



Determination Of Phenylurea Compounds In Drinking Water By Solid Phase Extraction And High Performance Liquid Chromatography With UV Detection

EPA Method 532 Revision 1.0

Part Number: **EUC18156** (6 mL cartridge with 500 mg C18)

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The Background

This is an HPLC method for the determination of phenylurea pesticides in drinking water. It is applicable to phenylurea compounds that are efficiently extracted from the water using a C18 solid phase cartridge. Accuracy, precision, and method detection limit (MDL) data have been generated for the following compounds* in reagent water and finished ground and surface waters:

Phenylurea Compounds

| Analyte | CAS # |
|---------------|------------|
| Diflubenzuron | 35367-38-5 |
| Diuron | 330-54-1 |
| Fluometuron | 2164-17-2 |
| Linuron | 330-55-2 |
| Propanil | 709-98-8 |
| Siduron | 1982-49-6 |
| Tebuthiuron | 34014-18-1 |
| Thidiazuron | 51707-55-2 |

Method Summary

A 500 mL water sample is extracted using an SPE cartridge containing a bonded C18 solid phase. The phenylurea pesticides and surrogate compounds are captured by the solid-phase. The analytes and surrogates are eluted from the solid phase with methanol, and the extract concentrated to a final volume of 1 mL. Analytes are then chromatographically separated by injecting an aliquot of the extract into an HPLC and detected using a UV/Vis detector. Identification of target and surrogate analytes and quantitation is accomplished by retention times and analyte responses using external standard procedures. Sample extracts with positive results are solvent exchanged and confirmed using a second, dissimilar HPLC column that is also calibrated using external standard procedures.

Safety

- Each chemical compound should be treated as a potential health hazard, and exposure to these chemicals should be minimized using proper handling technique

Sample Collection, Preservation and Storage

- Grab samples must be collected using 500 mL amber or clear glass bottles fitted with PTFE lined screw caps
- Prior to field shipment, sample bottles must contain, 0.25 g of cupric sulfate and 2.5 g of Trizma crystals to each bottle for each 500 mL of sample collected
- Alternately, the Tris buffer may be prepared by adding 2.35 g of Tris HCl and 0.15 g Tris to the sample bottle in addition to the 0.25 g of cupric sulfate
- When sampling from a cold water tap, remove the aerator so that no air bubbles will be trapped in the sample. Allow the system to flush until the water temperature has stabilized (usually about 3-5 minutes). Collect samples from the flowing system.
- When sampling from an open body of water, fill a 1 quart wide-mouth bottle or 1L beaker with sample. Carefully fill sample bottles from the container
- Sampling equipment, including automatic samplers, must be free of plastic tubing, gaskets, and other parts that may leach interfering analytes into the water sample
- Fill sample bottles, taking care not to over fill bottles so as not to flush out the sample preservation reagents. Samples do not need to be collected headspace free
- After collecting the sample, replace cover then agitate by hand for 1 minute. Keep samples sealed from collection time until extraction

Preservation Reagents

- Cupric Sulfate, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (ACS Grade or equivalent) – Added as a biocide to guard against potential degradation of method analytes by microorganisms
- Trizma Preset Crystals, pH 7.0 (Sigma # T 3503 or equivalent) – Reagent grade. A premixed blend of Tris [Tris(hydroxymethyl)aminomethane] and Tris HCL [Tris(hydroxymethyl)aminomethane hydrochloride]
- Alternatively, a mix of the two components with a weight ratio of 15.5/1; Tris HCL/Tris may be used. These blends produce a pH of 7.0 at 25°C in reagent water. Tris functions as a buffer, binding free chlorine in chlorinated finished waters and prevents the formation of a copper precipitate

Sample Shipment and Storage

- All samples should be iced during shipment and must not exceed 10° C during the first 48 hours after collection. Samples should be confirmed to be at or below 10°C when they are received at the laboratory
- Samples stored in the lab must be held at or below 6° C until extraction, but should not be frozen

Sample and Extract Holding Times

- Method analytes are stable for 14 days in water samples that are collected, dechlorinated, preserved, shipped and stored as described in above
- Samples must be extracted within 14 days
- Sample extracts may be stored in methanol at 0°C or less for up to 21 days after extraction. Samples that are exchanged into reagent water/acetonitrile (60/40) for confirmational analysis may be stored 7 days at 0° C or less. The combined extract holding time should not exceed 21 days

