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Simultaneous Quantification of Acetanilide Herbicides and Their Oxanilic and Sulfonic Acid Metabolites in Natural Waters

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This paper describes a procedure for simultaneous enrichment, separation, and quantification of acetanilide herbicides and their major ionic oxanilic acid (OXA) and ethanesulfonic acid (ESA) metabolites in groundwater and surface water using Carbopack B as a solid-phase extraction (SPE) material. The analytes adsorbed on Carbopack B were eluted selectively from the solid phase in three fractions containing the parent compounds (PCs), their OXA metabolites, and their ESA metabolites, respectively. The complete separation of the three compound classes allowed the analysis of the neutral PCs (acetochlor, alachlor, and metolachlor) and their methylated OXA metabolites by gas chromatography/mass spectrometry. The ESA compounds were analyzed by high-performance liquid chromatography with UV detection. The use of Carbopack B resulted in good recoveries of the polar metabolites even from large sample volumes (1 L). Absolute recoveries from spiked surface and groundwater samples ranged between 76 and 100% for the PCs, between 41 and 91% for the OXAs, and between 47 and 96% for the ESAs. The maximum standard deviation of the absolute recoveries was 12%. The method detection limits are between 1 and 8 ng/L for the PCs, between 1 and 7 ng/L for the OXAs, and between 10 and 90 ng/L for the ESAs.

Acetanilide herbicides are one of the major classes of pesticides applied worldwide. The detoxification pathway from plants and soil microorganisms via glutathione conjugation forms ionic and highly water-soluble metabolites.[1] These oxanilic acid (OXA) and ethanesulfonic acid (ESA) derivatives (for structures see Figure 1) have been found in groundwater in the United States with more frequency and at higher concentrations than their parent compounds.[2,3] Consequently, the possible pollution and accumulation of these compounds in the environment must be studied. Thus, analytical methods for the detection of the parent acetanilide herbicides should include the OXA and ESA metabolites. A multiresidue trace analytical method will allow researchers (i) to monitor these compounds in groundwater, surface water, and drinking water, (ii) to study the fate and behavior of these substances, and (iii) to assess their effects on ecosystems. Although Swiss water samples do not currently contain these compounds in high concentrations, it is known that the application of acetanilide herbicides in Switzerland is increasing.

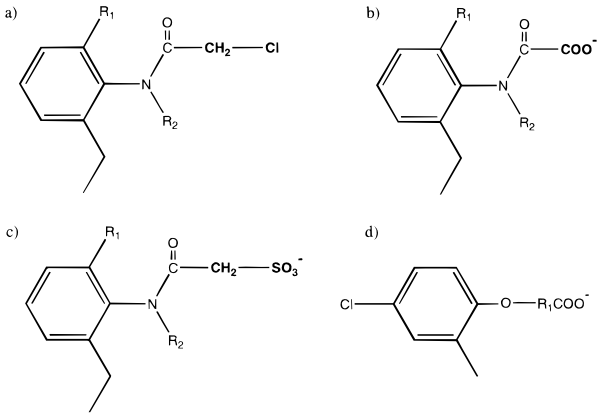

Figure 1. Structures of the parent acetanilide herbicides (a), their oxanilic acid (b) and ethanesulfonic acid (c) metabolites, and the volumetric standards (d), used for GC/MS and HPLC quantification. The substituents R_1 and R_2 are shown in Table 1.

The development of an SPE and separation method for the *simultaneous* analysis of nine target compounds, i.e., alachlor, acetochlor, and metolachlor and their corresponding OXA and ESA derivatives, is a challenging task because of the following reasons. Two of the compounds and their derivatives, i.e., alachlor and acetochlor, are very *similar* in structure and have identical molecular masses. Further, the hindered rotation around the amide bond of the metabolites leads to diastereomers[4] which are partially separated by HPLC at room temperature. Therefore, HPLC has to be carried out at elevated temperatures to obtain one single peak for each metabolite.[5] Further, due to the similarity

