Multiresidue HPLC Methods for Phenyl Urea Herbicides in Water

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High-performance liquid chromatography (HPLC) methods for the determination of phenyl urea herbicides in water are described. The target compounds include chlortoluron, diuron, fluometuron, isoproturon, linuron, metobromuron, metoxuron, monuron, neburon, and siduron. Water was subjected to solid phase extraction (SPE) using either automated SPE with 47 mm C_{18} Empore disks or on-line precolumn concentration. Herbicides were separated on a C_{18} reversed phase column with an acetonitile−water gradient and were detected with either a diode array detector (DAD) or a postcolumn photolysis and derivatization (PPD) detector system. Photolysis converted the phenyl ureas to monoalkylamines that were derivatized to fluorescent isoindoles by reaction with *o*-phthalaldehyde and 2-mercaptoethanol. The DAD monitoring at 245 nm was linear over three decades with instrument detection limits of ∼0.01 mg/L. SPE efficiency was between 48 and 70% in laboratory reagent water, but use of the internal standard quantitation method improved accuracy. High total dissolved solids and total organic carbon values in surface water improved recoveries relative to laboratory reagent water for all of the phenyl ureas. In Colorado River water spiked at 1 or 50 μg/L, mean recoveries ranged from 74 to 104%. Method detection limits (MDLs) ranged from 4 to 40 ng/L (parts per trillion) with the DAD instrument. PPD detection was highly specific but resulted in a slight loss in chromatographic efficiency and average MDLs ∼5 times higher using a single set of detection conditions. The study indicates that methods based on SPE followed by HPLC with diode array or PPD detection have practical utility for trace analysis of phenyl ureas in drinking water or surface waters.

Keywords: *Phenyl urea herbicides; analysis; water; solid phase extraction (SPE); diode array detection; postcolumn photolysis and derivatization detection*

INTRODUCTION

Phenyl ureas are selective herbicides used in various crops including cereals (chlortoluron and isoproturon), cotton (fluometuron), potatoes (monolinuron), and strawberries (chloroxuron) (Hance and Holly, 1990). Another phenyl urea, diuron, is used as a nonselective herbicide. Phenyl ureas are principally absorbed via the roots and are translocated to the site of action where they block photosynthetic electron transport (Buchel, 1977; Devine et al., 1993).

Phenyl ureas enter the environment by various means including spray drift, runoff from treated fields, and leaching into groundwater. Although photochemically unstable, phenyl ureas can persist in water for periods of days to weeks depending on the temperature and pH (Sanchis-Mallols et al., 1998). Although phenyl ureas generally exhibit low mammalian toxicity, some have been reported to be carcinogenic in experimental animals. A preliminary assessment by the California Environmental Protection Agency suggests that 10 μg/L may be a suitable drinking water health advisory for diuron. Diuron and linuron are unregulated contaminants that must be monitored in drinking water under the Safe Drinking Water Act (*Federal Register* 64, 180, pp 50556−50620). The European Union (EU) established 0.1 μg/L as the permissible limit for any individual herbicide in drinking water (Sanchis-Mallols et al., 1998).

Sensitive and specific methods are needed for the determination of diuron and its analogues in water. Phenyl urea residues in food and water have been analyzed both by gas chromatography (GC) and by high-performance liquid chromatography (HPLC) (Balinova, 1993; Parrilla et al., 1993; Gennaro et al., 1995; Field et al., 1997). GC methods usually do not allow analysis of intact phenyl ureas due to their thermal instability—the HN−CO bond breaks, giving the corresponding aniline in the heated inlet. U.S. Environmental Protection Agency (U.S. EPA) Method 632 utilizes HPLC separation with UV detection for the determination of seven phenyl ureas in wastewater (U.S. EPA, 1997). Particle beam HPLC-MS also has been used for the determination of diuron, linuron, monuron, and siduron in U.S. EPA Method 553. Although the particle beam method is very selective, the method detection limits (MDLs) are relatively high (e.g., 2−30 μg/L) (U.S. EPA, 1992).

Luchtefeld (1987) developed a novel HPLC method for the analysis of phenyl urea herbicides in food based on postcolumn photodecomposition followed by derivatization of the primary amine photoproducts with *o*-phthalaldehyde (OPA) and 2-mercaptoethanol (MERC). The isoindole products shown in equation 1 are detected at low concentrations using a fluorescence detector.

