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Determination of Sulfonylurea Herbicides in Water and Food Samples Using Sol−Gel Glass-Based Immunoaffinity Extraction and Liquid Chromatography−Ultraviolet/Diode Array Detection or Liquid Chromatography-Tandem Mass Spectrometry

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Immunoaffinity supports (IAS) were prepared using broad specific polyclonal anti-sulfonylurea (SU) antibodies immobilized in sol−gel glass. Two different kinds of supports were applied, crushed sol−gel monoliths and sol−gel-coated highly porous silica particles. Both were used for the quantitative enrichment of SUs in natural water and food samples followed by high-performance liquid chromatography−ultraviolet/diode array detection (HPLC-UV/DAD) and tandem mass spectrometry (LC-MS/MS), respectively. Loading, washing, and elution conditions of IAS were optimized. The capacity of supports was determined for 30 SUs and compared with the cross-reactivity pattern of the direct competitive enzyme-linked immunosorbent assay. The capacities correlated well with the affinity to individual SU compounds. Even analytes to which the polyclonal antibodies showed only a lower cross-reactivity could be enriched to a certain degree, if a sufficient capacity of IAS was provided. The IAS could be reused at least 10 times without a loss of effectiveness. Recovery of 16 selected SUs extracted from spiked water and food samples was dependent on the affinity of both immobilized antibodies to single compounds and matrix interferences. In water, 13 SUs showed recoveries higher than 80% when immunoaffinity extraction was used in combination with LC-UV/DAD. On the basis of the enrichment of 200 mL of aqueous sample, corresponding limit of detection (LOD) values ranged between 20 and 100 ng/L. The recoveries of 10 SUs, which were extracted from 10 g of potato spiked at a 10 μg/kg level, were higher than 75%. For grain samples, recoveries were at the same order for at least five SU herbicides. The LOD of LC-MS/MS measurements was about 1 order of magnitude higher, i.e., gave LODs between 1.1 and 6.9 μg/kg of food sample, depending on the compound and extraction procedure. These LODs provide evidence that the main advantage of the prepared IAS is their high selectivity for group specific recognition of SUs as compared to other nonspecific solid phase extraction materials.

KEYWORDS: Immunoaffinity supports; sol−gel glass; sulfonylurea herbicides; water samples; food samples; LC-UV/DAD; LC-MS/MS

INTRODUCTION

Sulfonylureas (SUs) are a class of herbicides introduced in 1982 by Dupont Agricultural Products (*1*). They are used for weed control in cereals such as wheat, barley, oats, rice, corn, and other crops such as potatoes, sugar beets, and turnips. As compared with other herbicides, SUs have much lower application ranges, situated between 10 and 100 g of active ingredient per hectare. In addition, they show a more rapid degradation in the environment. Therefore, the concentration of SUs usually found in environmental and food samples is about 100−1000-fold lower as compared to other herbicides. The molecular structures of SUs are very similar, and they may be present as a mixture of several compounds in commercial formulations, e.g., metsulfuron/flupyrsulfuron (Ciral) and metsulfuron/thifensulfuron (Concert). For these reasons and because of their chemical and thermal instability, simultaneous monitoring of a series of these herbicides in environmental and food samples is a particularly challenging problem (*2*).

Therefore, sensitive and reliable analytical methods are needed to evaluate the presence of SUs in environmental and

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food samples at ppb or ppt levels. Various methods for their determination have been published up to now. While gas chromatography (GC) is generally preferred in pesticide residue analysis, the polar SUs are not directly amenable to GC, because of their low volatility and thermal instability. However, after derivatization, for example, with diazomethane, GC analysis is reported (*3*). Further methods are high-performance liquid chromatography (HPLC) (*4*), capillary electrophoresis (*5*), and enzyme-linked immunosorbent assay (ELISA) (*6*−*8*). Most of the applications known are based on HPLC using reversed phase columns followed either by ultraviolet (UV) (*9*) or mass spectrometric (MS) detection (*10*). For both UV and MS detection usage, there is an increasing demand for group specific enrichment and precleaning methods, which can also be implemented as a module for multicomponent analysis to meet high sensitivity and selectivity.

Generally, the trace analysis of complex environmental and food samples needs pretreatment steps in order to reduce matrix interferences and enrich trace level analytes. This is traditionally performed by liquid−liquid extraction (LLE) or more rapid and economic solid phase extraction (SPE) or dispersive solid phase extraction (DSPE). Materials such as RP18, ion exchangers, mixed mode phases, graphitized carbon, and polystyrene divinylbenzene supports have been shown to be valuable sorbents for sample enrichment of various SUs in water. These sorbents, however, are rather nonspecific in nature. Using SPE supports, the detectability of trace analytes can be greatly enhanced by applying a large sample volume, but the high matrix load may also give rise to the partial coextraction of interfering substances with similar polarities, due to the very limited selectivity of the sorbent materials. Therefore, an increase in sensitivity may not be obtained. Moreover, the high matrix loads would inevitably affect the performance of the extraction sorbent and result in frequent exchange of the SPE material, which may be especially a problem in on-line applications.

DSPE materials were often used for the extraction of food samples, as a quick and convenient replacement method to LLE, where extraction takes place on the surface of a hydromatrix (*11*). This method removes quite effectively many polar matrix components such as organic acids, certain polar pigments, and sugars to some extent from food extracts but often also needs further enrichment and cleaning steps, especially when UV detection is used. Therefore, there is an increasing interest in the development of alternative sorbents, which have high extraction selectivity for single analytes or classes of compounds and result in an efficient sample cleanup for the monitoring of trace analytes in complex environmental or food samples.

A high selectivity may be obtained by using molecularly imprinted polymers (MIPs), which are artificial receptors, gained by synthetic cross-linking of a macroporous polymer complementary to the template molecule both in shape and in the arrangement of functional groups. The formed selective recognition sites in a stable polymer matrix allow the selective rebinding of template from a sample. The preparation and application of such a kind of synthetic antibody mimic for SUs was recently published by our group (*12*). The advantages of MIPs are their high stability and selectivity. Especially because of their compatibility with organic solvents, they have attracted considerable attention for molecularly imprinted SPE, also in trace analysis of pesticides (*13*). According to our experience, the development of high-quality MIPs needs considerable time, effort, and know-how.

