Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pestcide Name: Simazine

MRID #: 406144-14

Matrix: Soil

Analysis: HPLC/UV

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APPENDIX 1

EN-CAS Method No. 87-1

Determination of Hydroxy-Simazine
in Soil Samples

EN-CAS METHOD NO. 87-1	AUTHOR(S) Wayne Barker	DATE ISSUED: 12/10/87
		REVISIONS
TITLE: Determination of Hydroxy-Simazine in Soil Samples	QA APPROVAL	
	MGMT APPROVAL	

1.0 Scope

This method is used for the determination of hydroxy-simazine residues in soil samples. This method is especially applicable to soils from which OH-simazine is difficult to extract using conventional soil extraction solvents (e.g. methanol-water or acetonitrile-water).

2.0 Principle

Hydroxy-simazine (OH-simazine) is extracted from soil by refluxing the soil in 80/20 methanol/concentrated hydrochloric acid. Prior to acid extraction, the soil is solvent washed to remove any parent simazine so as to preclude conversion of the parent to OH-simazine under the hot acid extraction conditions. The acidic extract is neutralized with ammonium hydroxide and concentrated by rotary evaporation until essentially aqueous remains. The aqueous concentrate is cleaned up using a C-18 Bond Elut cartridge. The cartridge eluent is analyzed by HPLC.

3.0 Detection Range

This method is effective to a screening level of 0.05 ppm in soil. Since recoveries generally fall in the 40-80% range, instrument sensitivity should be adjusted to allow detection of the analyte at 40% of the screening level.

4.0 Analysis Time

A sample set of twelve, including controls and fortifications, require approximately three man-days to complete.

5.0 Interferences

Appearance of interferences at the OH-simazine retention time varies among soil types and depths. When interferences are observed, a change in the liquid chromatography (L.C.) mobile phase can usually be used to separate the OH-simazine from the interfering components. In particular, increasing the 1-octane sulfonic acid content selectively moves the OH-simazine retention time outward without significantly affecting most other peaks.

6.0 Safety Precautions

Normal safety precautions, including the wearing of gloves and safety glasses, should be taken whenever handling organic solvents. When handling concentrated hydrochloric acid for acid reflux, additional precautions are advised, including the wearing of goggles or a face shield, or working behind a safety shield. Both the acid reflux and the neutralization should be performed in a fume hood.

7.0 Apparatus

7.1 Bottle, 500 ml, French square with Teflon-lined cap (or equivalent)

7.2 Buchner funnel, 9 cm

7.3 Sidearm flask, 500 ml

7.4 Shaker, reciprocating - New Brunswick scientific G10 gyrotory or equivalent

7.5 Filter paper, glass fiber, 12.5 cm (Whatman 934-AH or equivalent)

7.6 Filter paper, glass fiber, 9.0 cm (Whatman GF/C or equivalent)

7.7 Erlenmeyer flask, 500 ml

7.8 Hot plates (Corning PC-100 or equivalent)

7.9 Condensers, reflux

7.10 Glass beads, hollow, 4 mm

7.11 pH meter (Beckman Zeromatic SS-3 or equivalent)

7.12 Rotary evaporator (Buchi or equivalent)

7.13 Boiling flask, round, flat bottomed, 1000 ml

7.14 Vac-Elut processing station (Analytichem Int. SPS 24)

7.15 Bond Eluts, C18, 3 cc (Analytichem Int. #607303)

7.16 Reservoir, 75ml capacity (Analytichem Int. # 607500)

7.17 Test tube, 15 ml, graduated

7.18 Graduated cylinder, 100 ml

7.19 L.C. pump (Waters model 510 or equivalent)

7.20 L.C. autoinjector (Waters WISP 710B or equivalent)

7.21 L.C. detector (Kratos Spectroflow 783 variable wavelength or equivalent)

7.22 L.C. column, Whatman Partisil 5, ODS-3, 25 cm

7.23 L.C. autosampler vials.

8.0 Reagents

8.1 Acetonitrile, HPLC grade

8.2 Acetonitrile, pesticide grade

8.3 Ammonium chloride, ACS grade

8.4 Ammonium hydroxide, ACS grade

8.5 Citric acid, monohydrate (granular) ACS grade

8.6 Ethyl acetate, pesticide grade

8.7 Hexane, pesticide grade

8.8 Hydrochloric acid, concentrated, ACS grade

8.9 Hyflo Super Cel (Fisher # H333)

8.10 Methanol, HPLC grade

8.11 Methanol, pesticide grade

8.12 Milli-Q (deionized) water

8.13 1-octane sulfonic acid, sodium salt, HPLC grade (Eastman Kodak)

9.0 Procedure

9.1 Preparation of Sample

9.1.1 Weigh ten grams of well-homogenized, air dried soil into a 500 ml French square bottle fitted with a Teflon-lined cap.