The goal of the current work is to develop rugged multiresidue methods for the determination of phenyl

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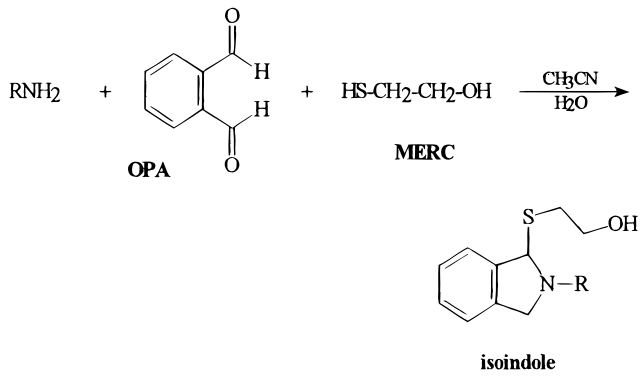


ureas in water at sub parts per billion (ppb) concentrations. HPLC methods using both the diode array detector (DAD) and postcolumn photolysis and derivatization (PPD) detection were investigated. PPD was of interest because of its improved specificity relative to single-wavelength absorbance detection. Solid phase extraction (SPE), used increasingly in drinking water compliance monitoring, was selected because of the need for low detection limits.

MATERIALS AND METHODS

Chemicals and Reagents. Phenyl urea herbicides were obtained as high-purity solids from the U.S. EPA (Research Triangle Park, NC). Primary standards (1.00 mg/mL) were prepared in methanol, and secondary and working calibration standards were obtained by serial dilution in methanol. Reagent water for mobile phases and other uses was obtained from a Barnstead Nanopure Infinity water purifier with charcoal cartridges and a UV cell (Barnstead-Thermolyne, Dubuque, IA). HPLC grade acetonitrile (ACN) was purchased from Baker. The OPA−MERC reagent was prepared in a 1 L volumetric flask by adding 50 mL of 1 M pH 10.4 borate buffer diluent, ~900 mL of reagent water, 250 mg of OPA dissolved in 5 mL of methanol, 2 mL of MERC, and reagent water to volume. The OPA−MERC reagent is prepared each day as it decomposes in the presence of oxygen. Postcolumn derivatization reagents and diluents were obtained from Pickering Laboratories (Mountain View, CA). Total dissolved solids (TDS) and total organic carbon (TOC) in Colorado River water were determined using Standard Methods 2540 (APHA, AWWA, and WEF, 1998) and 5310B (APHA, AWWA, and WEF, 1989), respectively. All samples and sample extracts were stored in amber glassware at 4 °C.

Instrumentation. A Hewlett-Packard (HP) model 1100 HPLC system (Wilmington, DE) was used. This HPLC was equipped with both G1315A diode array (DAD) and G1321A fluorescence detectors (FLD). The instrument had an autosampler and a mobile phase degasser and used a Hewlett-Packard Chemstation for data acquisition and processing.

Postcolumn photolysis and derivatization (PPD) was performed with a PHRED photoreactor (Aura Industries, New York) equipped with a 254 nm Hg vapor lamp in line with a Pickering postcolumn derivatization apparatus and the HP FLD. A schematic diagram for the apparatus is shown in Figure 1. After separation on the analytical column, the analytes pass through a knitted coil of Teflon tubing (5 m × 0.5 mm i.d.; 1 mL total volume) wrapped around the Hg vapor lamp. The photolysis products eluting are mixed with the OPA−MERC reagent, and the mixture is conducted to the FLD. The system consists of a variable flow rate reagent pump, a pulse dampener or flow conditioner, a mixing tee, and a reaction coil and pressure relief valve to avoid overpressuring the FLD flow cell. The OPA−MERC reagent is metered into the mobile phase through a tee at a rate of 0.5 mL/min and allowed to react in the 1 mL Teflon tubing reaction coil (16 m × 0.28 mm i.d.). The FLD used an excitation wavelength of

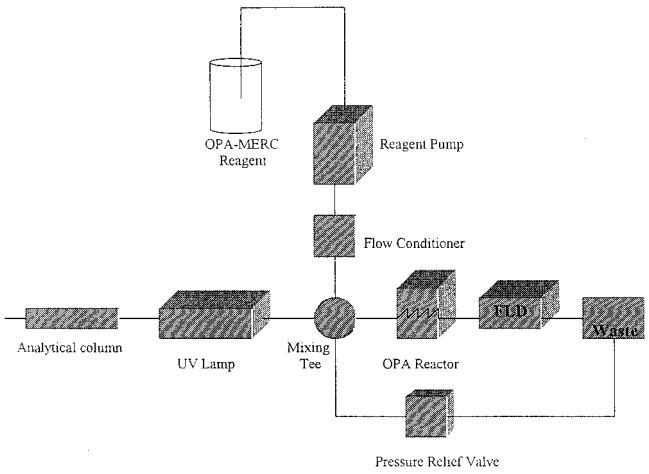


Figure 1. Schematic diagram of postcolumn photolysis and derivatization detector.