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of alachlor metabolites and acetochlor metabolites, some of the compounds coelute. On the other hand, the neutral parent compounds and the ionic metabolites exhibit very *different* physicochemical properties. For example, using C18 material in the SPE procedure allowed only the enrichment of the ESA derivatives from a small sample volume (20 mL) without large breakthrough.[5] Some attempts have been made to overcome these problems. An HPLC method with mass spectrometric (MS) detection was developed by Ferrer et al.[6] This method allowed the quantification of all oxanilic acid metabolites and metolachlor ESA. Even though acetochlor OXA and alachlor OXA were not separated chromatographically, they could be individually quantified by MS due to different fragmentations. However, the coeluting acetochlor and alachlor ethanesulfonic acids have identical fragmentation patterns and could not be separately quantified. The LC/MS/MS method, recently published by Vargo,[7] allowed the quantification of all ethanesulfonic acid metabolites but did not include the oxanilic acid metabolites.

The objective of this work was to develop an analytical method for the simultaneous enrichment of parent acetanilide herbicides (acetochlor, alachlor, and metolachlor) and their OXA and ESA derivatives and the quantification of each of these compounds in groundwater and surface water in a concentration range of a few nanograms per liter to micrograms per liter. Therefore, the dual-mode (hydrophobic interactions for the neutral compounds and cationic sites for the metabolites) SPE material Carbopack B was evaluated for (i) the simultaneous enrichment and (ii) the step by step selective elution of the parent compounds (first), the OXA derivatives (second), and the ESA derivatives (third). This approach allowed the quantification of the parent compounds and the OXA derivatives (after methylation) by GC/MS and the pure ESA fraction by HPLC.

EXPERIMENTAL SECTION

Materials. Metolachlor, alachlor, acetochlor, and MCPB (4-(4-chloro-2-methylphenoxy)butanoic acid), which was used as the volumetric standard for HPLC quantification, are commercially available from Riedel-de Haën (Seelze, Germany). The $^{13}C_6$-ring-labeled metolachlor from Cambridge Isotope Laboratories (Andover, MA) and the trideuterio-ring-labeled MCPA ((4-chloro-2-methylphenoxy)acetic acid), purchased from Dr. Ehrenstorfer (Augsburg, Germany), were used as volumetric standards for GC/MS quantification of PCs and OXAs, respectively. Standards of alachlor ESA, alachlor OXA, and acetochlor OXA were obtained from Monsanto Chemical Co. (St. Louis, MO). Metolachlor ESA and metolachlor OXA were donated by Novartis (Basle, Switzerland), and acetochlor ESA was taken from an earlier work.[4] Carbopack B cartridges (Supelclean ENVI-Carb SPE tubes, 6 mL, 0.25 g) were purchased from Supelco (Bellefonte, PA). Methanol (MeOH), methylene chloride ($MeCl_2$), and ethyl acetate (EA), all HPLC grade, and ascorbic acid (>99.5%) were obtained from Fluka AG (Buchs, Switzerland). HCl (37%), chloroacetic acid (ClA, >98%), and ammonium acetate (AA, >98%) were purchased from Merck (Darmstadt, Germany). The nitrogen gas (99.995%) was from Carbagas (Rümlang, Switzerland). All chemicals were used as obtained; standard stock solutions were prepared in methanol. Diazomethane (approximately 0.4 M in diethyl ether) was produced in our laboratory as described by de Boer and Backer[8] and stored at −20 °C for no longer than 1 week; residues were destroyed by adding acetic acid. ***Caution! Special care is required in the handling of diazomethane because it is carcinogenic and, under certain conditions, explosive. All procedures should be carried out in a hood and with great care.***

Sampling and Sample Preparation. Lake water samples were collected from Greifensee (northeast of Zürich, Switzerland) and Murtensee (close to Bern, Switzerland; for details see Müller et al.[9]). Groundwater samples were taken from the catchment area of Greifensee and Murtensee. All samples were stored at 4 °C in the dark. Prior to SPE, surface water samples were filtered with cellulose nitrate filters, ∅ 50 mm, pore size 0.45 μm (Sartorius, Goettingen, Germany), to avoid clogging of the SPE cartridges. The exact volumes (about 1 L) of all samples were determined. For recovery studies, groundwater and lake water samples were spiked with standard mixtures of all investigated compounds and the resultant samples were shaken vigorously before extraction.