9.2 Extraction

9.2.1 Parent simazine may be converted to OH-simazine under acid conditions and therefore must be removed prior to the acid reflux step. Two alternate techniques have been devised to remove the parent simazine from the sample. One of these two procedures should be selected based on the extractability of OH-simazine from the specific soil type.

9.2.1.1 For soils from which OH-simazine is more readily extractable, add 250 ml of 75/25 ethyl acetate/hexane. Shake the mixture for two hours at 200 rpm on the gyrotory shaker. Vacuum filter through a 12.5 cm glass fiber filter contained in a 9 cm Buchner funnel. Rinse the filter with 50 ml of 75/25 ethyl acetate/hexane. Discard the filtrate, which should contain only parent (<2% of the OH-simazine present is extracted under these conditions). Using a tweezer, carefully transfer the filter containing the soil into a 500 ml Erlenmeyer flask. The filter will have to be rolled up to do this. The larger filter (12.5 cm) size facilitates transfer of the filter without loss of soil. Proceed to Step 9.2.2.

9.2.1.2 For soils from which OH-simazine is less readily extractable, add 150 ml of 80/20 acetonitrile/water to the soil sample and shake for one hour at 200 rpm on the gyrotory shaker. Filter as in 9.2.1.1 but use 50 ml of 80/20 acetonitrile/water to rinse the filter. Do not discard the filtrate and rinsate. They will be combined with the acid extract at a later step. Transfer the filter and soil to a 500 ml Erlenmeyer flask as described in 9.2.1.1.

9.2.2 Add 150 ml of 80/20 methanol/concentrated hydrochloric acid to the 500 ml Erlenmeyer flask containing the soil and the glass fiber filter and reflux for one hour. Add fifteen to twenty 4 mm hollow glass beads to facilitate refluxing. Allow samples to cool briefly, then while still warm, vacuum filter through a Whatman 9 cm GF/C glass fiber filter. Rinse the filter with ~50 ml methanol and combine the rinsate with the acidic extracts. Note: The filtration must be done while the reflux mixture is still warm. Some soils have been shown to reabsorb the analyte when the reflux mixture was allowed to stand and cool. This absorption increases with increasing standing time after cessation of heating.

9.3 Neutralization

9.3.1 Pour the acid extract into a 500 ml beaker. Using a suitable pH meter, adjust the pH to 6.5 - 7 with 25-30 ml of concentrated ammonium hydroxide, while stirring fairly vigorously with a magnetic stirrer. Precipitation should begin at a pH of 3 - 4. This should be completed by ~ pH6.

9.3.2 Remove the pH electrodes and add 15 grams of Hyflo Super Cel (Fisher # H333) to the beaker while continuing stirring. After approximately two minutes of stirring, filter through a Whatman 9 cm GF/C glass fiber filter using a 9 cm Buchner funnel and a 500 ml sidearm flask. As the filtration proceeds, a filter cake will form, and filtration will cease shortly thereafter. Gently break up the filter cake with a spatula. Rinse the sample beaker with ~50 ml of 80/20 acetonitrile/water and pour onto the filter cake to re-suspend the solids. Combine the rinsate with the first filtrate.

9.4 Sample Concentration

If Step 9.2.1.2 (for difficult-to-extract soils) was followed, transfer the acetonitrile/water filtrate from that step and the neutralized filtrate from Step 9.3.2 to a 1000 ml round bottom flask. Rotary evaporate the combined solution to less than 50 ml at which point only aqueous should remain. Bring the volume up to 50 ml with water, in a 100 ml graduated cylinder. If necessary, saturate the aqueous solution with 5 grams of ammonium chloride, (if a precipitate is already present, this step is unnecessary). If Step 9.2.1.1 was followed, concentrate the neutralized filtrate directly to less than 50 ml on a rotary evaporator. Bring the volume back to 50 ml with water and proceed with Step 9.5.