Immunoaffinity extraction (IAE) is mainly applied in clinical chemistry (*14*). However, during the past decade, there is also a growing number of applications described in food and environmental analysis (*15*). For example, methods for trace analysis of different pharmaceuticals (*16*), anabolic substances (*17*), endocrine-disrupting compounds (*18*), and veterinary drugs using IAE (*19*) were published. Classical target analytes for IAE in foods are mycotoxins (*20*), but there is also a growing number of support for the enrichment of pesticides (*21*). Up to now, immunoaffinity supports (IASs) for pesticide analysis, e.g., atrazines or phenylureas, mainly contained polyclonal antibodies covalently immobilized on sepharose or silica supports. Agarose-based IASs for the enrichment of SUs from soil were described for triasulfuron (*22*) and chlorimuron-ethyl (*23*). The development of sol−gel glass supports and their application to environmental samples such as surface water, seawater, and rainwater so far were reported only for polycyclic aromatic hydrocarbons (*24*), triazine herbicides (*25*), malathion (*26*), and isoproturon (*27*). Corresponding supports for food analysis were described for isoproturon (*27*), 1-nitropyrene (*28*), and bisphenol A (*29*, *30*).

In the present investigation, for the first time, sol−gel IASs as crushed monoliths or coated highly porous silica particles were prepared with broad specific polyclonal anti-SU antibodies and used for class selective enrichment of analytes from water and food samples.

MATERIALS AND METHODS

Chemicals. All reagents were of analytical grade unless specified otherwise. The analytical standards of amidosulfuron, cinosulfuron, nicosulfuron, primisulfuron-methyl, and prosulfuron were generously supplied by Syngenta (Basel, Switzerland). The standards flucarbazon and propoxycarbazon were a gift from Bayer AG (Leverkusen, Germany). All other SU standards were purchased from Dr. Ehrenstorfer (Augsburg, Germany), except for flazasulfuron, which was obtained from Riedel-de Häen (Seelze, Germany). Tetramethoxysilane (TMOS) was purchased from Fluka (Buchs, Switzerland); acetonitrile (MeCN), methanol (MeOH), dichloromethane (DCM), trifluoroacetic acid (TFA), and LiChrolut SPE cartridges were purchased from VWR International (Darmstadt, Germany). Stock standard solutions of SUs (1.0 mg/mL) were prepared in MeCN:water (1:1, v/v) and stored at 4 °C. HighTrap Protein A columns were from Amersham Biosciences (Freiburg, Germany), humic acid sodium salt was purchased from Carl Roth GmbH (Karlsruhe, Germany), and bovine serum albumin (BSA) was from Sigma-Aldrich (Steinheim, Germany). Oasis HLB SPE cartridges were obtained from Waters (Eschborn, Germany), and Chem Elut cartridges (unbuffered; volumes, 5 and 20 mL) were from Varian (Darmstadt, Germany). All of the solvents were of HPLC quality. Ultrapure water used for sample preparation was obtained by reverse osmosis including UV treatment (Milli-RO 5 Plus, Milli-Q_{185} Plus, Millipore, Eschborn, Germany). Highly porous silica particles (diameter between 70 and 200 μm; pore size, 15, 150, or 250 nm) were a gift from Grace (Worms, Germany).

Preparation of IASs: Crushed Sol−Gel Monoliths and Sol−Gel-Coated Silica Particles. For both kinds of supports, an isolated immunoglobulin (IgG) fraction from polyclonal rabbit anti-SU antiserum (pAb-R03) was used. The generation of this serum and the development of a class specific ELISA ("sulfuron screen") were published recently (*31*). The isolation of IgG was performed using a protein A column according to the instructions given by the supplier. Sol−gel monoliths were prepared by a two-step procedure, in which hydrolysis of TMOS was followed by polymerization in the presence of biomolecules. For that, 1.7 mL of TMOS, 0.1 mL of MeOH, 0.3 mL of water:glycerol (50:50, v/v), 0.25 mL of water, and 0.1 mL HCl (0.04 M) were repeatedly mixed under ice cooling for about 10 min until the acidic silica sol gave a homogeneous clear solution. The purified antibody fraction was dissolved at a concentration of 2.0 mg/mL in phosphate-buffered saline (PBS), which was 0.08 M sodium phosphate (pH 7.6) containing 0.15 M sodium chloride, cooled on ice, mixed with an equivolume amount of the silica sol, and immediately

poured into a Petri dish. The sol−gel was allowed to age at 4 °C for about 24 h until a loss of weight of about 55% occurred. Afterward, the monolith was ground in a mortar, sieved to remove particles smaller than 32 μm, and packed into 8 mL glass columns (VWR International). PTFE frits (porosity, 10 μm; VWR International) were placed above and below the sorbent bed. The columns were washed with 10 mL of PBS and stored at 4 °C.

For the preparation of sol−gel-coated silica particles, 295 μL of TMOS, 400 μL of antibody solution containing 2 mg of IgG, 50 μL of water, and 50 μL of water:glycerol (50:50, v/v) were mixed intensively for 5 s at room temperature and then poured into a polypropylene (PP) container, which contained 0.6 g of silica particles. The closed PP container was shaken thoroughly. The gel formation took about 15 min. After 24 h, the PP container was opened and the sol−gel glass air-dried. Thereafter, the material was successively washed with 5 mL portions of water, MeCN:water (30:70, v/v), and PBS under sonication and ice cooling for 15 min, followed by sieving to remove solvent and particles smaller than 32 μm in size. Finally, the coated material was filled into 8 mL glass columns or self-made stainless steel columns (diameter and length 1 cm each) and stored at 4 °C in PBS. In parallel, different types of blank supports were prepared in the same way but replacing anti-SU antibodies by nonspecific IgGs such as commercial bovine IgG (Serva, Heidelberg), rabbit polyclonal anti-diclofenac and anti-BTEX (benzene, toluene, ethylbenzene, and xylenes) antibodies from own production (*32*, *33*), BSA, or protein-free PBS buffer.

Characterization of IASs: Antibody Leaching, Capacity, Selectivity, Recovery, and Reusability. Antibody leaching from sol−gel immunosorbent was assessed by rinsing prepared columns five times with 1 mL of water, followed by five times with 1 mL of MeCN:water (30:70, v/v). Each fraction was tested for the presence of proteins by the Micro BCA assay according to the instructions given by the supplier and/or the presence of specific anti-SU antibodies by a direct competitive ELISA as described earlier (*31*). The column capacity was evaluated by overloading the immunosorbent with 10 mL of aqueous solution containing 1 μg of metsulfuron-methyl (MSM) or other SU compounds and analyzing eluted fractions by HPLC-UV/diode array detection (DAD) as described below. The selectivity was tested by percolating defined mixtures of two or more (up to 13) SUs through the column (sol−gel-coated highly porous silica particles) at a concentration of 50 ng each in 10 mL of water and determination of the individual compounds in the eluates by HPLC-UV/DAD. Comparable experiments were performed by LC-MS/MS, loading only 10 ng per compound of 16 SUs on the IAE cartridge. The recovery rates were calculated in dependence on sample matrix like pure water, tap water, surface water, food extracts (barley, oats, and potato), sample pH, humic acid concentration, and herbicide load. Loading, elution, and analysis were performed as described in the following section. The reusability of the cartridges was tested by submitting both types of supports to either 20 or 30 consecutive cycles of loading, washing, elution, and equilibration with the use of MSM as a target analyte.

