer's dial setting (generally 200-250 RPM) to effect proper mixing. Allow layers to separate and drain lower layer into the original receiving flask. Discard upper hexane layer.

BES

SOP# METH-65, Page 7

2. Add two drops of phenolphthalein indicator solution to the extract. Add 50% sodium hydroxide, dropwise (swirling occasionally), until the extract remains pink, then add two more drops. Chill basic aqueous extract in a cold water bath. This may take approximately 10-20 minutes. Return chilled extract to the separatory funnel.
3. Extract two times with 50 mL of nano-hexane, using a Lab-Line orbit shaker, shaking for two minutes per extraction. Drain lower layer to the original Erlenmeyer flask and the upper hexane layer into a clean flask or beaker. After the second extraction, pass combined extracts through approximately 50 g of sodium sulfate (to remove any water that is present) into a Zymark tube. Rinse the sodium sulfate with 20 mL nano-hexane.
4. Evaporate extract to approximately 3 mL using a Zymark Turbo-Vap evaporator at 34 °C. During evaporation, gradually adjust the nitrogen pressure from a minimum level (when the sample volume is relatively high) to a maximum of 10 psi.

Note: Use of the Zymark evaporator eliminates the need to use a "keeper" solution.

5. For soil samples that generally do not require further cleanup, transfer the concentrated extract to a test tube calibrated to the desired final volume. Rinse the Zymark tube with a minimum of 0.5 mL hexane and transfer rinsings to the sample test tube. Adjust the final volume with hexane. Use nitrogen and a heating block set at no more than 34 °C if additional adjustment of the final volume is necessary. Submit for GC analysis.

Bond Elut Column Cleanup

1. From step 4 of "3,4-DCA Extraction" section, transfer the concentrated extract (at approximately 3 mL) from the Zymark tube to a labeled test tube marked at approximately 5 mL. Use hexane for rinsings and transfers so that the final volume of the extract is approximately 5 mL. Mix gently but thoroughly.
2. Place empty labeled sample test tube (calibrated to a final volume designated for the sample) into the collection rack of the Vac Elut apparatus. Adjust apparatus to the "waste" position.
3. Attach Bond Elut column to designated Vac Elut position for column cleanup. Prewash column with 5 mL of hexane using gentle pressure to aid in elution. From this point on, insure that the column packing does not go dry. Discard this wash.

BEST AVAILABLE COPY

SOP# METH-65, Page 8

4. Load sample extract at 5 mL onto the Bond Elut column and allow extract to elute to just above column packing with the aid of gentle pressure. Discard this elution.
5. Wash the column with 10 mL of hexane with the aid of gentle pressure. Elute to just above column packing. Discard this wash.
6. Adjust the Vac Elut apparatus to the "collect" position and elute 3,4-DCA with 5 mL of benzene (without the aid of the pressure pump) into a designated test mbe.
7. Evaporate eluate using the Reacti-Vap at 35 °C to the desired final volume for GC analysis.

Caution: 3,4-DCA extracts cannot be evaporated to less than 1 mL without significant losses or erratic results.

Gas Chromatographic Analysis

3,4-dichloroaniline is chromatographed intact (underivatized) using halogen or nitrogen specific detection.

Note: Historically, the column and conditions stated below are satisfactory for the matrix being analyzed. The specific column packing, column temperature, carrier gas, and flow rate listed are typical conditions for this analysis. Specific conditions used will be noted on each chromatographic run and will not otherwise be documented.

Gas Chromatograph: MicroTek MT-220 (or equivalent) equipped with a Coulson electrolytic conductivity detector in the halogen mode. (The nitrogen mode can be used if interferences pose a problem in the halogen mode.)

Gas Chromatographic
Columns and Conditions:

Primary - 4' x 1/4" o.d. x 5/32" i.d. glass column packed with 10% SP-2100 on 80/100 Supelcoport. (Supelco Catalogue #1-2140, Tel. #800/247-6628)

Temperatures: Column - 144 °C
Injector - 240 °C

Carrier Gas
& Flow Rate: Hydrogen at 80 mL/min

888

SOP# METH-65, Page 9

Alternate - 3 1/2' x 1/4" o.d. x 5/32" i.d. glass column packed with 20% OV-11 on 100/120 mesh Chromosorb WHP. (Custom made by Supelco)

Temperatures: Column - 185 °C
 Injector - 240 °C

Carrier Gas
& Flow Rate: Hydrogen at 80 mL/min

Prepare a four-point standard curve by injecting constant volumes of 3,4-DCA at the following suggested concentrations: 0.045 µg/mL, 0.2 µg/mL, 0.4 µg/mL and 0.8 µg/mL. Plot nanograms 3,4-DCA injected versus the peak height response (in mm). Inject a constant volume of sample extract into the instrument. The concentration of sample extract submitted for GC analysis is generally 10 g of sample per mL of sample extract. Sample responses not bracketed by the standard curve require dilution and reinjection. Inject a standard per every 4-5 sample injections.

Calculations:

Sample responses are compared to the 3,4-DCA standard curve that was generated. The amount of analyte present in the sample (in ppm) is calculated as follows:

$$\text{ppm analyte} = \frac{\text{ng 3,4-DCA} \times \text{MWCF}}{\text{mg sample injected}}$$

where:

1. ng 3,4-DCA is derived from standard curve.
2. mg sample injected =

$$\text{concentration of sample (g/mL)} \times \text{volume of sample injected (}\mu\text{L)}$$

BEST AVAILABLE COPY

SOP# METH-65, Page 10

3. MWCF is the molecular weight conversion factor for converting 3,4-DCA to the compound analyzed.

Conversion Factors: Diron	= 144	R915	= 127
Lisiron	= 153	Z513	= 145
15654	= 135		

The limit of quantitation for this procedure is typically 0.81 ppm. For difficult matrices where a five gram sample is analyzed, the limit of quantitation is typically 0.05 ppm.

SOP prepared by Frances Brooker

APPROVED BY: [Signature]
DATE: 9/23/93

3