340 nm, and emission was monitored at three wavelengths (440, 480, and 500 nm) using a 380 nm cutoff filter.

HPLC Determination. A 25 cm × 4.6 mm 5 μm C_{18} Supelcosil LC-18 analytical column (Supelco, Bellefonte, PA) with a 5 μm C_{18} Supelco precolumn was used exclusively. Satisfactory resolution of the phenyl ureas and method internal standards (IS) required a 20 min linear ACN−water gradient from 10 to 70% strong solvent. To wash the column, the mobile phase was further ramped from 70 to 100% ACN over 2 min and held for 5 min. The flow rate was uniformly 1.5 mL/min. The instrument was operated as follows: injection volume, 25 μL; column oven, 20 °C; DAD detector λ, 245 nm. The injector was automatically washed between injections.

Solid Phase Extraction (SPE). Automated SPE used 47 mm C_{18} Empore disks (3M, St. Paul, MN) and a Tekmar Autotrace SPE workstation (Cincinnati, OH). The C_{18} SPE technique is similar to that used routinely for the determination of semivolatiles in drinking water as in U.S. EPA Method 525.2 (U.S. EPA, 1995). Briefly, 1 L water samples are dechlorinated with freshly prepared sodium sulfite and adjusted to pH <2 by adding 6 N hydrochloric acid. Methanol (4 mL) is added to the water sample prior to extraction, and disks are extracted with ethyl acetate followed by ethyl acetate−methylene chloride. The combined extracts are dried with sodium sulfate and reduced to a 1 mL ethyl acetate final volume in a nitrogen evaporator. Deuterated polycyclic aromatic hydrocarbon (PAH) internal standards, pyrene-d_{10} (DAD only), and phenanthrene-d_{10} (DAD or PPD), are added to the water sample at 10 μg/L prior to extraction. For spike−recovery studies the herbicides were added to water in microliter volumes of methanol.

On-Line SPE. In preliminary work on-line SPE was investigated using a manual injection instrument and procedure reported in detail elsewhere (Draper et al., 1999). A 4.6 mm C_{18} cartridge mounted on six-port valve was used as a concentrator column. Water samples (50 mL) were acidified with four drops of 6 N HCl. A 20 mL aliquot was pumped through the concentrator column (a C_{18} HPLC guard column) at 4 mL/min. The concentrator column was then backflushed onto the analytical column with the chromatographic mobile phase, an ACN−0.025 N phosphoric acid gradient with a 1.5 mL/min flow rate. Phenyl ureas were resolved using a 20 min gradient ramping from 10 to 60% strong solvent and were detected by absorbance at 254 nm. As above, the column was washed with ACN in a similar cycle.

RESULTS AND DISCUSSION

Chromatography. As seen in Table 1 the phenyl urea herbicides selected for study differ only slightly in structure. Six of the compounds are *N*-aryl-*N*′,*N*′-dimethylureas that varied only in the pattern of substitution on the benzene ring. Two of the compounds are

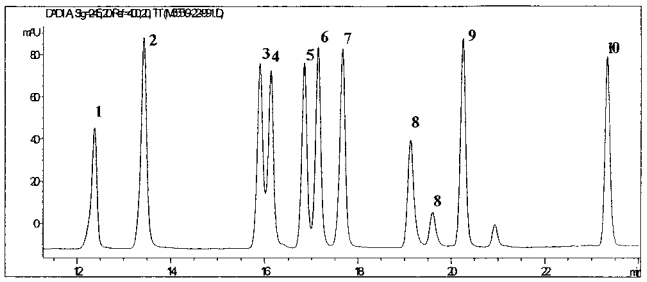
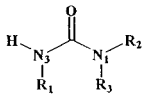
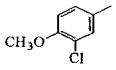
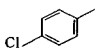
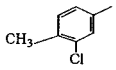


Figure 2. UV absorbance chromatogram (245 nm) of a 2.0 μg/mL standard solution containing metoxuron (1), monuron (2), chlortoluron (3), fluometuron (4), isoproturon (5), diuron (6), metobromuron (7), siduron (8), linuron (9), and neburon (10).

