LC/MS Analysis of Cyclohexanedione Oxime Herbicides in Water

LeEtta J. Marek*

Laboratory Services Division, Minnesota Department of Agriculture, 90 West Plato Boulevard, St. Paul, Minnesota 55107

William C. Koskinen

Agricultural Research Service, Soil and Water Management Research Unit, U.S. Department of Agriculture, 1991 Upper Buford Circle, No. 439, St. Paul, Minnesota 55108

Gail A. Bresnahan

Plant Sciences Department, North Dakota State University, Fargo, North Dakota 58103

A multiresidue method for the determination of alloxydim (methyl 2,2-dimethyl-4,6-dioxo-5-[1-[2-propenyloxy)amino]butylidene]cyclohexanecarboxylate), clethodim (*E,E*)-(±)-2-[1-[[3-chloro-2-propenyl)oxy]imino]propyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one), sethoxydim ((±)-2-[1-(ethoxyimino)butyl]-5-[2-ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one), and two metabolites, clethodim sulfoxide ((*E,E*)-(±)-2-[1-[[3-chloro-2-propenyl)oxy]imino]propyl]-5-[2-(ethylsulfinyl)propyl]-3-hydroxy-2-cyclohexen-1-one) and sethoxydim sulfoxide ((±)-2-[1-(ethoxyimino)butyl]-5-[2-ethyl-sulfinyl)propyl]-3-hydroxy-2-cyclohexen-1-one), in water by high-performance liquid chromatography/electrospray/mass spectrometry (LC/ES/MS) is reported. River water and distilled water were spiked at 0.08 and 0.8 μg L^{-1} with all three herbicides, which were then extracted from the water by C_{18}-SPE (SPE = solid-phase extraction). The herbicides and metabolites were quantified and confirmed using selected ion monitoring. The percent recoveries of the herbicides from water spiked at 0.8 μg L^{-1} were as follows: alloxydim, 117 ± 11%; clethodim, 96 ± 14%; sethoxydim, 89 ± 13%. There was no evidence of oxidation of clethodim and sethoxydim during the extraction to their respective sulfoxides. The limit of quantitation was <0.1 μg L^{-1}. We have shown that we can analyze and confirm three cyclohexanedione oxime herbicides and two metabolites in water by LC/ES/MS. This multiresidue method should also be appropriate for other cyclohexanedione oximes.

INTRODUCTION

Some of the newer classes of herbicides are polar, nonvolatile, and thermally labile, and applied at low rates of <300 g ha^{-1}. This combination of factors makes their analysis difficult at the trace levels (μg L^{-1}) necessary to monitor their environmental fate in soil, water, and air. For instance, cyclohexanedione oxime herbicides such as alloxydim (methyl 2,2-dimethyl-4,6-dioxo-5-[1-[2- propenyloxy)amino]butylidene]cyclohexa-necarboxylate), clethodim (*E,E*)-(±)-2-[1-[[3-chloro-2-propenyl)oxy]imino]propyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one), and sethoxydim ((±)-2-[1-(ethoxyimino)butyl]-5-[2-ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one) are applied at 100–300 g ha^{-1} to control annual and perennial grasses in dicotyledonous crops such as soybean [*Glycine max* (L.)], sugarbeet [*Beta vulgaris* (L.)], and oilseed rape [*Brassica napus* (L.)].

Early attempts to study the fate of these cyclohexanedione oxime herbicides in soil in the field have relied on bioassay techniques (Buhler and Burnside, 1984; Hsiao and Smith, 1983). Methods of analysis of these

same herbicides using HPLC with a UV detector have had limited success. For instance, clethodim degradation as a function of solution pH and light was analyzed by HPLC/UV in aqueous solution at relatively high concentrations, 10−100 mg L^{-1} (Falb et al., 1990, 1991). In another study, the limit of detection of sethoxydim was 5 ng on column by HPLC/UV; however, the maximum extraction efficiency from aqueous solution was 15%, and sethoxydim could not be extracted from freshly spiked soil at 1 mg kg^{-1} (Shoaf and Carlson, 1986). Complicating analysis is that these are labile chemicals and may degrade during the extraction process. For instance, sethoxydim degrades in methanolic solutions exposed to incandescent light (Shoaf and Carlson, 1992).