9.5 Sample Cleanup

Place C-18 Bond Eluts (Analytichem Int., 3 cc, # 607303) on a vacuum box or equivalent apparatus. Prime the Bond Eluts by eluting with 1 volume (~5 ml) of methanol followed by 1 volume (~5 ml) of Milli-Q water under vacuum (the flow rate should be approximately 4 ml/min). Do not allow the Bond Eluts to dry. Attach a 75 ml capacity reservoir tube (Analytichem Int. # 607500) tube to the Bond Elut. Pour the sample into the reservoir atop the Bond Elut. Apply vacuum. The 50 ml volume should elute in 10-15 minutes. After the 50 ml has completely passed through the Bond Elut, elute with 9 ml of 25/75 methanol/water (first rinsing the sample container). This eluent should contain the OH-Simazine. Adjust volume to 10 ml with 25/75 methanol/water.

Samples are now ready for liquid chromatography.

10.0 Liquid Chromatography Conditions

10.1 Detector: Kratos Spectroflow 783 variable wavelength detector, set at 240 nm.

Pump: Waters model 510, set at 1.0 ml/min.

Injector: Waters WISP 710B

Column: Whatman Partisil 5, ODS-3 25 cm (Whatman Cat. # 4238 001)

Mobile Phase: 27/73 acetonitrile/water with 10 grams/liter of 1-octane sulfonic acid and 2.2 grams/liter citric acid as a buffer

Attenutation: 0.05 AUFs (or attentuation sufficient to detect 0.015 ug/ml OH-simazine when 60 ul of sample injected)

Retention Time: Approximately 18.5 minutes

11.0 Calibration

Known quantities of OH-simazine are injected throughout the L.C. run at selected intervals. A linear curve is constructed using peak height in millimeters vs nanograms injected. The sample nanograms are determined by inserting the sample peak height values into the standard curve linear regression equation. The nanograms of standard injected range from 0.75 to 60.0.

12.0 Preparation of Standards

12.1 Weigh 0.025 grams of hydroxy-simazine and place in a 250 ml volumetric flask. Add approximately 150 ml of 0.1N hydrochloric acid and dissolve with the aid of an ultrasonic bath. Bring up to the mark with 0.1N hydrochloric acid. This yields a solution of 100 ug/ml.

Make serial dilutions from the above stock solution with 25/75 methanol/water. Useful standard range is from 0.015 ug/ml to 1.0 ug/ml hydroxy-simazine.

13.0 Calculations

13.1 The ppm value for each sample is determined by:

ppm = ng found (from std curve)/milligrams of soil equivalents injected

Where milligrams of soil equivalents injected

$$\frac{\text{mg sample wt. taken through method x ul inj.}}{\text{ul final volume x dilution factor}}$$

The ppm value is then corrected for the method recovery of the fortified samples, if the recovery is less than 100%. No correction is made for recoveries above 100%.

$$\text{corrected ppm} = \frac{\text{ppm found}}{\text{average \% recovery (as a decimal)}}$$

13.2 Moisture Calculations

Moisture values are determined by weighing out 10 grams of soil in duplicate. This is then dried overnight in an oven at 110 C.

% moisture in a sample

$$\frac{\text{wet soil wt - dry soil wt}}{\text{wet soil weight}} \times 100 = \% \text{ water in sample}$$

Since the samples are air dried prior to analysis, the necessary conversion is from dry weight to wet weight basis.

ppm wet = ppm dry X (1- decimal % moisture)

If the samples were not air dried, dry weight ppm can be determined by:

$$\text{ppm dry} = \frac{\text{ppm wet}}{(1-\text{decimal \% moisture})}$$

13.3 Conversions to parent-simazine ppm equivalent

$$\text{molecular weight conversion factor} = \frac{\text{MW parent simazine}}{\text{MW OH-simazine}}$$

$$= 201.67/183.2 = 1.10$$

ppm OH-simazine X 1.1 = ppm as parent simazine

14.0 Results

This method has been validated on soil samples from two widely separated areas, at 3 different depths. See Table 1 for percent recovery.