IAE. Standard solutions containing up to 16 SUs were used for loading. Ten milliliters of pure water containing 10 or 20 ng (using LC-MS/MS), respectively, or 50 or 100 ng (using HPLC-UV/DAD) of SU standards and 100 mM NaCl were passed through the column at a flow rate of ∼1 mL/min followed by a washing step with 5 mL of bidest water or PBS buffer. The retained analytes were eluted from the cartridge with 2.0 or 10 mL of MeCN:water (30:70, v/v) for HPLC-UV/DAD and LC-MS/MS detection, respectively. Five hundred microliters of eluting fractions was directly injected into a 500 μL sample loop and analyzed by LC-UV/DAD, and 20 μL was injected into LC-MS/MS. Both of the samples that passed through the affinity cartridge and the washing fractions were collected and loaded onto commercial RP18-SPE cartridges to check for analyte loss of the IAE method.

RP18-SPE of SU Herbicides in Food Extracts. To draw a comparison with IAE, RP18-SPE was used as a sample preparation with food extracts. For that, a 20 mL aliquot of the aqueous food extract was acidified with 125 μL of phosphoric acid (1%, v/v) and 0.625 mL of phosphate buffer (0.1 M, pH 3.0). Then, the sample was loaded onto a 3 mL Oasis HLB SPE cartridge, which had been preconditioned with 3 mL of MeOH and water, respectively. The sample passed through the column at a flow rate of about 3 mL/min. After it was washed with 5 mL of water, the cartridge was dried in a gentle stream of nitrogen. The analytes were eluted with 3 mL of MeOH, and the extract was dried with nitrogen. The residue was reconstituted in HPLC eluent.

HPLC-UV/DAD Analysis. Analysis was performed using a Shimadzu LC system equipped with a SCL-6B controller, two LC-6A pumps, photodiode array UV−visible detector SPD-M6A, and CTO-10A column oven (Shimadzu, Duisburg, Germany). Chromatographic separations were carried out with a Prontosil 120-3-C18-AQ column (Bischoff NC, Leonberg, Germany), 250 mm × 4.6 mm i.d. (3.0 μm particle size). The analytical column was protected by a C18 precolumn, 4 mm × 3 mm i.d. (Phenomenex, Aschaffenburg, Germany), and inline filter, 2.0 μm (Alltech, Unterhaching, Germany). Injection was performed with a model 7125 injector (Rheodyne, Cotati, CA) equipped with a 500 μL high volume sample loop. Analysis was carried out using a gradient solvent program. The initial composition of the mobile phase was 32% of MeCN and 68% of water; both solvents contained 3.0 mmol/L of TFA. For separation of 13 SUs, the initial mobile phase composition of 32% MeCN was increased linearly to 62% in 40 min. To clean the column, the amount of MeCN was increased from 62 to 90% within 5 min and kept constant for 3 min. The initial mobile phase composition was restored, and the column was equilibrated for 10 min. On the other hand, for only separation of selected SUs with retention times lower than 20 min, a linear gradient was used to increase the amount of MeCN from 32 to 50% in 24 min and then from 50 to 90% in 5 min. The flow rate was 0.8 mL/min, and the column temperature was 25 °C. The UV detector was set at 227 nm wavelength. Data were acquired and evaluated by using the CSW v.1.7 package (DataApex, Prague, Czech Republic). Peak areas were used for quantification. The calibration curve of each of the SUs was used to calculate the recoveries of the analytes.

HPLC-MS/MS Analysis. A Waters Alliance HT 2795 LC system combined with a Quadrupole MS/MS Ultima Pt detector was used for LC-MS/MS measurements. Chromatographic separation was carried out with an Inertsil C18 column, 150 mm × 3.0 mm ODS 3 (5.0 μm particle size), from MZ-Analytical (Mainz, Germany). The LC time program was started with 100% of eluent A (MeCN:water, 10:90, v/v), containing 1% formic acid; over 5 min eluent B (MeCN) was linearly increased to 92%, held for another 5 min at this concentration, and then decreased within 5 min to 0%. Twenty microliters of sample was injected by an autosampler. Electrospray ionization MS was performed using Micromass Z-spray source in positive ion mode. The spray capillary voltage was set to 0.5 kV, the cone voltage was set to 60 V, the temperature of the heated inlet capillary was set to 140 °C, and the electron multiplier voltage was set to 650 V. Nitrogen served as both the sheath (694 L/h) and the auxiliary gas (75 L/h). The product ion spectra were recorded using argon as a collision gas at a pressure of 4.35 e^{-3} mbar and a collision energy of 20 eV in the positive mode, scanning a mass range from 80 to 400 Da, with a scan duration of 0.1 s. Multiple reaction monitoring experiments in the positive mode were performed using protonated parent ions and one selected daughter ion. Prior to measurements, mesosulfuron-methyl was added at a concentration of 10 ng/mL as an internal standard and calibration curves were prepared in different sample extracts to control matrix effects. Evaluations were made using Micromass MassLynx 4.0 software. Analyte specific parameters of 16 SUs are listed in **Table 1**.

Surface Water Samples. Surface water from rivers and lakes was collected in brown bottles (1 L) in agricultural areas located in Bavaria (Germany). A short sensory analysis of fresh samples including appearance and odor as well as pH measurement and determination of the dissolved organic carbon (DOC) was made. Tap water was taken from the municipal water supply of the laboratory. Additional samples from rivers were kindly provided by Dr. M. Lackoff from the Bavarian Water Management Agency in Munich. While tap water was used without any preparation, surface water was filtered over a glass microfiber filter (GF/C, Whatman, Maidstone, England) to remove particles larger than 1.2 μm and kept at 4 °C in the dark until analysis. For recovery studies, water samples were spiked with SUs at concentrations of 50 and 100 ng/L.