Sample Concentration and Preseparation by SPE. After filtration, the samples were extracted according to Berg et al.[10] The analytes were eluted sequentially from the cartridge. First, the PCs were eluted with 1 mL of MeOH and 6 mL of $MeCl_2$/MeOH (80:20; v/v) (eluent I). Second, the OXAs were eluted with 6 mL $MeCl_2$/EA (80:20; v/v) which was acidified with 50 mM ClA (eluent II). Third, the ESAs were eluted with 6 mL of $MeCl_2$/MeOH (80:20; v/v) containing 50 mM AA (eluent III). Each fraction was collected in conical 7.5 mL reaction vessels from Supelco (Bellefonte, CA) and concentrated by evaporating the solvent with a gentle nitrogen stream at 40 °C to volumes of 100 ± 50 μL for fractions 1 and 2, and <100 μL for fraction 3. For the determination of absolute recoveries, 75 ng of metolachlor-$^{13}C_6$ was added to the first fraction as a volumetric standard for GC/MS quantification (besides their functions as volumetric standards, metolachlor-$^{13}C_6$ and MCPA-d_3 are suitable internal standards for quantification of PCs and OXAs, respectively). To remove the remaining MeOH, 300 μL of EA was added and the volume was again reduced to 200 ± 50 μL. After addition of 75 ng of MCPA-d_3 as the volumetric standard, the second fraction was derivatized with diazomethane. Derivatization was carried out as described by Bucheli et al.[11] by slowly adding diazomethane to the extract until the yellow color of the derivatization reagent was maintained for at least 15 min. Then the volume was carefully reduced again to 200 ± 50 μL before the solution was passed through a 0.45 μm filter (Spartan 13) from Schleicher & Schuell (Kassel, Germany). A 500 ng quantity of MCPB, a compound that is not found in natural water samples in Switzerland, was added to the third fraction as the volumetric standard before the extract was dissolved in 200 μL of 25 mM phosphate buffer (pH 7)/MeOH (75:25; v/v). Because of the different adsorption behaviors of carboxylic and sulfonic acids on Carbopack B, MCPB is not

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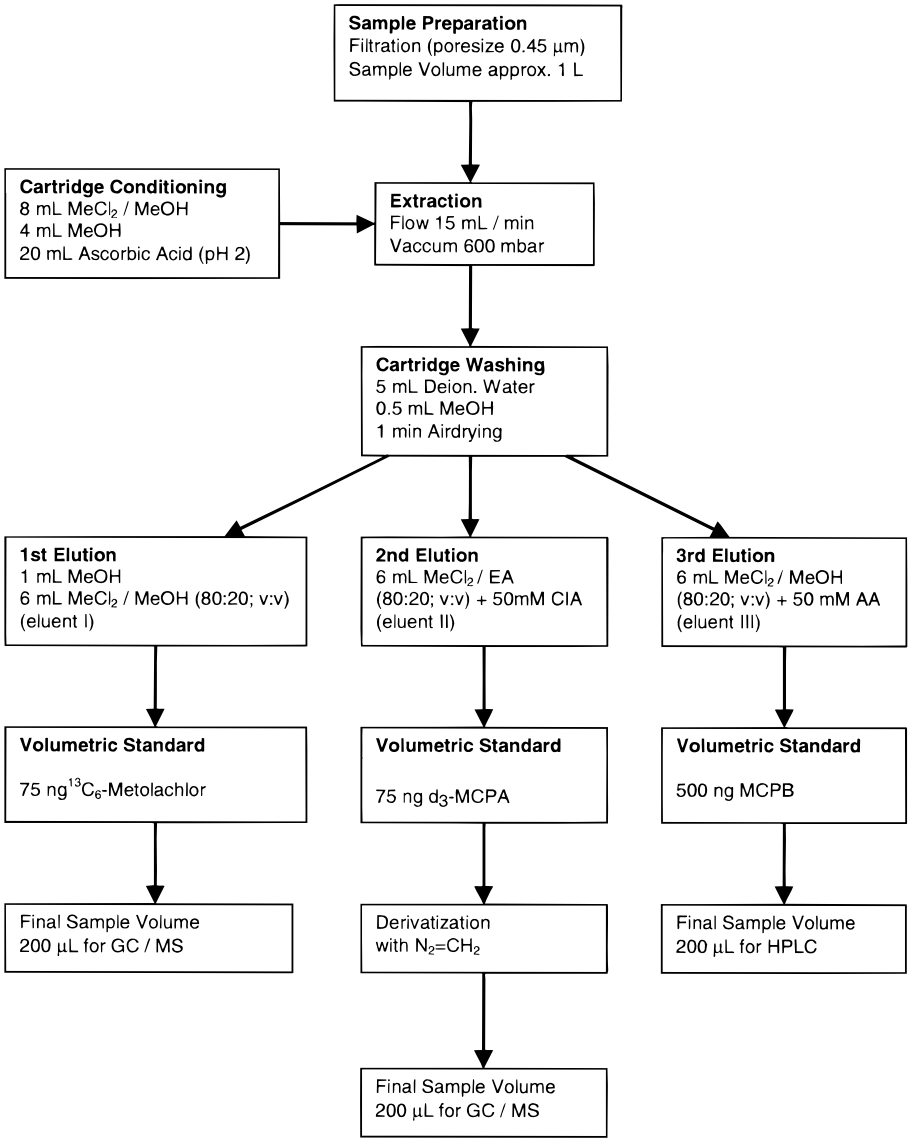