In previous work, analysis of sethoxydim residues in soil simultaneously quantified the parent and eight metabolites by their conversion to a common volatile end product, 3-[2-(ethylsulfonyl)propyl]pentanedioic acid dimethyl ester, which entailed extensive cleanup and derivatization and analysis using gas chromatography with a flame photometric detector (Koskinen et al., 1993). Although relatively sensitive (10−20 μg kg^{-1}), this method is nonselective and cannot distinguish between parent sethoxydim and its major metabolites. In addition, the results would also include clethodim, a

* To whom correspondence should be addressed. Phone: (651) 297-3093. Fax: (651) 297-8787. E-mail: Leetta.Marek@state.mn.us.
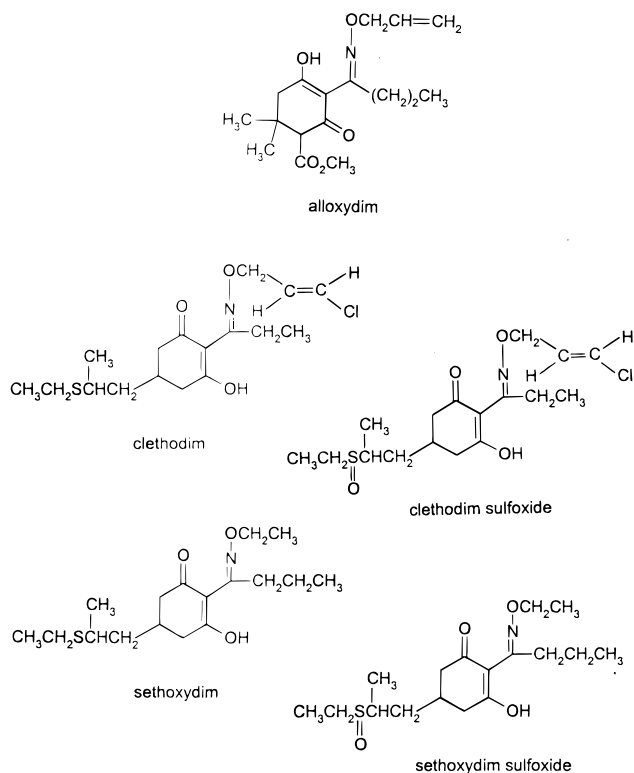
2798 *J. Agric. Food Chem.,* Vol. 48, No. 7, 2000

Marek et al.

Figure 1. Structures of alloxydim, clethodim, clethodim sulfoxide, sethoxydim, and sethoxydim sulfoxide.

related herbicide, and its metabolites, if present in soil or water.

A limitation with all of the above methods is the lack of confirmation of the herbicide or metabolite, which would require a second analytical method. A promising multiresidue method for these newer classes of herbicides is HPLC/electrospray/MS (LC/ES/MS). For instance, multiresidue methods have been recently reported for the determination of trace levels of sulfonylurea herbicides in water (Volmer et al., 1995) and soil (Marek and Koskinen, 1996) and aryloxyphenoxypropionic herbicides in water (D'Ascenzo et al., 1998) and soil (Lagana et al., 1998). Separation of extracted analytes by HPLC with detection and quantitation by mass spectrometry required no derivatization or special cleanup steps, other than solid-phase extraction (SPE) as part of a concentration step. Ionization of the analytes provided mass spectra with at least three ions necessary for confirmation of each in environmental samples. In this paper, we report the extraction, separation, quantitation, and confirmation of three cyclohexanedione oxime herbicides and two metabolites in water by LC/ES/MS.

MATERIALS AND METHODS

Reagents. Analytical grade standards (98% purity) of alloxydim, clethodim, and sethoxydim were obtained from ChemService (West Chester, PA). Clethodim sulfoxide (*E,E*)-(±)-2-[1-[[3-chloro-2-propenyl)oxy]imino]propyl]-5-[2-(ethylsulfinyl)propyl]-3-hydroxy-2-cyclohexen-1-one) (99% purity) and sethoxydim sulfoxide ((±)-2-[1-(ethoxyimino)butyl]-5-[2-ethylsulfinyl)propyl]-3-hydroxy-2-cyclohexen-1-one) (99% purity) were obtained from the U.S. Environmental Protection Agency and BASF, respectively. Chemical structures are shown in Figure 1.