Table 1. Analyte Specific Parameters of 16 Selected SU Herbicides from LC-MS/MS

SU compound	RT[a] (min)	PT[b] (*m*/*z*)	ST[c] (*m*/*z*)
chlorimuron-ethyl	8.71	415.1 → 186.1	415.1 → 83.0
imazosulfuron	8.39	413.3 → 153.1	413.3 → 232.0
chlorsulfuron	7.86	357.8 → 140.7	357.8 → 167.0
cyclosulfamuron	8.89	422.2 → 199.1	422.2 → 224.0
pyrazosulfuron-ethyl	8.71	415.2 → 182.0	415.2 → 214.0
sulfosulfuron	8.14	471.1 → 211.0	471.1 → 260.8
triasulfuron	7.67	402.1 → 167.0	402.1 → 141.1
MSM	7.61	382.0 → 167.0	382.0 → 199.0
iodosulfuron-methyl	8.46	508.1 → 167.1	508.1 → 141.0
prosulfuron	8.55	420.2 → 141.0	420.2 → 167.0
thifensulfuron-methyl	7.52	388.0 → 167.0	388.0 → 205.0
cinosulfuron	7.52	414.2 → 183.1	414.2 → 215.1
sulfometuron-methyl	7.77	365.0 → 149.6	365.0 → 199.0
triflusulfuron-methyl	8.64	493.1 → 264.1	493.1 → 96.0
ethoxysulfuron	8.74	399.2 → 261.1	399.2 → 279.1
nicosulfuron	7.08/8.58	411.0 → 181.7	411.0 → 213.0
mesosulfuron-methyl[d]	7.64	504.1 → 182.0	504.1 → 156.0

[a] Retention time. [b] Primary (quantitative) transition. [c] Secondary (confirmatory) transition. [d] Internal standard.

Food Samples. The grain samples (barley, sort "Scarlet", and oats, sort "Jumbo") were grown in the north of Bavaria using the commercial herbicide formulation Concert (DuPont) according to the guidelines of the manufacturer and containing the active ingredients MSM and thifensulfuron-methyl. The potato sample (sort "Granada") was not treated with any SUs during cultivation. The samples were minced in a kitchen mixer and stored in laboratory containers either at room temperature (grain samples) or in the freezer at −18 °C (potato samples) until use. Spiked samples were prepared by placing 10.0 g of food sample into a flask followed by the addition of SU standards. Samples were allowed to stand for 1 h before extraction. In addition, a set of blank sample extracts was spiked directly prior to IAE to evaluate the performance of immunoextraction without being affected by possible losses of herbicides during preceding sample extraction methods. Two different extraction methods were compared. One method was performed according to Klein and Alder (*34*) with a minor modification of sample pH prior to DSPE. In detail, water (pH 4.0) was added to 10.0 g of spiked food sample to obtain a total volume of 10 mL. While to potato samples (water content 70%) only 3 mL of water had to be added, a much higher volume of 9 mL was necessary for grain samples (water content 10%). These suspensions were then blended (Ultra-Turrax T25 basic, IKA Labortechnik, Staufen, Germany) in beakers with 20 mL of MeOH for 2 min, and the homogenate was centrifuged or filtered over a 1.2 μm glass microfiber filter. A 6 mL aliquot of the extract was thoroughly mixed with 2 mL of NaCl solution (20 g of NaCl per 100 mL of water). A 5 mL aliquot was transferred to an acidified Chem Elut column and equilibrated for 5 min, and then, the column was eluted with 15 mL of DCM. The eluate was evaporated to dryness at 40 °C, and the residue was redissolved in 100 μL of MeOH. After the addition of 10 mL of water, the solution was loaded on the immunoaffinity column.

A second extraction method was performed according to Powley (*2*), using a 20 mM potassium phosphate buffer, pH 6.0, which has the advantage that the aqueous buffer solution is directly amenable to IAE. For that process, 10 g of crop samples was allowed to soak for 60 min in 90 mL of buffer before homogenization in order to hydrate the matrix. After blending using an ultra-turrax, samples were centrifuged and the supernatant was separated. The residue was homogenized a second time with 90 mL of buffer, and supernatant was removed. Both extracts were combined and adjusted to 200 mL with water. A 20 mL aliquot of the extract was spiked with SUs and loaded on the IAE column. Samples were passed through the cartridge at a flow rate of ∼1 mL/min. For both methods, the washing and elution procedures of IAE were the same as described above. Unspiked food samples were treated accordingly and served as blanks.

RESULTS AND DISCUSSION

Optimization of IAE Performance. As experienced from earlier experiments, crushed sol−gel glass monoliths may give rise to some problems especially in on-line applications by increasing back pressure after long-term use of the material. The phenomenon is not yet fully understood. While very small particles can be removed from the crushed xerogel rather easily by sieving just before packing of the IAE column, succeeding clogging of prefilters by glass particles cannot be avoided at present. Possibly, their appearance is caused by abrasion from irregularly ground material by high pressure. The hardness of the sol−gel glass could be drastically increased by a sintering process as it is applied for the preparation of commercially available Chromolith columns (Merck); however, this is not compatible with the enclosed biomolecules. As an alternative approach, we prepared sol−gel glass-coated highly porous silica particles based on a method that was reported for the immobilization of lipoxygenases on different silica materials (*35*). The mechanical stability of these new support materials was found to be better, and material properties, therefore, improved for future on-line applications. However, the problem could not yet be solved completely. The method needs further optimization first of all to circumvent the formation of glass plaques on the surface of the silica particles, which still seem to be a reason for this problem (**Figure 1**). So far, in regard to reaching the highest activity of the encapsulated antibodies and sol−gel glass formation largely within the pores of silicates, a moderate aging process (weight loss only about 30−40%), a water:silan ratio (*R* value) of 14:1, and silicate carrier particles with large pores (150 or 250 nm) were revealed as the most promising. In addition, after sonication of the coated particles with a mixture of MeCN:water (40:60, v/v) just before packing of the IA cartridge, a further reduction of clogging was evident. As a disadvantage, coated particles lost biological activity faster than crushed monoliths when applied consecutively in IAE.

Because of a diffusion-limited process, gradual loading of IAS with a lower flow rate of ∼1 mL/min is important to obtain high recoveries. The addition of NaCl (100 mM in sample) was found positive for maintaining biological activity of the entrapped antibodies. Especially remarkable was the absence of any detectable nonspecific binding of SUs to different types of sol−gel glass blanks prepared with different unspecific antibodies such as bovine IgG, anti-diclofenac, anti-BTEX antibodies, and other serum proteins such as BSA or cartridges without any protein.

It is well-known that the molecular recognition of antigens by antibodies is based on different types of interaction. Concluding from this, elution of an analyte in IAE needs a break of the binding forces as mild as possible in order not to denature the biomolecules irreversibly and allow frequent repeated usage of the sorbent but, on the other side, harsh enough to elute the analyte preferably quantitatively. Typically, buffers of extreme pH value, chaotropic ions, reagents of high ionic strength, and organic solvents are used (*36*). In the present work, it was shown that 3 mL of 30% of MeCN or 2 mL of 40% MeCN, respectively, was the optimal eluent for an efficient, i.e., rapid and low volume elution for most of the SU compounds. For washing of the support after addition of sample, pure water was revealed as the solvent of choice. The presence of only 5% of MeCN lead to a loss of loosely bonded analytes.

Capacity, Recovery, and Selectivity. The binding characteristics of the polyclonal antibody (pAb-R03) used in this work were previously evaluated in this laboratory based on cross-reactivities against 30 SUs in direct competitive ELISA (*31*).