Figure 2. Schematic description of the solid-phase extraction (SPE) procedure.

suitable as an internal ESA standard. The sample was filtered in the same manner as the second fraction, and the filtrate was placed in a 200 μL HPLC insert vial. The SPE procedure is schematically described in Figure 2.

Separation and Detection with GC/MS. The instrumental setup consisted of a GC 8060 instrument with an autosampler (A 200 S) and a quadrupole MD 800 detector, all from Fisons Instruments (Beverly, MA). A 2 μL portion of the extract was injected in the splitless/split mode at 250 °C. The split was opened after 1 min. All compounds were separated on an RTX-5MS column (DB5 equivalent, 30 m, 0.25 mm i.d., 0.25 μm film thickness) and a deactivated precolumn (3 m, 0.32 mm i.d.) with the following temperature program: initial temperature of 100 °C for 1 min; temperature gradient of 10 °C/min to 180 °C, which was held for 25 min, followed by a gradient of 30 °C/min to 260 °C; final temperature (260 °C) held for 5 min. Full-scan spectra were acquired to select two or three major ions per compound for further single-ion monitoring. After it was established that there were no overlapping peaks or matrix effects from natural water samples, the ion with the highest abundance for each compound was used for quantification (see Table 1).

Separation and Detection of ESA Metabolites by HPLC. The HPLC system consisted of an autosampler (Gina 50), a high-precision pump (model 480), and a diode array detector (UVD 340), all from Gynkotek (Germering b. München, Germany). A Discovery column (150 × 4.6 mm) from Supelco (Bellefonte, PA) was used for separating the compounds. The mobile phase was a 25 mM phosphate buffer (pH 7)/methanol/acetonitrile mixture (70:20:10; v/v/v), which was prepared and filtered through a nylon membrane, Ø 47 mm, pore size 0.45 μm, from Alltech Associates (Deerfield, IL), immediately before use. After injection of 20 μL of the extract, an isocratic elution with a 1 mL/min flow rate was carried out. The column was kept at 60 °C (for details see ref 4). The quantification wavelength was 205 nm.

Calibrations and Determination of Absolute Recoveries. For each compound class, an external three-point calibration curve was prepared. The levels were 25, 250, and 500 ng of the PCs dissolved in 200 μL of EA with 75 ng of metolachlor-$^{13}C_6$ as the

Table 1. Structures, Retention Times, and Monitored Ions of the Investigated Compounds

compound	substituent[a]		retention time (min)			acquired ions (*m*/z)	
	R_1	R_2	PCs[b]	OXAs[b]	ESAs[c]	PCs[e]	OXAs[d,e]
acetochlor	CH_3	$CH_2OCH_2CH_3$	26.3	24.3	16.2	**146**, 162, 174	146, 162, **174**
alachlor	CH_2CH_3	CH_2OCH_3	27.7	25.5	15.1	146, **160**, 188	146, 160, **188**
metolachlor	CH_3	$CH(CH_3)CH_2OCH_3$	33.7	30.5	17.6	162, **238**	146, 188, **248**
metolachlor-$^{13}C_6$[f]	CH_3	$CH(CH_3)CH_2OCH_3$	33.7			168, **244**	
MCPA-d_3[g]	CH_2			13.1			**217**, 219
MCPB[h]	$CH_2CH_2CH_2$				18.8		