Other reagents included ACS/HPLC/GC grade acetonitrile (Mallinckrodt Ultim AR), high-purity solvent methanol and water (Burdick and Jackson), nanograde ethyl acetate (Mallinckrodt), glacial acetic acid (aldehyde free, Baker Analyzed Reagent) (J. T. Baker), and ammonium acetate (J. T. Baker).

Instrumentation. For analysis by LC/ES/MS, a Hewlett-Packard HP5989B mass spectrometer engine equipped with a Hewlett-Packard HP1090M Series II high-performance liquid chromatograph and a Hewlett-Packard electrospray MS interface (Palo Alto, CA) was used. The high-performance liquid chromatograph included a binary solvent system, autosampler, heated column compartment, and diode array detector. The column was a Zorbax RX-C8 2.1 × 150 mm × 5 μm (MAC-MOD Analytical, Chadds Ford, PA).

For LC/ES/MS analysis the mobile-phase gradient elution was 30% acetonitrile:70% formic acid (0.1%) (v/v) from 0 to 4 min, increasing to 60% acetonitrile at 6 min, and increasing to 90% acetonitrile at 12.5 min. The total run time was 15 min. The mobile-phase flow rate was 0.2 mL min^{-1}. The sample injection volume was 100 μL.

MS conditions were as follows: capillary exit and entrance voltages were optimized at *m/z* 105, 164, 206, 268, and 360; electron multiplier voltage 2500 mV; MS quad temperature 120 °C; positive ion electrospray ionization mode; full scan spectra, 100–500 amu, acquired at 2 scans s^{-1}. For confirmation and quantitation by SIM, the ions monitored (including [MH]$^+$) are listed in Table 1.

For analysis by HPLC/UV, a Hewlett-Packard 1050 high-performance liquid chromatograph was used with a Zorbax RX-C-8 column, 4.6 × 250 mm, at 30 °C with the detector set at a wavelength of 254 nm. The mobile phase was 68.6:30:1.4 acetonitrile/water/glacial acetic acid with a flow of 1.0 mL min^{-1}. The injection volume was 100 μL. The retention times were (alloxydim) 17.6 min, (clethodim) 23.4 min, and (sethoxydim) 25.5 min.

Water Fortification and Extraction-LC/MS. Creek water and tap water (250 mL) were spiked at 0.08 and 0.8 μg L^{-1} with all three herbicides, which were then extracted from the water by C$_{18}$-SPE (MegaBondElut, 1 g/6 mL, Varian). The C$_{18}$-SPE cartridges were preconditioned with 10 mL of methanol followed by 6 mL of distilled water. After the chemicals were extracted from the water, they were eluted from the cartridges with 10 mL of methanol. The samples were evaporated to dryness (bath temperature <40 °C) and then redissolved in 3 mL of methanol, and then added to SAX cartridges (Mega BondElut, 1 g/6 mL, Varian), which had been preconditioned with 10 mL of methanol. The SAX cartridges were then rinsed with 10 mL of methanol, and the total methanol solution was collected. The samples were evaporated to dryness again using a rotary evaporator (bath temperature <40 °C). The samples were redissolved in 0.5 mL of acetonitrile, by swirling, agitating with a vortex mixer, and then sonicating for 5 min. After addition of 1.5 mL of 10 mM ammonium acetate and mixing again, a portion of the solution was passed through a 13 mm, 0.2 μm Gelman Acrodisc PTFE syringe filter and placed into a vial for analysis.