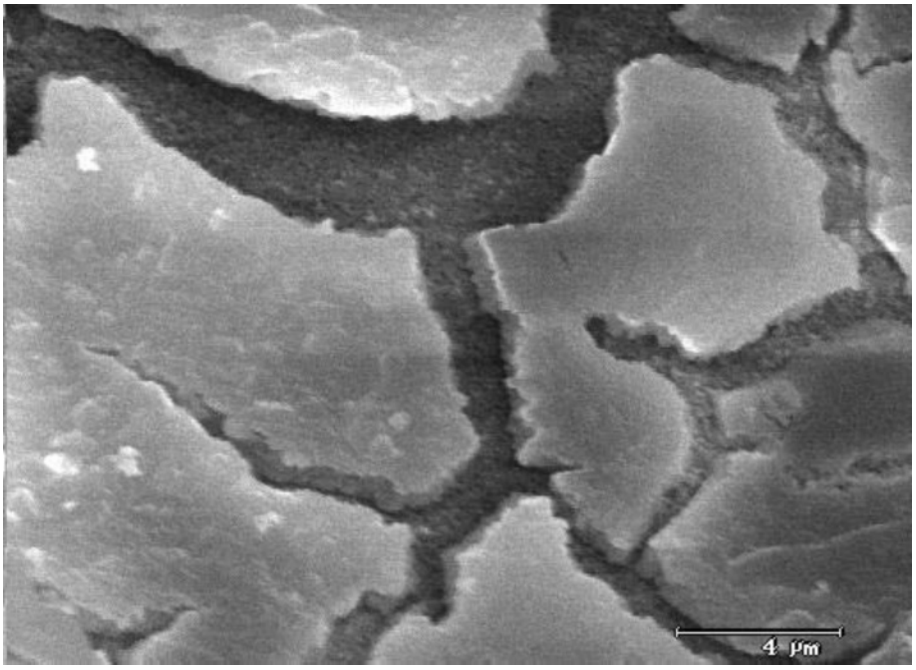

Figure 1. SEM image of sol–gel glass-coated highly porous silica particles (particle size, 70–200 μm; porosity, 15 nm).

It was shown that among seven antisera, pAb-R03 exhibited the best class specific recognition with cross-reactivities higher than 15% for 19 SUs. In addition, affinity to 16 SUs was sufficiently high to detect these herbicides at relevant concentrations of 0.1 μg/L or lower in tap water within a single assay.

In accordance with results of the ELISA, the present investigation using the prepared sol−gel glass-coated particles showed that all SUs were bonded on the support (**Table 2**). Depending on the amount of immobilized antibodies, the SUs were captured at different extents, i.e., high binding capacity for highly cross-reactive SUs and vice versa. For performing immunoextraction, antibodies with medium affinity are preferred because both high and low affinity antibodies may cause analyte losses. The extraordinary high affinity of antibody pAb-R03 to mesosulfuron-methyl lead to very strong binding of this analyte on the IAS and thus required MeCN:water (80:20, v/v) for elution. However, the immobilized antibodies could not withstand these harsh conditions. As a consequence, this SU herbicide was not considered for spiking experiments. Furthermore, LC-MS/MS experiments revealed that for quantitative elution of small loaded amounts of 10 ng per compound of analytes cyclosulfamuron, imazosulfuron, and iodosulfuron-methyl, which displayed a rather strong antibody binding as well, 10 mL of 30% MeCN was necessary. Other SUs could be eluted with much less eluent volumes. While sulfosulfuron-methyl and thifensulfuron-methyl required 4 mL, for all other compounds, an eluent volume of 2−3 mL was totally sufficient.

Table 3 shows the recoveries of selected SUs under optimal conditions for both detection methods. Samples were free of matrix, except for the addition of 100 mM NaCl, and a total capacity on the support was provided, which was theoretically more than double as high than the total amount of loaded analytes. On the average, recovery rates were somewhat higher with MS detection. These results demonstrate that the IAE yields high recovery rates close to 100% for most of the selected SU compounds, regardless of lower CR in ELISA. There are only a few exceptions. Regardless of the detection method, imazosulfuron and iodosulfuron-methyl were recovered clearly below 60%. In addition, the recovery of cyclosulfamuron was below 70% using LC-MS/MS. The low recovery rates for these three compounds were confirmed in all later experiments with spiked samples. Possibly, the eluent strength was not sufficiently high to break the antibody−analyte bond. This cannot be explained on the basis of the determined CR for these compounds in ELISA. However, while the ELISA was performed with the nonpurified antiserum, IAE cartridges were prepared with an isolated IgG fraction. It is well-known that a polyclonal

Table 2. Specificity of Antibody pAb-R03 as Compared to the Capacity of Immunoaffinity Sorbent (Sol–Gel Glass-Coated Particles; 2 mg of Immobilized Antibody, $n = 3$ Replicates)

SU compound	CR[a] in direct ELISA (%)	binding capacity (ng)
mesosulfuron-methyl	1050	459 ± 65
chlorimuron-ethyl	277	440 ± 37
imazosulfuron	229	402 ± 26
chlorsulfuron	197	391 ± 31
cyclosulfamuron	153	455 ± 69
pyrazosulfuron-ethyl	144	457 ± 66
sulfosulfuron	128	423 ± 36
triasulfuron	110	494 ± 41
MSM	100	392 ± 47
halosulfuron-methyl	75	313 ± 21
ethametsulfuron-methyl	68	326 ± 33
iodosulfuron-methyl	66	267 ± 19
primisulfuron-methyl	60	321 ± 40
flazasulfuron	57	246 ± 29
prosulfuron	48	287 ± 27
thifensulfuron-methyl	33	208 ± 12
cinosulfuron	20	284 ± 38
azimsulfuron	16	212 ± 19
sulfometuron-methyl	16	323 ± 24
flupyrsulfuron-methyl-sodium	3	143 ± 18
tribenuron-methyl	2	82 ± 11
triflusulfuron-methyl	1	211 ± 25
amidosulfuron	<0.5	83 ± 34
ethoxysulfuron	<0.5	71 ± 12
flucarbazon	<0.5	84 ± 14
nicosulfuron	<0.5	108 ± 26
oxasulfuron	<0.5	73 ± 43
rimsulfuron	<0.5	98 ± 14
bensulfuron-methyl	n.r.	36 ± 21
propoxycarbazon	n.r.	11 ± 10

[a] Cross-reactivity (MSM was set 100%).