[a] Substituents of the structures in Figure 1. [b] Acquired by GC/MS. [c] Acquired by HPLC. [d] Masses of the methyl esters of the OXA compounds. [e] Bold numbers: ions used for quantification. The traces of the quantifier masses of a groundwater sample, spiked with 25 ng/L of all PCs and OXAs, are shown in Figure 3. [f] Ring-labeled volumetric standard for acetanilide parent compounds. [g] Ring-labeled volumetric standard for oxanilic acid metabolites. [h] Volumetric standard for ethanesulfonic acid metabolites. Chromatograms of a spiked groundwater and a spiked lake water sample are shown in Figure 4.

volumetric standard. For the OXAs, 25, 250, and 500 ng were used with 75 ng of MCPA-d_3 as the volumetric standard. The compounds were derivatized, and the volumes were reduced. Then, EA (approximately 200 μL) was added, and the solutions were filtered as described above. The ESA calibration was made with 250, 500, and 1000 ng levels, and to each vial was added 500 ng of MCPB as the volumetric standard. These calibration standards were reconstituted in 200 μL of 25 mM phosphate buffer (pH 7)/MeOH (75:25; v/v).

For the determination of absolute recoveries of PCs, OXAs, and ESAs in natural waters, two groundwater and two surface water samples from different sources were (i) analyzed as collected and (ii) spiked with 25 and 250 ng of PCs and OXAs and with 250 and 1000 ng of ESAs. The unfortified samples of these groundwaters and surface waters served as blanks. Triple samples were extracted, and each sample was measured once by GC/MS (PCs and OXAs) and once by HPLC (ESAs).

RESULTS AND DISCUSSION

SPE Enrichment and Sequential Elution of Acetanilides and Their OXA and ESA Metabolites. The SPE methods for the analysis of neutral (acetanilides and triazines) and acidic pesticides (phenoxy acids), developed by Bucheli et al.,[11] which allowed the sequential elution of the neutral pesticides and the acidic phenoxy acids, and the method of Altenbach et al.,[12] which allowed the enrichment and elution of sulfonated compounds on Carbopack B, were used as starting points for this work. The function of Carbopack B as a sorbent to isolate neutral and ionic analytes from water samples is based on hydrophobic and electrostatic interactions. The ionic interactions of the OXAs and the ESAs with the sorbent are stronger than the hydrophobic interactions of the neutral compounds. This difference was used to elute the neutral parent herbicides acetochlor, alachlor, and metolachlor (PCs) first with a neutral solvent mixture. For elution of the OXAs, the eluent $MeCl_2$/EA was acidified with chloroacetic acid (ClA). We found that this gives good recoveries for the OXAs without early elution of the ESAs. This can be explained by (i) the acidification of eluent II (the pK_a of ClA is 2.86) leading to protonated neutral OXA compounds and/or (ii) the competition of $ClCH_2COO^-$ anions with the OXAs at the cationic sites of the Carbopack B surface. The ethanesulfonic acids (pK_a < 1) show

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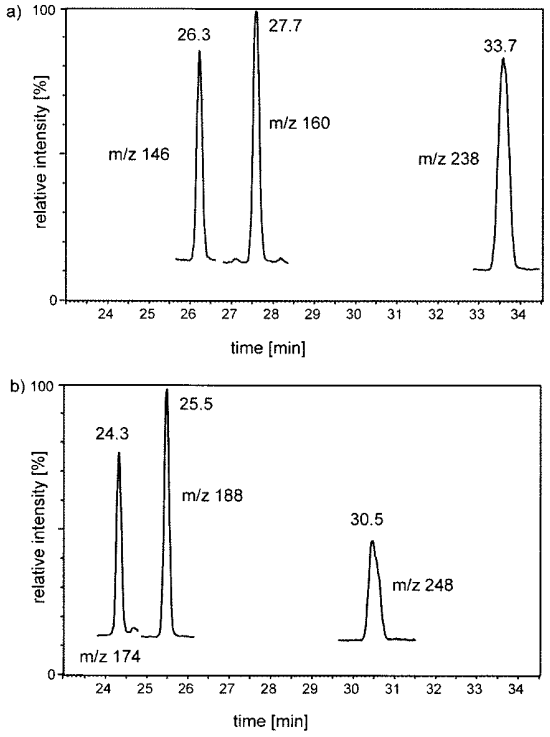