Water Fortification and Extraction-HPLC/UV. Duplicate 60 mL distilled water samples were spiked at 17 mg L^{-1} alloxydim and clethodim and 20 mg L^{-1} sethoxydim, and at 85 mg L^{-1} alloxydim and clethodim and 98 mg L^{-1} sethoxydim. The water samples were loaded onto BondElut C$_{18}$-SPE cartridges (Analytichem International), which had been preconditioned with 5 mL of methanol followed by 10 mL of distilled water. The herbicides were eluted from the cartridges with 10 mL of methanol and the samples evaporated just to dryness with a rotary evaporator (bath temperature <40 °C). The samples were redissolved in 5 mL of 60% acetonitrile/40% ammonium acetate (10 mM, pH 7) (v/v) buffer, and a portion was placed into a vial for HPLC analysis.

RESULTS AND DISCUSSION

LC/ES/MS. LC/ES/MS has been shown to provide the information necessary to meet the minimum criteria for positive confirmation for a variety of pesticides prior to

LC/MS Analysis of Cyclohexanedione Oxime

J. Agric. Food Chem., Vol. 48, No. 7, 2000 **2799**

Table 1. Fragmentation of Alloxydim, Clethodim, Clethodim Sulfoxide, Sethoxydim, and Sethoxydim Sulfoxide

| chemical	m/z^a	fragment	relative abundance
alloxydim	**324**	$[MH]^+$	100
	266	$[M - OCH_2CH{=}CH_2]$	25
	234		12
	206	$[M - OCH_2CH{=}CH_2 - CO_2CH_3]$	
clethodim	**360**	$[MH]^+$	100
	268	$[M - OCH_2CH{=}CHCl]$	34
	240	$[M - OCH_2CH{=}CHCl - CH_2CH_3]$	
	206	$[M - OCH_2CH{=}CHCl - SCH_2CH_3]$	
	164	$[M - OCH_2CH{=}CHCl - CH_2CH(CH_3)SCH_2CH_3]$	61
clethodim sulfoxide	**376**	$[MH]^+$	100
	270	$[M - CH_2CH_3 - SOCH_2CH_3]$	
	206	$[M - OCH_2CH{=}CHCl - SOCH_2CH_3]$	34
	164	$[M - OCH_2CH{=}CHCl - CH_2CH(CH_3)SOCH_2CH_3]$	38
sethoxydim	**328**	$[MH]^+$	100
	282	$[M - OCH_2CH_3]$	28
	220	$[M - OCH_2CH_3 - SCH_2CH_3]$	
	178	$[M - OCH_2CH_3 - CH_2CH(CH_3)SCH_2CH_3]]$	46
sethoxydim sulfoxide	**344**	$[MH]^+$	100
	298	$[M - OCH_2CH_3]$	
	266	$[M - SOCH_2CH_3]$	
	220	$[M - OCH_2CH_3 - SOCH_2CH_3]$	24
	178	$[M - OCH_2CH_3 - CH_2CH(CH_3)SOCH_2CH_3]$	36