Table 1. Phenyl Urea Structures and Retention Time Windows



Compound	R_1	R_2	R_3	tR (min) ± 95% Confidence Limit
Metoxuron		CH_3	CH_3	12.43 ± 0.011
Monuron		CH_3	CH_3	13.50 ± 0.012
Chlortoluron		CH_3	CH_3	15.99 ± 0.012
Fluometuron		CH_3	CH_3	16.22 ± 0.009
Isoproturon		CH_3	CH_3	16.93 ± 0.013
Diuron		CH_3	CH_3	17.22 ± 0.012
Metobromuron		OCH_3	CH_3	17.78 ± 0.013
Siduron			H	19.25, 19.73 ± 0.014
Linuron		OCH_3	CH_3	20.38 ± 0.013
Neburon		CH_3	$CH_3(CH_2)_3$	23.48 ± 0.014

N-aryl-*N*′-methoxy-*N*′-methylureas. Despite the minor structural differences, the standards were satisfactorily resolved using the C_{18} reversed phase column (Figure 2). Analytes eluted from the column in a 10 min window with metoxuron eluting first at 12.5 min and neburon eluting last at 23 min. Siduron's diastereoisomers also are resolved in the separation. The most difficult pair to separate on the C_{18} column is chlortoluron and fluometuron, but using the conditions described there was a 20% valley between the two chromatographic peaks. The column selected has good performance characteristics for a wide variety of HPLC analytes with a chromatographic efficiency of ~19000 theoretical plates according to manufacturer specifications.

Retention times were highly reproducible using the automated HPLC system, even over an extended time period. During the course of the MDL determinations, for example, t_R values showed negligible drift. After 1 week of operation, the retention time 95% confidence intervals were in the range of 0.009−0.014 min. Identification of the target compounds is highly reliable with this type of instrument performance because the retention time windows are on the order of only ±1 s.

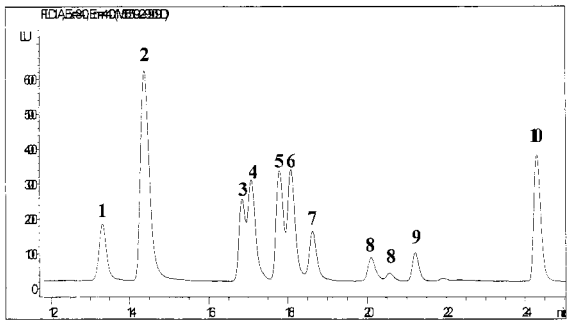


Figure 3. HPLC chromatogram of a 10 μg/mL phenyl urea standard solution using postcolumn photolysis and derivatization detection. Excitation and emission wavelengths for the isoindole derivatives are 340 and 440 nm, respectively. The elution order is indicated in Figure 2.

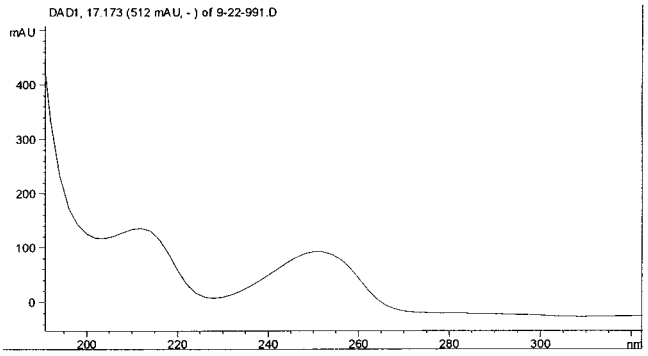


Figure 4. Diuron absorption spectrum acquired using the diode array detector.

The chromatogram obtained with PPD detection was similar with the exceptions that (a) the retention times were ~0.7 min longer due to the added hold up volume and (b) the chromatographic efficiency was slightly lower. In the PPD chromatogram diuron and metobromuron also represent a difficult to resolve pair (Figure 3). Extracolumn band broadening appears to be an important consideration with PPD detection and, therefore, selection of the optimal column type will be more critical in this system. The slight loss in chromatographic efficiency may be associated, in part, with mixing and diffusion in the knitted photolysis cell and reaction coil.

Detection of Phenyl Ureas. The phenyl urea herbicides exhibit two absorption maxima in the ultraviolet region. For example, diuron has absorbances centered at 212 and 250 nm (Figure 4), each with approximately the same extinction coefficient. The longer wavelength band for each of the phenyl ureas is near its maximum absorbance at 245 nm, and this wavelength was selected for use in the multiresidue method. Although the shorter wavelength band usually is more intense (Table 2), interferences are less often encountered at the higher wavelength. The ratio of absorbances for the two bands can be used to confirm compound identity in sample extracts or to evaluate peak purity.