Figure 3. Quantification mass traces of the parent acetanilides (a) and their oxanilic acid metabolites (b), acquired from a groundwater sample, spiked with 25 ng/L of all PCs and OXAs. For details see Table 1.

even stronger electrostatic affinities for the SPE material and hence are retained well under these conditions. Protonation of these compounds would need a very strong acid. Therefore, ammonium acetate was added to $MeCl_2$/MeOH, which leads to acetate anions competing with the sulfonic acids on the cationic sorption sites and NH_4^+ ions allowing ion-pairing with the sulfonic acids which are then displaced from the sorbent surface.

Separation and Detection of Parent Acetanilides and OXA Metabolites with GC/MS. For quantification of PCs and derivatized OXAs, a GC/MS method was developed. All acquired quantification and qualifier masses of the PCs and methylated OXAs and the corresponding retention times are given in Table 1. Figure 3 shows the mass traces of the quantification ions of all PCs and OXAs in a groundwater sample spiked with 25 ng/L of each compound. The shoulder of the metolachlor OXA peak (see Figure 3b, *m*/*z* 248) is the result of the partial separation of

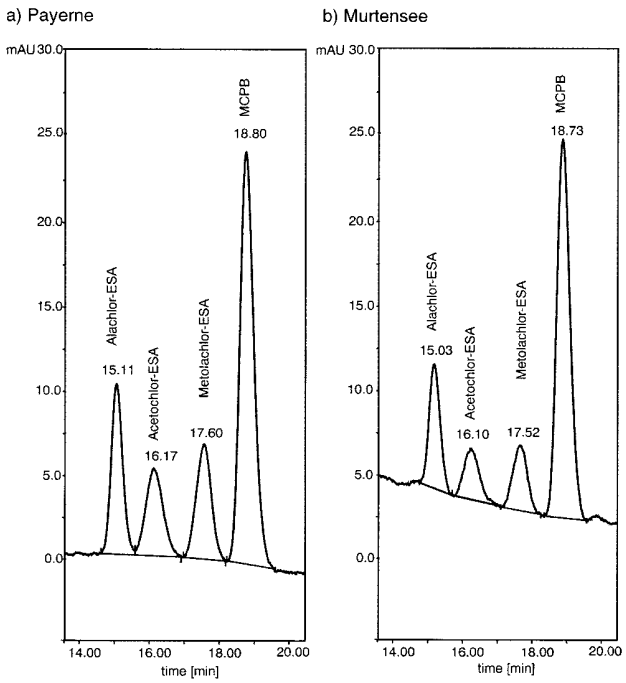

Figure 4. HPLC chromatograms of a groundwater sample (a) and a surface water sample (b). Both samples were spiked with 250 ng/L before extraction. All ESA compounds are baseline separated. The fourth peak (MCPB) refers to the internal standard used for quantification.

diastereomers of this compound.[4] However, the quantification of metolachlor OXA is thereby not affected because the total integrated area was used for calculations. Although the PCs and OXAs could be separated completely by GC/MS, simultaneous elution of PCs and OXAs in SPE was not desired because significant losses of PCs were observed during derivatization of OXAs with diazomethane (data not shown). Thus, it was essential to separate PCs and OXAs before derivatization. Furthermore, because of overlapping peaks in HPLC, the OXAs were preseparated from the ESAs by sequential elution and derivatized for GC/MS analysis, to provide not only lower detection limits but also for the unequivocal identification and individual quantification of all compounds.

External calibration proved to be linear within 5−500 ng/200 μL for all PCs and within 50−5000 ng/200 μL for all OXAs. As approximately 200 μL is the final extract volume of a 1 L water sample, the linear range of the instrumental analysis is at least up to 500 ng/L for PCs and 5000 ng/L for OXAs.