^{a} m/z values in bold were used for SIM.
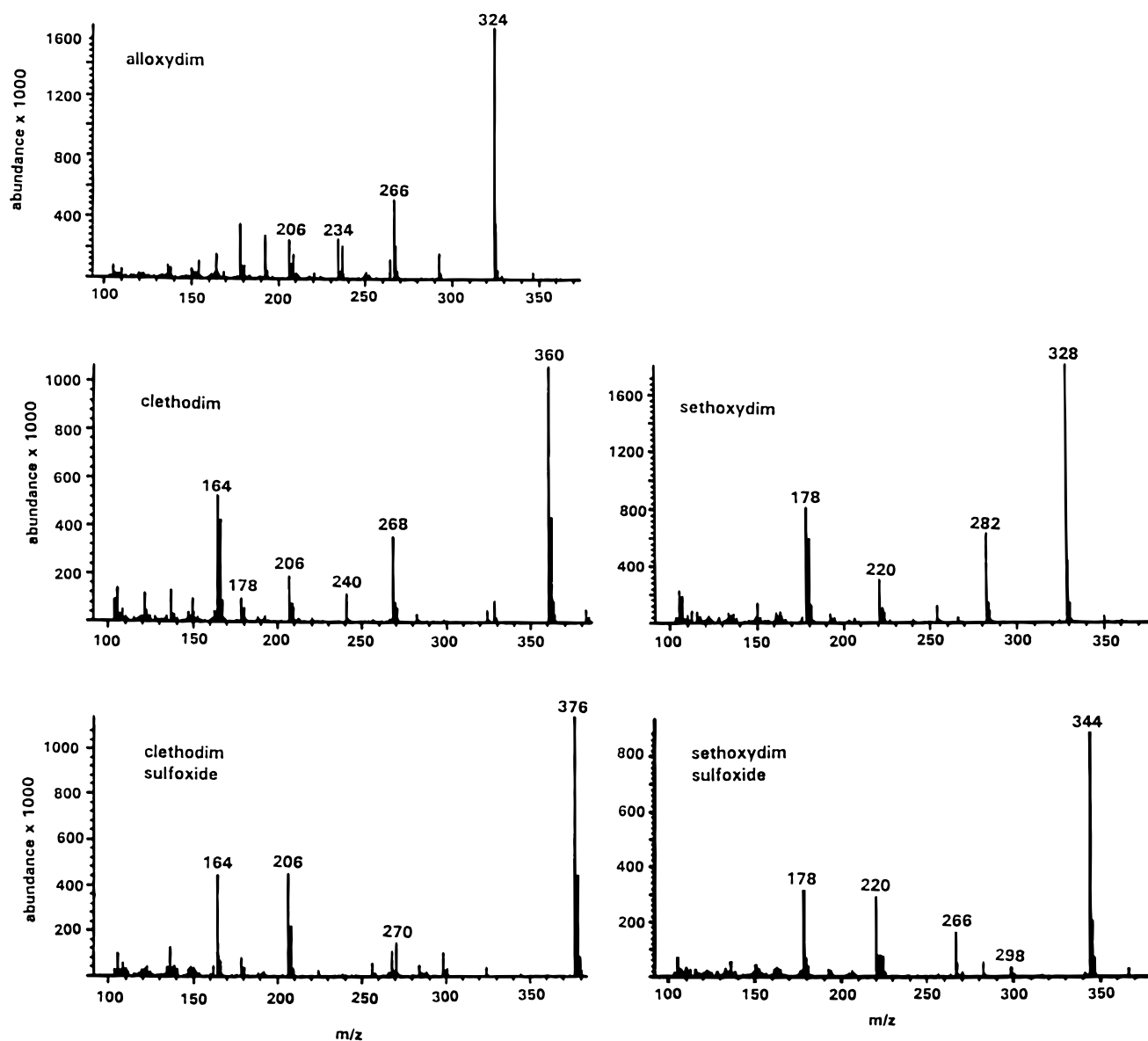
Figure 2. Positive mode electrospray mass spectra of alloxydim, clethodim, clethodim sulfoxide, sethoxydim, and sethoxydim sulfoxide.
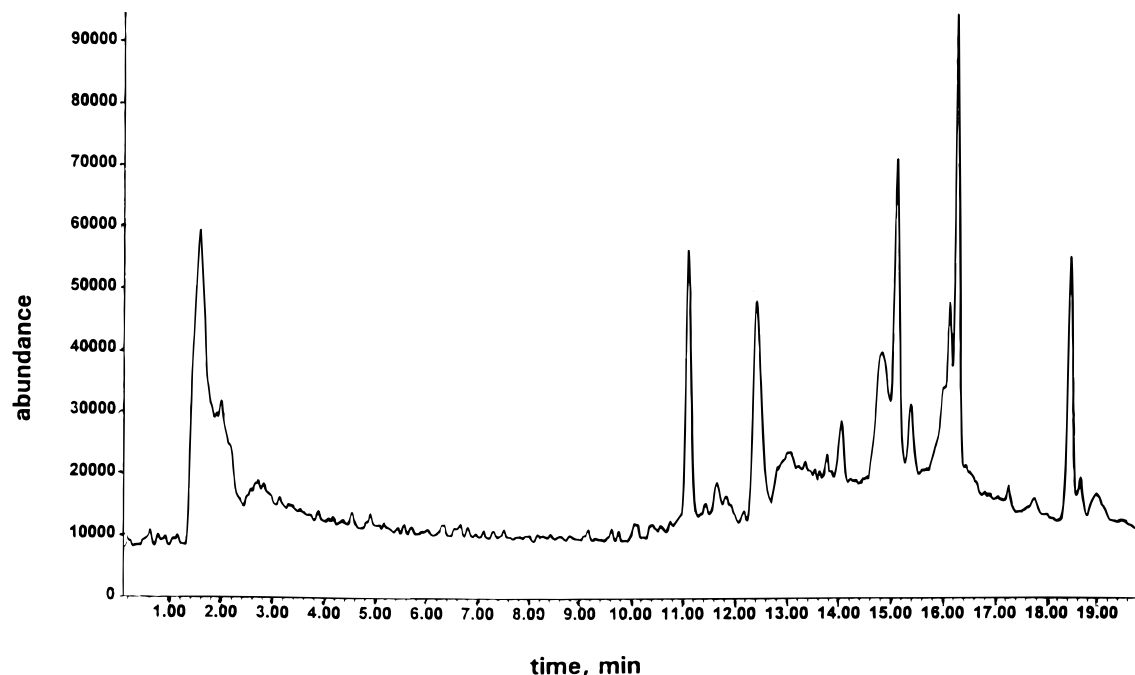
2800 *J. Agric. Food Chem.,* Vol. 48, No. 7, 2000