The diode array instrument was calibrated with eight herbicide mixtures between 0.2 and 50 mg/L, demonstrating excellent linearity with correlation coefficients

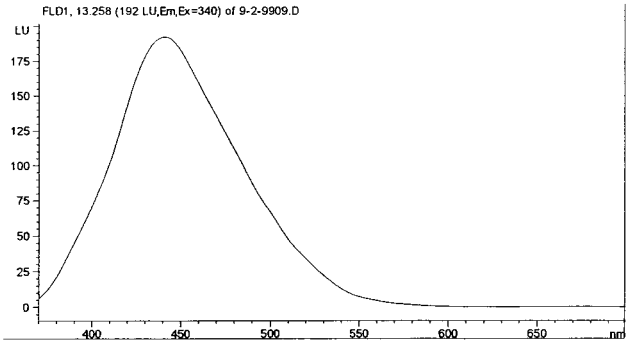


Figure 5. Emission spectrum of the OPA−MERC adduct from metoxuron.

Table 2. Absorbance Maxima for Phenyl Urea Herbicides

compound	t_R (min)	λ_{max} (nm) A	λ_{max} (nm) B	absorbance ratio (A/B)
chlortoluron	15.99	208	244	1.24
diuron	17.22	212	250	1.33
fluometuron	16.22	204	244	1.19
isoproturon	16.93	204	242	1.23
linuron	20.38	210	248	1.20
metobromuron	17.78	202	248	1.09
metoxuron	12.43	208	244	1.79
monuron	13.5	204	246	1.08
neburon	23.48	212	251	1.16
siduron (either peak)	19.25, 19.73	204	242	1.40

in all cases exceeding 0.999. Accordingly, the response factors were uniform over this concentration range, with response factor relative standard deviations (RSDs) averaging 4.6 and ranging from ~3 to ~8%. The instrument also was linear between 0.01 and 0.2 mg/L, although the sensitivity (slope) was slightly different. On the basis of these calibration data and 1000-fold concentration factors achieved by SPE, very low detection limits (low parts per trillion) were anticipated for the DAD-SPE method. At 245 nm the phenyl urea response factors were within a factor of 2 based on peak areas.

The response of the phenyl ureas to PPD detection is different for each of the compounds due to differences in the rate of light absorption and the quantum yield for formation of the monoalkylamine product. The reactivity of phenyl ureas depends on the N_1 nitrogen substituents and to a lesser extent on the N_3 nitrogen substituents. According to Luchtefeld (1987), phenyl ureas with methoxy groups at N_1 are 2−5 times less responsive than those with *N*,*N*-dimethyl substitution. To optimize detection of each of the compounds, three emission wavelengths were monitored, 440, 480, and 500 nm. As seen in the emission spectrum of the isoindole formed by metoxuron photoproducts (Figure 5), the maximum emission occurs at ~440 nm and drops off by more than half at 500 nm. To obtain the widest linear dynamic range, phenyl ureas giving the lowest response were monitored at 440 nm and those with the highest molar response were monitored at 500 nm. In MDL determination where optimal sensitivity is needed, 440 nm was used as the monitoring wavelength. The internal standard phenanthrene-d_{10} was monitored by its fluorescence at 480 nm.

The PPD detector also was linear over a narrower calibration range, typically 0.5−20 μg/L and as much as 0.1−20 μg/L. Correlation coefficients ranged from 0.985 to 0.999, and response factor RSDs averaged ~15%. Thus, the PPD system exhibited a linear dynamic response that was completely satisfactory for quantitative analysis, but its linearity did not match that of the DAD. PPD calibration curves for the phenyl urea herbicides are shown in Figure 6.