Separation and Detection of ESA Metabolites with HPLC. As no derivatization procedure for trace determination of sulfonic acids was available, separation of ESAs was achieved by reversed-phase HPLC. Different columns, mobile phases, and temperatures were examined. Complete separation of all ESAs and the volumetric standard MCPB was achieved by an isocratic elution. Heating the column to 60 °C was essential because at ambient temperature the diastereomers of the compounds are partly separated, resulting in broad peaks (for details see ref 4). Figure 4 shows the chromatograms of spiked groundwater and surface water samples. The linear range of the HPLC analysis for the ESAs was between 200 and 5000 ng/200 μL (equivalent to the extract volume) using an external calibration curve.

Absolute Recoveries, Precisions, and MDLs. *Groundwater.* Table 2 summarizes the absolute recoveries of parent compounds, oxanilic acids, and ethanesulfonic acids from groundwater samples. These are within 70−96% for all compounds. Further, no significant difference in recoveries between the lower and the higher spike levels were observed. With external calibration, high precision of the whole method was achieved for all analytes of interest. In general, the standard deviations of three equivalent water samples were below 13%, often below 5%. This result is also reflected in the low method detection limits (MDLs) of <10 ng/L for PCs and OXAs and <100 ng/L for ESAs, respectively. Like the recoveries, the precisions are comparable for higher and lower concentrations.

Surface Water. The absolute recoveries of the neutral compounds from surface water samples were within 80−100%, comparable to the results from groundwater (see Table 2). However, in contrast to the recoveries of the groundwater samples, reduced recoveries for OXAs and ESAs, of 41−80% and 47−76%, respectively, were observed for surface waters. Additional experiments revealed that breakthrough of the OXAs and ESAs during the enrichment procedure as well as early elution of the OXA compounds with the parent compounds and the ESA compounds with the OXA compounds were the reasons for the lower recoveries. This can be explained by the DOC content of surface waters (approximately 10 mg/L in our samples), which competes with the anionic metabolites for the cationic sites. However, even for surface water samples and with external calibration, high precision of the method was achieved for all investigated compounds. Standard deviations below 12%, often below 5%, led to calculated method detection limits (MDLs) below 5 ng/L for PCs and below 10 and 60 ng/L for OXAs and ESAs, respectively. Also, in surface water, the precisions are in the same range for higher and lower concentrations. All data are shown in Table 2.

To compensate for losses during the enrichment and/or elution steps, metolachlor-$^{13}C_6$ and MCPA-d_3 could be used as internal standards for the entire analytical procedure. Unfortunately, no appropriate internal standard is currently available for ESA. Therefore, standard additions are indicated for quantification of ESA compounds if high contents of DOC are expected.

The use of 1 L water samples for extraction is important to compensate for the poor detection limit of the HPLC/UV system. The use of Carbopack B is essential not only for sequential elution but also for efficient recovery of the polar OXA and ESA compounds from large sample volumes.

CONCLUSIONS

The analytical procedure presented here illustrates the advantages of simultaneous enrichment of neutral and ionic compounds on Carbopack B and the selective, sequential elution of neutral, weakly acidic, and strongly acidic compounds into three fractions. The novelty of the method further consists of the application of GC/MS analysis not only to PCs but also to the carboxylic acid metabolites, which have not been measured by GC/MS to date. The derivatization with diazomethane enables their gas chromatographic separation without overlapping peaks, followed by MS detection, at a concentration below 10 ng/L for each compound.

Using a new column type, reproducible and stable baseline separation of the sulfonic acid metabolites was achieved by HPLC. Although the HPLC/UV technique does not achieve the sensitivity of GC/MS detection, the use of 1 L water samples alleviates this