Marek et al.

Figure 3. Selected ion monitoring chromatogram of alloxydim, clethodim, and sethoxydim extracted from creek water spiked at 0.08 μg L^{-1}.
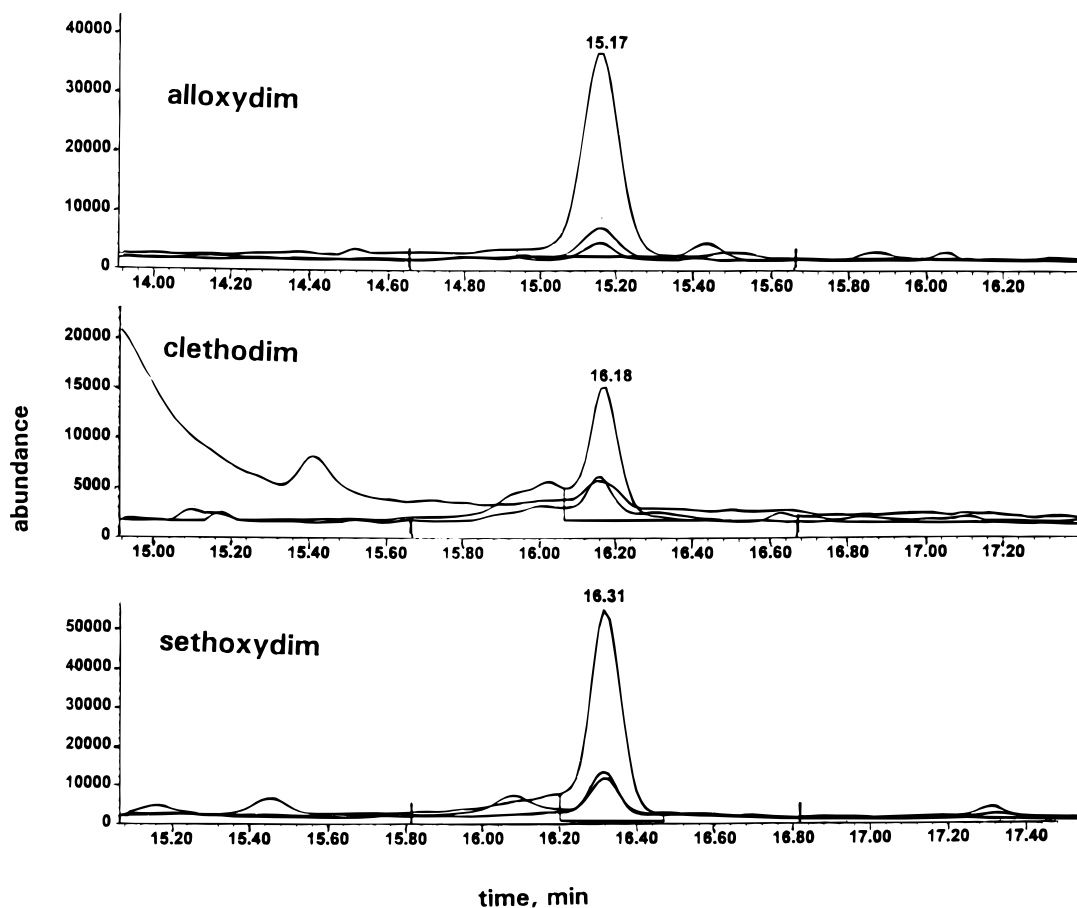