Table 3. Recovery of 16 Selected SU Herbicides in Spiked Water Samples (10 mL) under Optimal Conditions (Matrix-Free, Capacity Surplus[a]) Using (A) HPLC-UV/DAD (Standard Mixtures Contained Three Compounds, Each 5 ng/mL) and (B) LC-MS/MS (Standard Contained 16 Compounds, Each 1 ng/mL) in Combination with IAE (n = 3 Replicates)

	recovery mean ± RSD (%)	
SU compound	HPLC	LC-MS/MS
chlorimuron-ethyl	82.8 ± 6.3	103.7 ± 8.3
imazosulfuron	26.6 ± 8.0	53.5 ± 9.5
chlorsulfuron	95.2 ± 8.7	111.5 ± 5.8
cyclosulfamuron	96.1 ± 4.1	66.8 ± 4.0
pyrazosulfuron-ethyl	97.9 ± 11.9	100.7 ± 12.2
sulfosulfuron	92.3 ± 3.4	88.7 ± 6.4
triasulfuron	102.2 ± 4.1	101.5 ± 9.2
MSM	88.2 ± 9.4	115.7 ± 15.2
iodosulfuron-methyl	23.8 ± 5.0	56.0 ± 6.9
prosulfuron	88.2 ± 11.6	100.2 ± 13.1
thifensulfuron-methyl	95.6 ± 5.0	97.4 ± 2.6
cinosulfuron	82.6 ± 15.2	92.7 ± 3.3
sulfometuron-methyl	89.9 ± 7.0	118.4 ± 4.7
triflusulfuron-methyl	59.5 ± 3.1	86.8 ± 11.2
ethoxysulfuron	90.5 ± 7.6	97.8 ± 8.6
nicosulfuron	86.6 ± 4.8	94.0 ± 4.3
mean, all compounds	**81.1 ± 23.1**	**92.8 ± 8.6**

[a] The capacity was 670 ng/g of sorbent for MSM.

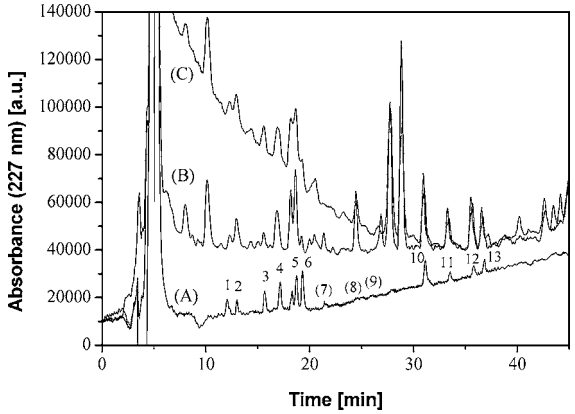

Figure 2. HPLC chromatograms after enrichment of SUs using (**A**) SGG-IAE (crushed monolith), (**B**) LiChrolut (Merck), (**C**) Oasis HLB 3 cm^3 (Waters) from 100 mL of river water, spiked with 50 ng of 13 SU standards: nicosulfuron (1), thifensulfuron (2), MSM (3), sulfometuron-methyl (4), triasulfuron (5), chlorsulfuron (6), amidosulfuron (7), rimsulfuron (8), tribenuron-methyl (9), prosulfuron (10), chlorimuron-ethyl (11), triflusulfuron-methyl (12), and primisulfuron-methyl (13). Measurement, HPLC-UV/DAD. Gradient solvent system: solvent A, bidest, 3 mmol/L TFA; solvent B, MeCN, 3 mmol/L TFA. RP18-Prontosil column, 3.0 μm particle size; injected volume, 500 μL.

antiserum contains a high number of analyte specific antibody subpopulations exhibiting different affinity constants. Possibly, during serum fractionation, antibodies of low binding affinity to the three SUs mentioned above were partially denatured. As a consequence, the analytes are more difficult to eluate from the remaining high affinity antibodies.

The prepared IASs, i.e., crushed sol−gel glass monoliths and coated porous silica particles, were compared with two different commercial C-18 SPE columns (LiChrolut and Oasis HLB) in regard to selectivity. River water samples (100 mL) were spiked with 50 ng each of 13 selected SUs, and eluates were analyzed off-line by HPLC-UV/DAD as described in the Materials and Methods. The resulting chromatograms are shown in **Figure 2**. No significant difference between both types of IASs in regard

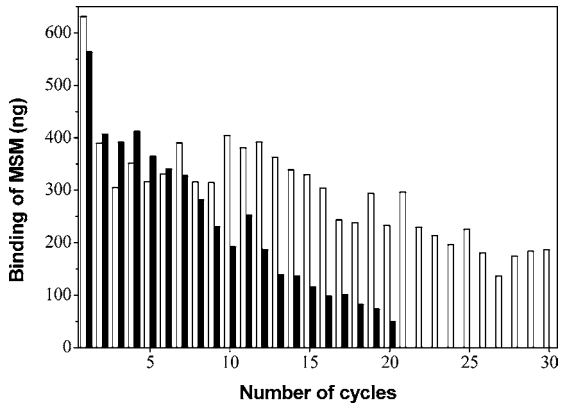

Figure 3. Stability of sol–gel glass immunosorbents prepared from crushed monoliths and coated highly porous silica particles. Twenty to 30 consecutive cycles of equilibration, loading, washing, and elution were performed, and the MSM binding capacity was determined by HPLC-UV/DAD. Cartridges were loaded with 10 mL of water, which contained 1 μg of MSM. Crushed monolith, □; coated silica particles, ■.

to selectivity was observed. The displayed chromatogram of the IAS prepared with the crushed monolith (curve **A** in **Figure 2**) is free of matrix interferences. In comparison, matrix compounds from river water were not separated sufficiently by both kinds of commercial supports to allow quantification without additional cleanup steps. Partial overlap of peaks makes quantification difficult or impossible. Therefore, the immunosorbent could prove its superior quality for selective enrichment of SUs from surface water. However, the commercial materials are very valuable in combination with IAE for on-line HPLC determination of SUs, i.e., for re-enrichment of analytes from diluted IAE eluates before separation on the analytical column (data not shown).

Reusability and Matrix Effects. To estimate the initial capacity of the prepared IAE cartridges, spiked water samples were loaded to reach saturation of antibody binding sides. After washing and elution steps, the effluent and washing and eluting fractions were analyzed by HPLC-UV.

In common, both supports lost about 30−40% of the initial capacity of approximately 600 ng of analyte after the first use (**Figure 3**). Then, for crushed particles, the capacity remained constant for at least 10−15 cycles before losing an additional 30% over the following 15 cycles. In comparison, an almost continuous damage of capacity was observed for coated particles starting already from the fifth use. After 20 cycles, the remaining capacity was only about 50 ng of analyte, i.e., 8% of the initial value. To draw a conclusion, despite problems of material stability, the biological activity of entrapped antibodies is better preserved in crushed monoliths as compared to sol−gel glass-coated porous silica, obviously.