Table 2. Absolute Recoveries, Standard Deviations (SDs), and Method Detection Limits (MDLs) in Groundwater and Surface Water ($n = 3$)[a]

		groundwater 1		groundwater 2		surface water 1		surface water 2	
compound	spike level (ng/L)	abs rec (SD) (%)	MDL (ng/L)	abs rec (SD) (%)	MDL (ng/L)	abs rec (SD) (%)	MDL (ng/L)	abs rec (SD) (%)	MDL (ng/L)
PCs									
acetochlor	0	n.d.		n.d.		n.d.		n.d.	
	25	81 (10)	8	88 (1)	1	80 (1)	1	85 (3)	2
	250	84 (3)		76 (5)		81 (4)		83 (6)	
alachlor	0	n.d.		n.d.		n.d.		n.d.	
	25	82 (9)	7	88 (2)	2	80 (3)	2	84 (4)	3
	250	84 (2)		77 (5)		81 (3)		84 (5)	
metolachlor	0	n.d.		n.d.		7[b] (0)	<1	n.d.	
	25	86 (4)	3	92 (3)	3	100 (2)		85 (4)	3
	250	93 (5)		84 (7)		87 (3)		87 (4)	
OXAs									
acetochlor OXA	0	n.d.		n.d.		n.d.		n.d.	
	25	91 (1)	1	82 (3)	2	62 (4)	3	53 (11)	5
	250	84 (4)		73 (5)		59 (2)		41 (4)	
alachlor OXA	0	n.d.		n.d.		n.d.		n.d.	
	25	88 (1)	1	80 (2)	2	64 (5)	4	51 (9)	6
	250	79 (5)		70 (5)		58 (3)		44 (4)	
metolachlor OXA	0	n.d.		n.d.		n.d.		n.d.	
	25	89 (1)	1	79 (3)	3	80 (4)	3	66 (10)	7
	250	76 (3)		70 (3)		64 (3)		51 (3)	
ESAs									
acetochlor ESA	0	n.d.		n.d.		n.d.		n.d.	
	250	82 (3)	20	78 (10)	80	50 (7)	50	47 (2)	10
	1000	92 (6)		86 (4)		71 (1)		56 (8)	
alachlor ESA	0	n.d.		n.d.		n.d.		n.d.	
	250	87 (4)	30	96 (10)	80	71 (5)	40	70 (2)	20
	1000	89 (6)		87 (4)		76 (0)		62 (5)	
metolachlor ESA	0	n.d.		n.d.		n.d.		n.d.	
	250	82 (4)	30	81 (12)	90	55 (6)	50	59 (8)	60
	1000	86 (6)		72 (3)		65 (1)		54 (4)	

[a] Method detection limits were derived from 3 times the SDs of identically spiked samples. For PCs and OXAs, the 25 ng/L level was considered, and for ESAs, the 250 ng/L level was considered. For PCs and OXAs, values were rounded to the next 1 ng/L, and for ESAs, values were rounded to the next 10 ng/L. n.d. = not detected. Groundwater 1: Payerne (catchment area of Murtensee). Groundwater 2: Oetwil (catchment area of Greifensee). Surface water 1: Murtensee. Surface water 2: Greifensee. [b] In just one unfortified surface water sample metolachlor was detected; this value was used for the MDL determination.

drawback. In contrast to previous methods,[6] all compounds could be separated, identified, and quantified individually using the three-step sequential elution. Another advantage of this method is the resulting clean extract for HPLC analysis. Due to two previous elution steps, the third eluate contains significantly lower interfering substances, which could otherwise obscure the results. Thus, the reliability of UV detection is enhanced and the method detection limits are improved. The MDLs for all ESAs are below 100 ng/L in natural water samples. In practice, this method should prove to be useful in the analysis of ESA metabolites which occur in higher concentrations in the environment than PCs and OXAs.[2,3,13] Furthermore, the SPE method will be valuable for investigations involving the enantioselectivity of the metabolism of acetanilide herbicides in the environment.[14] The separation and identification of the enantiomers and diastereomers of the acetanilides and their metabolites has been achieved with nuclear magnetic resonance spectroscopy and capillary zone electrophoresis (CZE).[4] For natural samples, a preseparation into compound classes as described here followed by CZE will result in fewer peaks in the electropherograms and will facilitate the identification and quantification of analytes.

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NOTE ADDED IN PROOF

Only recently we obtained the oxanilic acid and ethanesulfonic acid metabolites of dimethenamid, another important acetanilide herbicide from the manufacturer. We are currently trying to quantify these compounds with the method described above. Such a method would allow the simultaneous quantification of the four most important acetanilide herbicides and their most important metabolites in natural waters.

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