Figure 4. Extracted ion chromatograms of alloxydim, clethodim, and sethoxydim extracted from creek water spiked at 0.08 μg L^{-1}.

quantitation. In the present study, positive mode ES mass spectra of alloxydim (MW 323.4), clethodim (MW 359.9), clethodim sulfoxide (MW 375.9), sethoxydim (MW 327.5), and sethoxydim sulfoxide (MW 343.5) show the molecular ion [MH]$^+$ and numerous other fragments

(Figure 2, Table 1). The ions (*m/z*) in bold (Table 1) were monitored in SIM. The capillary exit and entrance voltages were selected to optimize formation of fragment ions while keeping [MH]$^+$ at 100% relative abundance. The relative abundance of fragment ions of the herbi-

LC/MS Analysis of Cyclohexanedione Oxime

J. Agric. Food Chem., Vol. 48, No. 7, 2000 **2801**

cides extracted from creek, lake, and distilled water samples were very similar to those found in a pure standard.

For confirmation of the chemicals, the criterion of retention times of the extracted analytes being ±2% of those of the corresponding standards was met. The retention times were (sethoxydim sulfoxide) 11.4 min, (clethodim sulfoxide) 11.9 min, (alloxydim) 15.2 min, (clethodim) 16.2 min, and (sethoxydim) 16.3 min. Favorable comparison of ion ratios (±30%) of three selected ions ([MH]$^+$ and two parent–daughter transitions at a signal-to-noise ratio (S/N) of >5) in the extracted samples and the known standards provided the second confirmation criterion.

Once confirmed, quantitation was based on the peak area of [MH]$^+$ for each chemical. No matrix effects (e.g., signal enhancement or suppression) were observed in creek water samples as compared to Nanopure water samples. The lack of matrix effects is presumably due to the C$_{18}$/SAX SPE cleanup steps. No concentration effects on ion ratios of the herbicides were observed at the two concentrations used in this study, 0.08 and 0.8 μg L^{-1}.

Average recoveries of eight samples of water (five distilled water, three creek water) spiked at 0.8 μg L^{-1} were (alloxydim) 117 ± 11%, (clethodim) 96 ± 14%, and (sethoxydim) 89 ± 13%. Comparable recoveries of triplicate samples of creek water spiked at 0.08 μg L^{-1} were obtained. A selected ion monitoring chromatogram (see Table 1 for ions) of the three herbicides spiked in creek water at 0.08 μg L^{-1} is shown in Figure 3. On the basis of extracted ion chromatograms (Figure 4), the minimum limit of quantitation (LOQ), assuming S/N >3, would be 0.08 μg mL^{-1} for clethodim, but could easily go lower for alloxydim and sethoxidim, ~0.04 μg L^{-1}.

Although we obtained quantitative recoveries of clethodim and sethoxydim, the less than 100% recovery could be due to either losses during SPE cleanup steps or decomposition. Clethodim and sethoxydim are labile compounds, easily oxidized to the sulfoxide and, under certain conditions, to the sulfone. However, using the present method for extraction and analysis, there was no evidence of oxidation of clethodim and sethoxydim to their respective sulfoxides. Therefore, losses, which are within acceptable recovery criteria, are attributed to the SPE steps of the method.