TABLE I

Hydroxy-Simazine Validation Data

Soil Depth and Location	Fortification Level	% Recovery	Average
0- 8" Oregon	0.05, 0.10, 1.0 ppm	63%, 46%, 74%	61%
0- 8" Oregon	0.05, 0.10, 1.0 ppm	63%, 69%, 78%	70%
8-16" Oregon	0.05, 0.10, 0.50 ppm	43%, 47%, 63%	51%
16-24" Oregon	0.05, 0.10, 0.50 ppm	64%, 54%, 59%	59%
16-24" Oregon	0.05, 0.10, 0.50 ppm	69%, 61%, 60%	63%
0- 8" Missouri	0.05, 0.10, 1.0 ppm	54%, 68%, 84%	69%
8-16" Missouri	0.05, 0.10, 0.5 ppm	63%, 67%, 65%	65%
16-24" Missouri	0.05, 0.10, 0.5 ppm	50%, 52%, 46%	49%

FLOW DIAGRAM FOR OH-SIMAZINE METHOD

MORE EXTRACTABLE SOILS

10 gram soil

↓

Shake out with 250 ml of 75/25 ETOAC/Hexane for 2 hours at 200 rpm to remove parent.

↓

Filter and discard filtrate

↓

Reflux soil (and filter) for 1 hour in 150 ml of 80/20 methanol/conc. HCl

↓

Filter (while still warm from reflux)

↓

Neutralize with NH4OH

↓

Add 15 grams Hyflo Super Cel

↓

Filter ----- Discard solids.

↓ (Filtrate)

Rotary evaporate to aqueous

↓

Pass through C-18 Bond Elut

↓

Elute with 9 ml of 25/75 methanol/water

↓

Liquid chromatograph

LESS EXTRACTABLE SOILS

10 gram soil

↓

Shake out with 150 ml of 80/20 CH3CN/H2O for 1 hour at 200 rpm

↓

Filter

↓

(Filtrate) (Soil and Filter)

Reflux soil (and filter) 1 hour in 150 ml of 80/20 methanol/conc. HCl

↓

Filter (while still warm from reflux

↓

Neutralize with NH4OH

↓

Add 15 grams of Hyflo Super Cel

↓

Filter ---- Discard solids

↓ (Filtrate)

