Chemistry and HPLC Analysis of Phenylurea Pesticides

Reliable Results with Restek Reference Mixes and HPLC Columns

Katia May, C. Vernon Bartlett, and Rebecca Wittrig

Restek Corporation, 110 Benner Circle, Bellefonte, PA 16823

Restek Corporation
www.restekcorp.com

Abstract

Phenylurea herbicides are a class of highly effective chemicals for agricultural weed control but when present in drinking water they are potential endocrine disrupters. GC analysis is not suitable for these herbicides because they are thermally unstable. Standard methods for determination of phenylurea herbicides in water samples involve solid phase extraction and high performance liquid chromatography (HPLC) using US EPA Method 532.

Phenylurea herbicides are light- and heat-sensitive and can degrade to their metabolites under these influences. The photochemical behavior of phenylurea herbicides in aqueous solution is dependent on the type and position of the substitution groups. Most of these herbicides have a methyl, halogen, or methoxy group as the substituent. When there is a methyl group substituent on the urea moiety, demethylation may occur. Methyl-containing diuron and fluometuron, for example, easily degrade to their metabolites. This paper discuses the stability of a phenylurea herbicides reference mix and the measures necessary for its storage. In addition, the solubility of a calibration solution and a surrogate standard for phenylurea herbicides was studied and the appropriate solvents were determined.

Method 532 requires two HPLC columns, a C18 column and a confirmation column. The C18 phase column used in this work is a high carbon load packing that offers higher retention. Several other phases were examined as possible secondary columns. The features and benefits of the columns are discussed and recommendations are offered for good resolution and fast analyses of phenylurea pesticides. A confirmation column with an alternate stationary phase and different elution order, relative to the C18 column, was selected.

Background

Phenylurea pesticides are used to control a wide range of broadleaf weeds, grasses, and mosses, for both selective and total weed control. While drinking water usually is free of pesticides and herbicides after treatment, when violations are reported they are mainly due to phenylurea, triazine, or phenoxyacid pesticides. Phenylurea pesticides in ground or drinking water are potential endocrine disrupters.

The US Environmental Protection Agency developed Method 532 for determining phenylurea compounds in drinking water. Solid-phase extraction (SPE) cartridges containing a bonded C18 organic phase are used to extract the pesticides from the sample, the analytes are eluted from the cartridges with methanol, and the concentrated extracts are analyzed by HPLC with ultraviolet detection. Phenylurea pesticides are not suitable for gas chromatography because they are thermally unstable.

Materials and Methods

Restek chemists have formulated a calibration solution and surrogate standard for determining target phenylurea pesticides in the latest version of EPA Method 532. The calibration mix contains 200µg/mL of each pesticide in acetonitrile, the organic mobile phase in the HPLC assay. Because diflubenzuron has limited solubility in acetonitrile, and thidiazuron is especially difficult to dissolve, we include a small amount of acetone in the formulation, to enhance solubility of these two compounds. The early-eluting acetone does not interfere with any of the analytes. Our surrogate standard contains monuron and carbazole at 500µg/mL each in 50:50 methanol/acetonitrile (monuron is soluble in methanol, carbazole is soluble in acetonitrile).

Results and Conclusions

Method 532 requires two HPLC columns: a C18 column plus a confirmation column with a dissimilar stationary phase. **Figure 1** shows an analysis of the phenylurea pesticides and surrogates on Restek's Ultra C18 column. The high carbon load of this column ensures excellent retention and selectivity. The phenylurea mix and the surrogates also are separated well, with one peak reversal on an Ultra Cyano cyanopropyl stationary phase – our recommendation for the confirmation column (**Figure 2**).

Figure 1. Phenylurea pesticides resolved in less than 20 minutes on an Ultra C18 column.

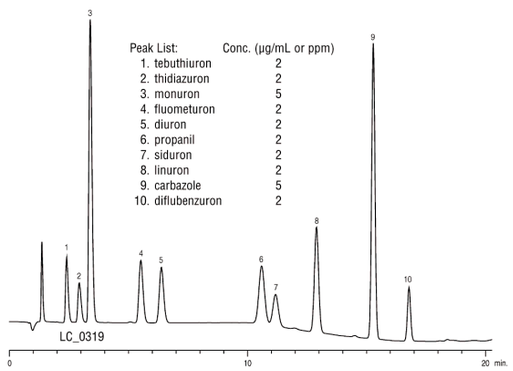

Figure 1 Conditions

Column: Ultra C18, 150 x 4.6mm; Particle/Pore Size: 5µm/100Å
Sample: 20µL; Conc.: see Figure 1; Diluent: acetonitrile
Conditions:
Mobile Phase: A: 25mM phosphate buffer, pH 2.5
B: acetonitrile

Time (min.)	**%B**
0	40
8	40
14	55
18	55
19	40

Flow: 1.5mL/min.; Temp.: ambient; Det.: UV @ 245nm

Figure 2. Monuron and thidiazuron reversed on an Ultra Cyano confirmation column.

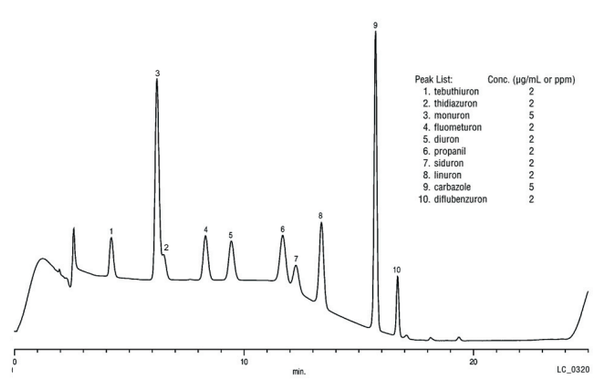

Figure 2 Conditions

Column: Ultra Cyano, 250 x 4.6mm; Particle/Pore Size: 5µm/100Å
Sample: 20µL; Conc.: see Figure 1; Diluent: acetonitrile
Conditions:
Mobile Phase: A: 25mM phosphate buffer, pH 2.5
B: acetonitrile

Time (min.)	%B
0	30
8	30
14	50
21	50
21.1	30

Flow: 1.5mL/min.; Temp.: ambient; Det.: UV @ 245nm