HPLC/UV. To further document benefits of using LC/ES/MS, we analyzed for the three parent herbicides by traditional HPLC/UV methods for comparisons to results from LC/ES/MS. Alloxydim, clethodim, and sethoxydim were extracted from water spiked at levels from 17 to 98 mg L^{-1} by C$_{18}$-SPE cartridges; recoveries averaged over concentrations were as follows: alloxydim, 116 ± 8%; clethodim, 83 ± 3%; sethoxydim, 86 ± 2%, similar to those using SPE/SAX SPE in the LC/ES/MS method. On the basis of the results from our low concentration, ~20 mg L^{-1}, it appears that we could have easily decreased the LOQ by decreasing the S/N (S/N was >10), increasing the amount of water extracted (we only extracted 60 mL of water), and decreasing the final solvent volume prior to placing the sample in the vial (the final sample solvent volume was 5 mL). However, no attempt was made to decrease the LOQ, since increasing the amount of water extracted would also increase the amount of interferences in the UV chromatogram and decreasing the final sample solvent

volume would increase the concentration of the interferences. Assuming we did not encounter these problems, our LOQ, ~1 mg L^{-1}, would be significantly higher than by LC/ES/MS. Also, decreasing the HPLC/UV LOQ would be a meaningless exercise without having appropriate confirmation.

Conclusions. We have developed a multiresidue method for the determination of alloxydim, clethodim, sethoxydim, and two metabolites, clethodim sulfoxide and sethoxydim sulfoxide, in water by LC/ES/MS. The method, which includes quantitative recoveries after C$_{18}$/SAX cleanup steps, has a limit of detection of <0.1 μg L^{-1}. There was no evidence of oxidation of clethodim and sethoxydim to their respective sulfoxides during the extraction and analysis. In contrast to analysis by HPLC/UV, LC/ES/MS provides the confirmation and LOQ necessary for a multiresidue method for environmental samples, and should also be appropriate for other cyclohexanedione oxime herbicides.

LITERATURE CITED

Buhler, D. D.; Burnside, O. C. Herbicidal activity of fluazifop-butyl, haloxyfop-methyl, and sethoxydim in soil. *Weed Sci.* **1984**, *32*, 824–831.

D'Ascenzo, G.; Gentili, A.; Marchese, S.; Perret, D. Determination of arylphenoxypropionic herbicides in water by liquid chromatography electrospray mass spectrometry. *J. Chromatogr.* **1998**, *813*, 285–297.

Falb, L. N.; Bridges, D. C.; Smith, A. E., Jr. Effects of pH and adjuvants on clethodim photodegradation. *J. Agric. Food Chem.* **1990**, *38*, 875–878.

Falb, L. N.; Bridges, D. C.; Smith, A. E., Jr. Separation of clethodim herbicide from acid and photodegradation products by liquid chromatography. *J.—Assoc. Off. Anal. Chem.* **1991**, *74*, 999–1002.

Hsiao, A. I.; Smith, A. E. A root bioassy procedure for the determination of chlorsulfuron, diclofop acid, and sethoxydim residues in soil. *Weed Res.* **1983**, *23*, 231–236.

Koskinen, W. C.; Reynolds, K. M.; Buhler, D. D.; Wyse, D. L.; Barber, B. L.; Jarvis, L. J. Persistence and movement of sethoxydim residues in three Minnesota soil. *Weed Sci.* **1993**, *41*, 634–640.

Lagana, A.; Fago, G.; Marino, A.; Mosso, M. Soil column extraction followed by liquid chromatography and electro-spray ionization mass spectrometry for the efficient determination of arylphenoxypropionic herbicides in soil samples at ng g^{-1} levels. *Anal. Chim. Acta* **1998**, *375*, 107–116.

Marek, L. J.; Koskinen, W. C. LC/MS analysis of 4-methoxy-6-methyl-1,3,5-triazin-2-yl containing sulfonylurea herbicides in soil. *J. Agric. Food Chem.* **1996**, *44*, 3878–3881.

Shoaf, A. R.; Carlson, W. C. Analytical techniques to measure sethoxydim and breakdown products. *Weed Sci.* **1986**, *34*, 745–751.

Shoaf, A. R.; Carlson, W. C. Stability of sethoxydim and its breakdown products in solution, in soil, and surfaces. *Weed Sci.* **1992**, *40*, 384–389.

Volmer, D.; Wilkes, J. G.; Levsen, K. Liquid chromatography/mass spectrometry multiresidue determintaion of sulfonylureas after on-line trace enrichment. *Rapid Commun. Mass Spectrom.* **1995**, *9*, 767–771.