During the evaluation of recoveries in spiked surface water samples, an average loss of 10−20% with an increasing DOC value of samples was noticed (data not shown). Humic acids typically compose about 50−90% of the DOC of an average surface water (*37*). Because of their intense UV/vis absorption, they can interfere with the HPLC-UV/DAD. Another problem that can be caused by humic material is the contamination of SPE columns and chromatographic systems, both leading to a significantly reduced performance of the method. This was confirmed using a water sample spiked at a concentration of 10 mg/L with a commercial humic acid and the SU standard mix. As a consequence, the determination of surface water samples with higher DOC values may give rise to some loss in

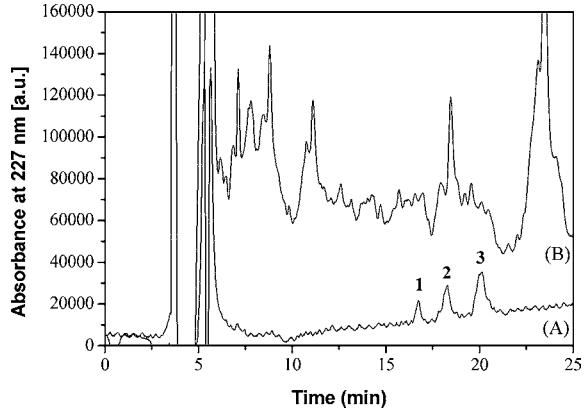

Figure 4. HPLC chromatograms after enrichment of SUs from potato samples using (**A**) SGG-IAE and (**B**) Oasis HLB 3 cm^3 (Waters). Ten grams of potato was spiked with three SU standards, thifensulfuron (1), MSM (2), and triasulfuron (3), at a 10 μg/kg level and extracted according to Klein and Alder (*34*) followed by IAE, respectively, SPE. The extract from **B** was diluted 1:2 prior to injection into HPLC. Measurement, HPLC-UV/DAD. Gradient solvent system: solvent A, bidest, 3 mmol/L TFA; solvent B, MeCN, 3 mmol/L TFA. RP18-Prontosil column, 3.0 μm particle size; injected volume, 500 μL.

recovery for some SUs. As an advantage, no effect of sample pH value in the range of 4−8 was observed.

Food extracts are even more complex than surface water samples and need extensive sample preparation, generally. The present experiments using either RP18-SPE or IAE, followed by HPLC-UV/DAD measurement, revealed that potato extracts can be cleaned efficiently using only IAE (**Figure 4**). In comparison, grain extracts contained interfering matrix compounds in immunoaffinity eluates as well, which made it difficult to estimate recoveries from corresponding chromatograms. Therefore, more selective and highly reliable LC-MS/MS detection was applied.

Precision and Limits of Detection (LODs). To demonstrate the applicability and reliability of this method for environmental and food chemistry, real samples such as tap water, surface water, and food samples (potatoes and grains) were selected and analyzed. After analysis by HPLC-UV/DAD, recovery, reproducibility, and LODs were calculated. The analyte recovery was higher than 80% in water samples. First of all, it was affected by the nature of the aqueous matrix, respectively, the DOC value of sample. As a consequence, the relative standard deviation ($n = 3$) between 2.1 and 13.4% was lower for tap water (DOC value < 0.10 mg/L) and between 2.3 and 23.1% for surface water (DOC value 1.19−2.85 mg/L), which is acceptable for real sample analysis. LOD values, calculated for several SUs after enrichment of 200 mL of spiked tap water, were between 20 and 70 ng/L, which meet the limit value of 100 ng/L set for pesticides in the European Council Directive 98/83/EC concerning the quality of water intended for human consumption (drinking water directive). LODs for surface water samples were similar and varied from 30 to 100 ng/L, mainly depending on UV absorbance characteristics of individual analytes.

The IAE cleanup of food samples could not remove all interfering compounds, especially from grain extracts, and thus did not allow quantification using HPLC-UV/DAD detection. Hence, there was a need for more selective detection like LC-MS/MS if additional cleanup steps should be avoided. Corresponding data were summarized in **Table 4**. No SUs were detected in nonspiked food samples. Experiments were performed using a combination of DSPE/IAE or only IAE. For the latter, recoveries in potato extracts were reduced for some SUs. Nevertheless, 13 compounds could be recovered higher than 75% (column C in **Table 4**). Results were very comparable to data from DSPE/IAE; that is, rather low recovery rates were found for only a few SU compounds mentioned above. Furthermore, for potato samples, no significant differences in recovery rates could be observed between spiked extracts (columns A and C in **Table 4**) and spiked food samples (column

Table 4. Recovery of 16 Selected SU Herbicides from Spiked Food Extracts (A, C) and Food Samples (B) (Standard Contained 16 SUs at a Concentration of 1 ng/mL per Compound) Using Two Different Extraction Procedures in Combination with IAE and LC-MS/MS Detection ($n = 3$ Replicates)