Interferences

- All glassware must be meticulously cleaned. Wash glassware with detergent and tap water, rinse with tap water. Follow by a reagent water rinse
- Non-volumetric glassware can be heated in a muffle furnace at 400° C for 2 hours.
- Method interferences may also be caused by contaminants in solvents, reagents (including reagent water), sample bottles and caps, and other sample processing hardware. This may lead to discrete artifacts or elevated baselines in the chromatograms
- All solvents and equipment must be routinely demonstrated to be free from interferences under the conditions of the analysis. **Subtracting blank values from sample results is not permitted in this method**
- Matrix interferences may be caused by contaminants that are co-extracted from the sample

Surrogate Analytes (SUR),

Monuron (CAS #150-68-5) & Carbazole (CAS# 86-74-8)

SUR STOCK SOLUTION (5 to 7 mg/mL)

- Accurately weigh 25 to 35 mg of the neat SUR to the nearest 0.1 mg into a tared, 5 mL volumetric flask. Dilute to the mark with the appropriate solvent, methanol for monuron and acetonitrile for cabazole
- Prepare each compound individually. They will be combined in the SUR primary dilution standard

SUR Primary Dilution Standard (500 µg/mL)

- Prepare the SUR Primary Dilution Standard (PDS) by dilution of the SUR stock standards. Add enough of each of the SUR stock standards to a volumetric flask partially filled with methanol to make a 500 µg/mL solution when filled to the mark with methanol

Analyte Stock Standard Solution

- Prepare analyte stock standard solutions for all compounds in methanol except thidiazuron and Diflubenzuron which are prepared in acetone
- Acetone elutes early in the chromatogram and will not interfere with compound quantitation
- Accurately weigh neat material to the nearest 0.1 mg into volumetric flasks, approximately 10 to 12 mg. Thidiazuron is difficult to dissolve but 10 mg of pure material should dissolve in a 10 mL of acetone. Sonication may be used to help dissolve these compounds.

Analyte Primary Dilution Standard (PDS, 200 µg/mL and 10 µg/mL)

- Prepare the Analyte PDS by dilution of the stock standards. Add enough of each stock standard to a volumetric flask partially filled with methanol to make a 200 µg/mL solution when filled to the mark with methanol
- Once prepared, a dilution of the 200 µg/mL solution may be used to prepare a 10 µg/mL solution used for low concentration spiking. The PDS's can be used for preparation of calibration and fortification solutions
- Use at least 5 calibration concentrations to prepare the initial calibration curve
- The lowest concentration of calibration standard must be at or below the MRL
- In this method, 500 mL of an aqueous sample is concentrated to a 1 mL final extract volume

The calibration standards for the development of this method are prepared as below

| Preparation of Calibration Curve Standards | | | | | |
|--|---------------------------|-----------------------------------|---------------------------|--|--|
| CAL Level | PDS Conc $\mu\text{g/mL}$ | Volume PDS Standard μL | Final Vol CAL Standard mL | Final Conc CAL Standard $\mu\text{g/mL}$ | Equivalent Conc in 500 mL sample $\mu\text{g/L}$ |
| 1 | 10 | 25 | 1 | 0.25 | 0.50 |
| 2 | 10 | 50 | 1 | 0.50 | 1.00 |
| 3 | 200 | 5.0 | 1 | 1.00 | 2.00 |
| 4 | 200 | 25 | 1 | 5.00 | 10.0 |
| 5 | 200 | 50 | 1 | 10.0 | 20.0 |
| 6 | 200 | 75 | 1 | 15.0 | 30.0 |

Analysis Procedure

1. Sample Extraction

- a. Assemble a glass block manifold. Automated extraction equipment may also be used
- b. Place a **EUC18156 C18** cartridge(s) on the manifold
- c. Rinse the cartridge(s) using 5 mL of methanol
- d. Slowly draw through leaving a layer covering the frit
- e. Repeat using a second 5 mL aliquot and slowly draw through. Leave enough methanol to cover the frit

Note: Do not let the cartridge dry out after addition of methanol otherwise repeat

- f. Add 5 mL of reagent water to the cartridge and draw through to the level of the frit
- g. Repeat with a second 5 mL aliquot of reagent water

2. Sample Extraction

- a. Add the water sample to the cartridge. Adjust vacuum setting for a flow rate of about 20 mL/minute
- b. Rinse the sample container with reagent water and add to the cartridge
- c. Once the sample has been extracted, turn vacuum setting to high and draw air through for 15 minutes to dry cartridge

3. Sample Elution

- a. Insert a clean collection tube in the vacuum manifold
- b. Add about 3-5 mL of methanol to each cartridge
- c. Draw through to the top of the frit then turn off vacuum
- d. Allow to soak for 30 seconds
- e. Add a second 2-3 mL of methanol and draw through cartridge as a drip
- f. Repeat methanol addition with a third 1-2 mL portion