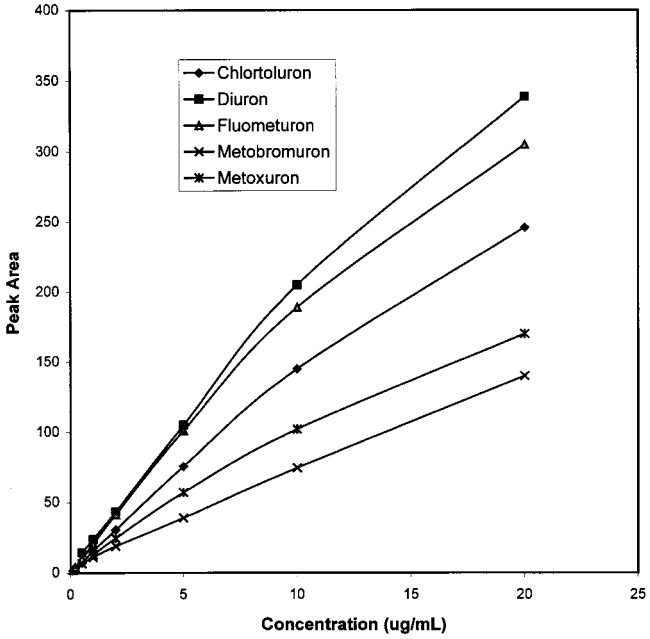


Figure 6. Postcolumn photolysis and derivatization calibration curves with isoindole fluorescence monitored at 480 nm.

Table 3. Recovery of Phenyl Urea Compounds from C_{18} SPE Disks and Recovery Relative to the Pyrene-d_{10} Internal Standard

compound	absolute accuracy (%)	accuracy relative to pyrene-d_{10} (%)
chlortoluron	63	92
diuron	63	93
fluometuron	62	92
isoproturon	65	93
linuron	62	92
metobromuron	59	88
metoxuron	53	77
monuron	48	71
neburon	54	79
siduron	70	99

Extraction Efficiency. C_{18} extraction disks were effective for each of the phenyl urea herbicides when the disks were properly conditioned and when the water was amended by acidification and methanol addition. The SPE conditions are similar to those used routinely for many semivolatile contaminants in drinking water. The absolute recovery of the phenyl ureas ranged from a low of 48% (monuron) to a high of 70% (siduron) in purified laboratory water at 1 μg/L. These recoveries were reproducible and are typical of many other semivolatile compounds extracted by this technique (unpublished results). The deuterated PAH internal standards used for quantitative analysis also have <100% SPE recovery, although their recovery exceeds that of the more polar phenyl ureas. Thus, quantitation using the internal standard method can improve accuracy. Either pyrene-d_{10} or phenanthrene-d_{10} was an appropriate internal standard for the DAD method, but only phenanthrene has sufficient photochemical stability with PPD detection. Absolute and relative recoveries are summarized in Table 3.

On-Line SPE Method. As an alternative method, coupled or on-line SPE-HPLC was investigated. This

Table 4. Method Detection Limits for Phenyl Urea Herbicides Determined by Automated SPE Followed by DAD-HPLC

analyte	spike concn (ng/L)	accuracy (%)	SD (ng/L)	MDL (ng/L)
chlortoluron	20 and 50	88	4.4	12
diuron	50 and 100	71	4.3	11
fluormeturon	20 and 50	81	3.4	9
isoproturon	50	85	3.3	10
linuron	50 and 100	76	5.0	13
metobromuron	50	73	3.5	11
metoxuron	20 and 50	68	1.3	4
monuron	20	72	1.4	5
neburon	50 and 100	80	6.7	18
siduron	50 and 100	74	13	35

Table 5. Method Detection Limits for Phenyl Urea Herbicides Determined by Automated SPE Followed by Postcolumn Photolysis and Derivatization HPLC

analyte	spike concn (ng/L)	accuracy (%)	SD (ng/L)	MDL (ng/L)
chlortoluron	100	64	9.3	30
diuron	50	86	13	40
fluormeturon	100	92	12	30
isoproturon	50	90	10	30
linuron	200	99	4.4	30
metobromuron	200	52	7.8	30
metoxuron	200	64	27	80
monuron	100	55	9.3	30
neburon	50	64	8.4	30
siduron	200	56	14	50

involved direct concentration of 20 mL water samples on a C_{18} cartridge—the sorbed compounds were backflushed onto the analytical column at the start of the analytical separation. The HPLC separation was similar to that used with off-line SPE, that is, C_{18} reversed phase, ACN−0.025 N phosphoric acid mobile phase gradient and UV absorbance detection. Substituting water for phosphoric acid did not affect the peak shape or elution order of the phenyl ureas. The absolute recoveries were improved with on-line extraction, that is, 80% on average for diuron versus 63%. This may be attributed to the minimal sample handling for this technique or possibly the high surface area of the packing material in the concentrator column.

Detection Limits. Phenyl urea detection limits were determined using the method detection limit (MDL) protocol established for use in drinking water compliance monitoring (*Federal Register*, 1984). Briefly, laboratory reagent water was fortified with the herbicides at a small multiple of the instrument detection limit—in the current study the pesticides were spiked at <5 × S/N level. Over a period of days the water is analyzed so as to accumulate a minimum of seven separate results. The MDL is the product of the *t*-statistic and the standard deviation and represents "the minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero." The MDL determination is repeated at several spike concentrations, and data from multiple concentrations are pooled when the variances at two levels are similar.