	recovery mean ± RSD (%)		
SU compound	A[a]	B[a]	C[b]
	potato		
chlorimuron-ethyl	90.6 ± 26.4	69.4 ± 5.0	76.3 ± 12.4
imazosulfuron	35.3 ± 14.7	22.4 ± 3.4	42.0 ± 8.5
chlorsulfuron	96.6 ± 4.8	94.4 ± 11.2	90.7 ± 12.2
cyclosulfamuron	43.0 ± 9.5	27.2 ± 4.5	53.0 ± 11.3
pyrazosulfuron-ethyl	80.7 ± 21.7	77.5 ± 2.9	85.0 ± 15.6
sulfosulfuron	75.7 ± 6.5	61.6 ± 2.3	82.0 ± 14.1
triasulfuron	95.6 ± 4.7	86.6 ± 11.8	100.6 ± 0.8
MSM	115.8 ± 19.6	103.6 ± 16.0	113.5 ± 30.3
iodosulfuron-methyl	36.7 ± 24.8	26.7 ± 5.2	50.5 ± 12.0
prosulfuron	69.5 ± 22.2	75.4 ± 4.4	91.1 ± 2.9
thifensulfuron-methyl	98.7 ± 10.7	100.3 ± 11.4	86.0 ± 21.6
cinosulfuron	89.7 ± 7.8	96.5 ± 7.0	83.5 ± 12.0
sulfometuron-methyl	108.1 ± 5.5	83.9 ± 15.6	93.3 ± 20.9
triflusulfuron-methyl	101.7 ± 19.6	104.8 ± 7.9	89.0 ± 11.3
ethoxysulfuron	72.3 ± 15.3	68.0 ± 4.5	96.5 ± 12.0
nicosulfuron	105.0 ± 21.5	98.9 ± 1.6	88.9 ± 21.6
mean, all compounds	**82.2 ± 24.5**	**74.8 ± 26.9**	**82.6 ± 18.4**
	oats		
chlorimuron-ethyl	51.8 ± 14.0	28.5 ± 31.0	63.0 ± 4.5
imazosulfuron	45.3 ± 37.8	6.2 ± 8.5	6.0 ± 8.0
chlorsulfuron	75.8 ± 27.2	40.8 ± 4.5	90.8 ± 7.6
cyclosulfamuron	19.3 ± 17.4	4.2 ± 5.7	60.1 ± 5.7
pyrazosulfuron-ethyl	78.5 ± 7.9	76.3 ± 23.3	90.5 ± 0.7
sulfosulfuron	63.7 ± 21.4	54.8 ± 4.1	52.1 ± 7.1
triasulfuron	88.3 ± 13.1	83.4 ± 3.5	102.2 ± 3.1
MSM	87.3 ± 22.5	93.8 ± 18.1	84.5 ± 0.5
iodosulfuron-methyl	33.7 ± 26.2	4.8 ± 0.8	7.5 ± 0.7
prosulfuron	80.9 ± 8.5	73.4 ± 27.4	90.0 ± 14.1
thifensulfuron-methyl	58.6 ± 35.2	16.0 ± 1.4	50.7 ± 6.2
cinosulfuron	86.0 ± 14.4	82.9 ± 8.3	86.1 ± 4.2
sulfometuron-methyl	90.9 ± 14.6	53.6 ± 15.8	97.2 ± 2.1
triflusulfuron-methyl	89.0 ± 11.5	77.9 ± 23.5	95.5 ± 3.5
ethoxysulfuron	80.7 ± 7.6	67.6 ± 24.3	90.5 ± 12.1
nicosulfuron	83.2 ± 14.5	21.0 ± 2.8	75.9 ± 12.7
mean, all compounds	**69.6 ± 21.2**	**49.1 ± 30.7**	**71.4 ± 28.9**
	barley		
chlorimuron-ethyl	39.2 ± 20.9	20.7 ± 2.5	11.6 ± 0.7
imazosulfuron	32.0 ± 23.7	18.3 ± 4.7	5.0 ± 1.4
chlorsulfuron	104.0 ± 12.9	93.6 ± 5.7	64.2 ± 8.5
cyclosulfamuron	15.3 ± 11.8	15.6 ± 1.7	15.5 ± 4.9
pyrazosulfuron-ethyl	67.2 ± 2.3	67.3 ± 1.0	71.1 ± 3.0
sulfosulfuron	70.1 ± 9.5	32.7 ± 3.3	46.3 ± 7.1
triasulfuron	98.2 ± 9.9	94.5 ± 0.6	97.3 ± 5.4
MSM	80.1 ± 9.8	95.5 ± 11.7	75.4 ± 7.3
iodosulfuron-methyl	44.2 ± 32.0	11.8 ± 2.5	6.3 ± 1.4
prosulfuron	84.1 ± 8.8	81.0 ± 1.6	83.0 ± 14.3
thifensulfuron-methyl	74.2 ± 21.8	43.8 ± 11.3	28.8 ± 0.9
cinosulfuron	82.3 ± 6.0	80.4 ± 7.5	81.2 ± 1.4
sulfometuron-methyl	113.6 ± 6.5	73.0 ± 18.3	95.2 ± 12.6
triflusulfuron-methyl	76.3 ± 2.9	67.1 ± 5.2	74.2 ± 2.8
ethoxysulfuron	64.3 ± 3.1	64.1 ± 4.5	90.5 ± 21.1
nicosulfuron	74.3 ± 15.4	28.0 ± 9.1	55.5 ± 6.4
mean, all compounds	**69.9 ± 25.6**	**55.5 ± 29.6**	**56.3 ± 31.9**

[a] Extraction according to Klein and Alder (*31*). [b] Extraction according to Powley (*2*).

B in **Table 4**) (mean 82.2 ± 24.5% and 82.6 ± 18.4% vs 74.8 ± 26.9%). Concluding from this, no additional analyte losses were caused by the overall sample preparation. In grain samples, mean recoveries were about 10−25% lower than in potatoes. Accordingly, Klein and Alder (*34*) in their study on the applicability of LC-MS/MS to the simultaneous screening of about 100 pesticides in different crops such as tomato, lemon, raisins, avocado, and wheat flour observed the lowest mean recovery (67.2 ± 17.8%) for SUs in the latter. Whereas the DSPE/IAE method led to similar results with both types of grain, extraction with potassium buffer followed by IAE yielded a higher mean recovery in oats (71.4 ± 28.9%) as compared to barley (56.3 ± 31.9%). In detail, at least five SUs showed recoveries higher than 75%, independent of the extraction procedure and type of grain. Significant differences were noticed between spiked extracts and spiked food samples for only some SUs. This is true first of all for chlorimuron-ethyl, thifensulfuron-methyl, sulfometuron-methyl, and nicosulfuron. Regardless of sample extraction method and grain matrix, triasulfuron, MSM, prosulfuron, and cinosulfuron could be recovered higher than 80%. On the contrary, imazosulfuron, cyclosulfamuron, and iodosulfuron-methyl were only found at rates below 50%, which is partly caused by suppression of ionization by nonremoved matrix interferences. The LOD of LC-MS/MS measurements of food samples was about 1 order of magnitude higher as compared to water samples; that is, LODs were between 1.1 and 6.9 μg/kg, depending on compound and extraction procedure. Nevertheless, the reached sensitivity is sufficiently high to analyze samples for the presence of SUs according to the German Maximum Residue Guideline for vegetable foods. In this guideline, maximum residue levels of 10−100 μg/kg were set for 14 SUs. Whether the loss of SUs during enrichment may be due to interaction of analytes with matrix compounds or due to unspecific binding of matrix constituents to the IAS, both making analytes not available for antibody binding within the sol−gel glass IAS, cannot be answered presently.

Conclusion. In this work, the newly developed IAE method proved to be a powerful tool for the class selective enrichment of SUs from environmental and food samples. The mechanical stability of IAS was increased by implementation of a new preparation method using sol−gel-coated highly porous silica particles. As a disadvantage, the biological activity of the entrapped antibodies, i.e., reusability, was reduced to some extent as compared to crushed monolithic glass. Nevertheless, both kinds of supports showed similar characteristics and, therefore, allowed reliable and rapid analysis of SUs in complex matrices at trace levels. As the main advantage of the prepared IAS, its high selectivity for group specific recognition of SUs as compared to other nonspecific SPE materials became evident. Therefore, at least for less complex samples such as tap water and surface water, IAE can be applied in combination with less selective detection methods such as HPLC-UV/DAD. More complex matrices, e.g., food extracts, need more selective detection like LC-MS/MS.

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