Combine in 1000 ml flask and rotary evaporate to aqueous

↓

Pass through C18 Bond Elut

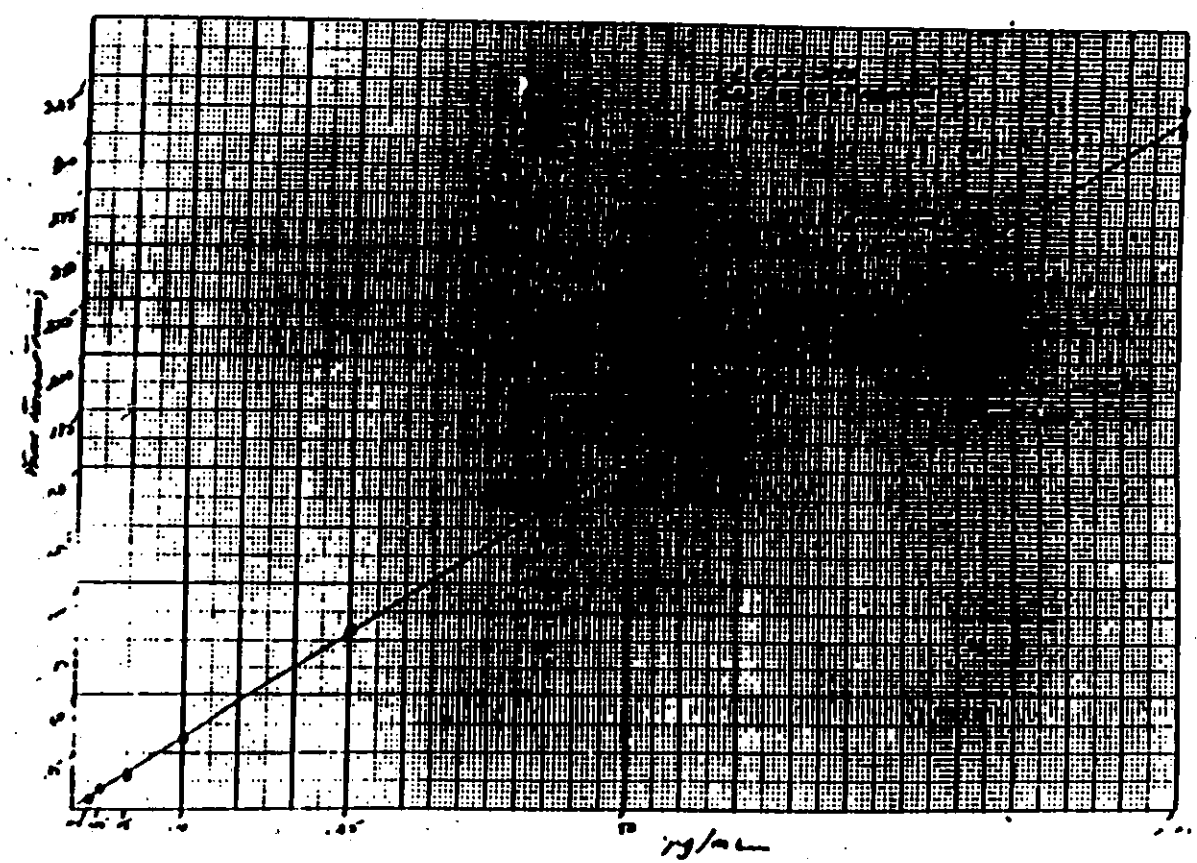
↓

Elute with 9 ml of 25/75 methanol/water

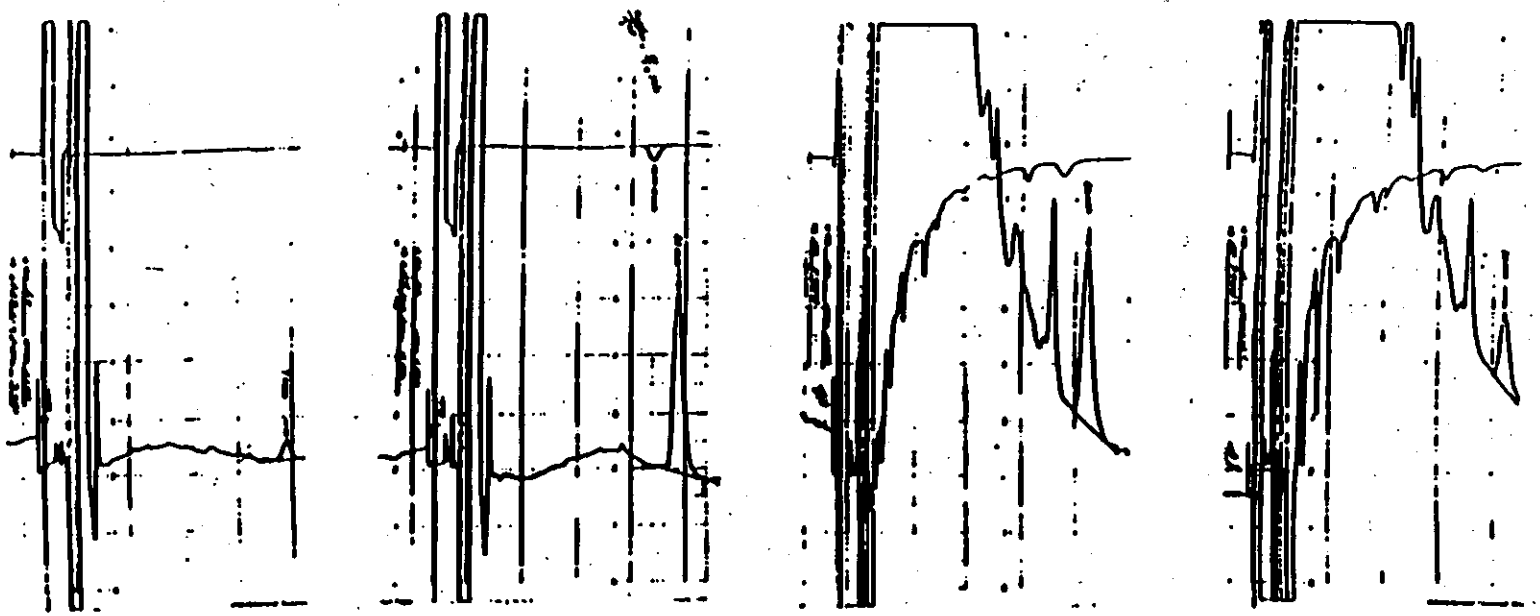
↓

Liquid chromatograph

TYPICAL CALIBRATION CURVE FOR HYDROXY-SIMAZINE
PEAK HEIGHT VS UG/ML



HYDROXY-SIMAZINE: TYPICAL CHROMATOGRAMS



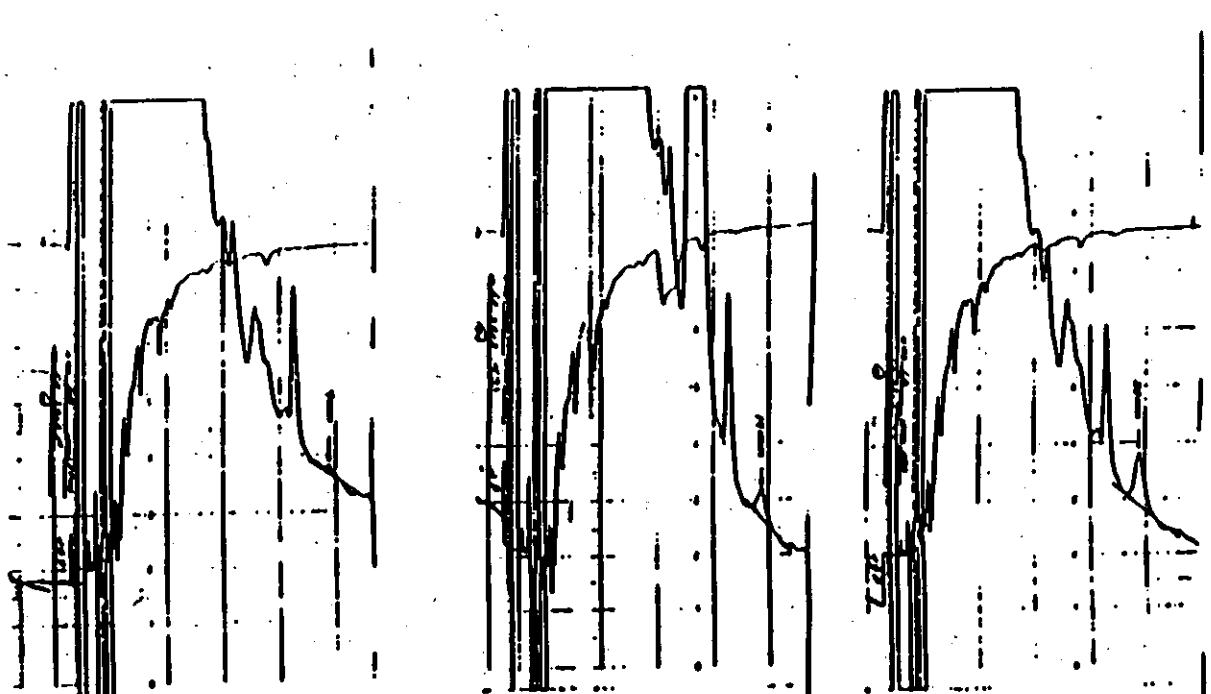
0.025 ug/ml (60 ul) Hydroxy-Simazine Standard

0.25 ug/ml (60 ul) Hydroxy-Simazine Standard

0-8" Oregon Soil 0.36 ppm Hydroxy-Simazine Residue

0-8" Oregon Soil 0.15 ug/ml Hydroxy-Simazine Residue

HYDROXY-SIMAZINE: TYPICAL CHROMATOGRAMS



EC7317C

0-8" Oregon Control Soil
<0.05 ppm Hydroxy-Simazine

EC7317 S1

0.05 ppm Fortification of 0-8" Oregon Control Soil. 63% Recovery

EC7317 S2

0.10 ppm Fortification of 0-8" Oregon Control Soil. 69% Recovery