4. Extract Concentration

- a. Concentrate the extract to about 0.5 mL in a warm water bath 4° C under a gentle stream of N₂
- b. Transfer to a 1 mL volumetric flask rinsing the collection tube with methanol
- c. Bring to volume with methanol
- d. Sample is ready for analysis

5. Solvent Exchange for Confirmation Analysis

- Samples to be confirmed must be exchanged into reagent water/acetonitrile (60/40)
- Transfer the remaining 980 uL of the extract to a 1 mL volumetric (or other appropriate collection tube)
- Mark the sample volume then take the extract to dryness in a warm water bath (at ~ 40° C) under a gentle stream of N₂
- Reconstitute the residue with a mixture of reagent water/acetonitrile (60/40) to the mark made before the extract was taken to dryness. Redissolve the film as thoroughly as possible. Use of a vortex mixer is recommended. Transfer to an appropriate autosampler vial
- The best recoveries are obtained when extracts are immediately reconstituted

6. HPLC Analysis—Primary Analysis

| HPLC Conditions | |
|---|------------------------|
| Solvent A | 25 mM phosphate buffer |
| Solvent B | Acetonitrile |
| 40% B | 0-9.5 minutes |
| Linear gradient 40-50% B | 9.5-10.0 minutes |
| Linear gradient 50-60% B | 10.0-14.0 minutes |
| Linear gradient 60-40% B | HPLC Conditions |
| Flow Rate | 1.5 mL/minute |
| Wavelength | 245 nm |
| Equilibration time prior to next injection = 15 minutes | |

Primary HPLC Column: Symmetry 4.6 x 150 mm with 3.5 μ m C18 phase or equivalent

Chromatographic Retention Time Data for the Primary Column

| Peak Number | Analyte | Retention Time (min) |
|-------------|-----------------|----------------------|
| 1 | Tebuthiuron | 2.03 |
| 2 | Thidiazuron | 2.48 |
| 3 | Monuron (SUR) | 2.80 |
| 4 | Fluometuron | 4.45 |
| 5 | Diuron | 5.17 |
| 6 | Propanil | 8.53 |
| 7 | Siduron A | 8.91 |
| 8 | Siduron B | 9.76 |
| 9 | Linuron | 11.0 |
| 10 | Carbazole (SUR) | 12.8 |
| 11 | Diflubenzuron | 13.9 |

7. HPLC Analysis--Confirmation Analysis

| Conditions | |
|---|---|
| Solvent A | 25 mM phosphate buffer |
| Solvent B | Acetonitrile |
| Linear gradient 20% B | 0-11.0 minutes, 1.5 mL/min flow rate |
| Linear gradient 20-40% B | 11.0-12.0 minutes, 1.5 mL/min flow rate |
| 40% B | hold to 16 minutes at 1.5 mL/min. flow rate, step to 2.0 mL/min flow rate at 16 min. and hold to 20 min |
| Linear gradient 40-20% B | 20.0-20.1 minutes, 2.0 mL/min. flow rate |
| Wavelength | 240 nm |
| Equilibration time prior to next injection = 15 minutes | |

Confirmation Column: Supelcosil 4.6 × 150 mm packed with 5 um cyanopropyl stationary phase or equivalent

Chromatographic Retention Time Data for the Confirmation Column

| Peak Number | Analyte | Retention Time (min) |
|-------------|-----------------|----------------------|
| 1 | Tebuthiuron | 2.56 |
| 2 | Thidiazuron | 3.98 |
| 3 | Monuron (SUR) | 4.93 |
| 4 | Fluometuron | 5.94 |
| 5 | Diuron | 7.67 |
| 6 | Propanil | 9.53 |
| 7 | Siduron A | 10.1 |
| 8 | Siduron B | 10.8 |
| 9 | Linuron | 12.2 |
| 10 | Carbazole (SUR) | 14.3 |
| 11 | Diflubenzuron | 15.2 |

*The analyst should refer to EPA Method 532 "Determination of Phenylurea Compounds in Drinking Water by Solid-Phase Extraction and High Performance Liquid Chromatography with UV Detection", Revision 1.0 Issued June 2000, by M. V. Bassett, S.C. Wendelken, T.A. Dattilio, and B.V. Pepich (IT Corporation) D.J. Munch (US EPA, Office of Ground Water and Drinking Water) EPA, National Exposure Research Laboratory, Office of Research and Development, US Environmental Protection Agency, Cincinnati, Ohio 45268

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