For the DAD, phenyl urea spike concentrations were between 20 and 100 ng/L (Table 4), giving experimental MDLs ranging from ∼4 to 40 ng/L. Laboratories reporting reliable quantitative data set the reporting limit at 3−10 times the MDL. A multiple of 5×, thus, yields reporting limits in the range of 20−200 ng/L. In this study the accuracy in the MDL determination was between 68 and 88%, although accuracy at these spike concentrations is often reduced.

The PPD HPLC was somewhat less sensitive and required MDL spike concentrations of 50, 100, and 200 ng/L depending upon the phenyl urea. In each case the fluorescence detector used an excitation λ of 340 nm and monitored at 440 nm, the emission maximum for the isoindole derivative. In all cases the MDL was lower using the DAD (Table 5). Nevertheless, the PPD MDLs were extremely low, varying between 30 and 80 ng/L and corresponding to reporting limits of between 0.15 and 0.4 μg/L (parts per billion).

The MDL for the on-line SPE method using a UV absorbance detector was roughly equivalent to that of the automated SPE method. Using 20 mL samples spiked with 300 ng/L, the diuron MDL was 18 ng/L. This is very similar to the 11 ng/L diuron MDL obtained by automated SPE using the 1000 mL samples spiked at 50 and 100 ng/L.

Method Validation. The available information suggested that methods based on automated SPE followed by either DAD-HPLC or PPD-HPLC would be useful for routine monitoring of phenyl ureas in water. To further validate the methods, accuracy and precision studies in reagent water and Colorado River water were conducted. The Colorado River water contained high dissolved solids (590 mg/L TDS) and total organic carbon (3.2 mg/L TOC) that could affect SPE recoveries or subsequent HPLC determinations. The phenyl urea compounds were spiked at multiple concentrations including 1.0, 10, and 50 μg/L, and spiked samples were analyzed up to 3 days after fortification. Accuracy and precision across this concentration range was acceptable, as seen in Table 6. The method was accurate and reproducible at the 1.0 and 10 μg/L spike levels with <10% bias for seven of the compounds and <30% bias for the other three. At these concentrations the RSD was <10% in all cases except for monuron at the low spike concentration. At 50 μg/L metoxuron and monuron had slightly higher biases of 27 and 37%, respectively, with ∼20% RSD.

For each of the 10 phenyl urea herbicides the recovery and accuracy were improved in the high TDS−high TOC surface water. This phenomenon was noted at both the high and low spike levels at which comparisons were possible. The improved recovery may be due to enhanced extraction efficiency associated with salting out of the herbicides by the TDS constituents. Another effect of the natural constituents was to improve the method precision at low concentrations, a phenomenon noted for all of the compounds except diuron at 1 μg/L. Surface water chromatograms are shown in Figure 7.

Preservation of Phenyl Ureas in Water. Preservation is often required to ensure that water samples analyzed in the laboratory are representive of those collected in the field. This is usually accomplished by refrigeration, pH adjustment, and/or elimination of any disinfectant residual with a reducing agent such as thiosulfate, ascorbate, or sulfite. In the current study, samples were dechlorinated by the addition of 50 mg/L of sodium sulfite and by reducing the pH to <2 with added 6 N hydrochloric acid. To examine the stability of the phenyl ureas in the presence of these preservatives, three sets of samples were analyzed: laboratory reagent water; tap water containing ∼2 mg/L of combined chlorine; and laboratory reagent water containing ∼2 mg/L of free chlorine prepared by the addition of 80 μL of chlorine bleach (NaOCl) per liter. These solutions

Table 6. Method Accuracy and Precision in Laboratory Reagent Water and Surface Water Using Automated SPE with Quantitation by DAD-HPLC

	concn found (μg/L)				
	laboratory reagent water			Colorado River water	
compound	1.0 μg/L	10 μg/L	50 μg/L	1.0 μg/L	50 μg/L
chlortoluron	0.97 ± 0.08	10.1 ± 0.44	48.0 ± 2.31	0.98 ± 0.02	50.0 ± 3.73
diuron	0.89 ± 0.02	9.4 ± 0.41	43.5 ± 2.13	0.92 ± 0.02	45.2 ± 3.58
fluometuron	0.93 ± 0.03	10.1 ± 0.45	46.1 ± 2.55	0.99 ± 0.02	48.9 ± 3.76
isoproturon	0.92 ± 0.02	9.9 ± 0.35	48.0 ± 2.27	0.97 ± 0.02	49.9 ± 4.37
linuron	0.92 ± 0.03	9.0 ± 0.40	41.5 ± 2.01	0.95 ± 0.01	43.4 ± 3.12
metobromuron	0.90 ± 0.04	9.7 ± 0.47	44.3 ± 2.13	0.93 ± 0.03	46.4 ± 3.86
metoxuron	0.74 ± 0.06	8.0 ± 0.39	36.5 ± 6.32	0.85 ± 0.03	41.4 ± 2.11
monuron	0.67 ± 0.12	7.3 ± 0.41	31.5 ± 6.70	0.77 ± 0.05	37.0 ± 0.95
neburon	0.79 ± 0.04	8.5 ± 0.36	40.0 ± 2.00	0.84 ± 0.03	41.9 ± 3.07
siduron	1.01 ± 0.03	9.9 ± 0.26	47.5 ± 2.42	1.04 ± 0.02	49.8 ± 3.66

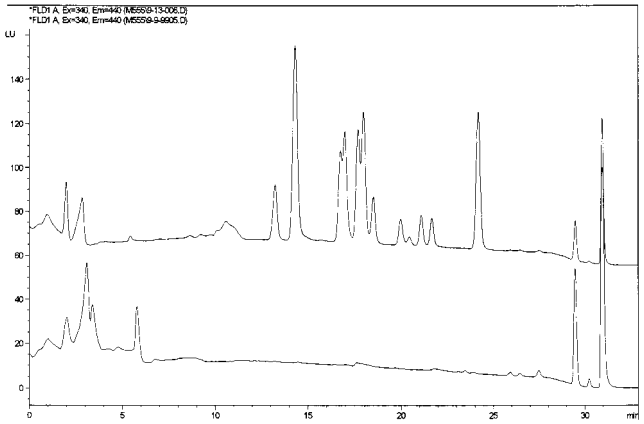


Figure 7. Colorado River water (lower trace) and Colorado River water containing 12.5 μg/L each of the 10 phenyl urea herbicides analyzed by PPD-HPLC. The two late eluting compounds are internal standards.

Table 7. Recovery of Phenyl Ureas after Preservation (Sulfite Treatment and pH Adjustment) after 14 Days of Storage at 4 °C

	mean recovery after preservation (%)		
compound	reagent water	tap water containing 2 ppm combined chlorine	reagent water containing 2 ppm free chlorine
chlortoluron	111	104	102
diuron	111	102	101
fluometuron	109	102	101
isoproturon	111	103	102
linuron	109	102	101
metobromuron	110	101	102
metoxuron	97	109	107
monuron	94	121	115
neburon	110	103	101
siduron	110	104	101

were fortified with each phenyl urea at a concentration of 5 μg/L and immediately treated with the reducing agent and acid. After 14 days at 4 °C, the samples were analyzed in triplicate to determine whether recoveries were affected or whether the analysis was otherwise impaired.

The results of this preservation study are summarized in Table 7. The data indicate that parts per billion concentrations of the target compounds are determined accurately after preservation with sulfite treatment and pH adjustment. Although the current study was designed to evaluate the applicability of preservation, we have no information suggesting that phenyl ureas are prone to decomposition in the presence of free or combined chlorine at concentrations used in drinking water treatment. These agents can reduce recoveries in SPE and on-line SPE.

Conclusion. Sensitive and accurate methods have been developed for the determination of phenyl urea herbicides in water using automated SPE and HPLC determination with DAD or PPD detection. The methods are applicable to all of the 10 phenyl ureas studied including chlortoluron, diuron, fluometuron, isoproturon, linuron, metobromuron, metoxuron, monuron, neburon, and siduron. The available information on detection limits and accuracy and precision indicate that the methods are reliable for the determination of these herbicides at parts per trillion and parts per billion concentrations in reagent water or natural water. Surface water with high TDS and TOC values is analyzed without dilution, and analytical recoveries and precision are improved, conceivably due to salting out of the pesticides in the extraction step. Water samples can be stored for 14 days at 4 °C after preservation without any detectable loss of target compounds. The specificity of the method is optimal using PPD detection, which relies on photodecomposition of the phenyl ureas to primary amines that are subsequently converted to fluorescent isoindoles for detection. Detection by diode array offers slightly lower detection limits and a wider linear calibration range and provides sharper chromatographic peaks. With conventional multiple-wavelength UV detectors, absorbance ratios can be use as an alternate means of analyte confirmation.

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