# Development of a Magnetic Particle-Based Automated Chemiluminescent Immunoassay for Triasulfuron

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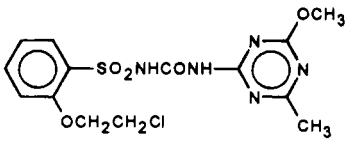
A fully automated immunoassay with high sample throughput for the quantitative analysis of triasulfuron in soil has been developed. The competitive assay was run on the Ciba-Corning ACS: 180 benchtop immunoassay analyzer and used a combination of hapten-bound paramagnetic particles and acridinium ester-labeled monoclonal antibody. Different solid-phase antigens for assay optimization were compared. Three consisted of protein–hapten conjugates covalently linked to primary amine-coated particles, and one corresponded to the chloroethoxy phenylsulfonylurea moiety of triasulfuron with an extended aminopentyl spacer specially designed to improve assay sensitivity and to allow a single step coupling of the hapten to the particles without protein carrier. In the latter assay format, the limits of detection of triasulfuron were 0.02 μg/L in aqueous media and 0.05 μg/kg in soil. Specificity studies indicated that among several structurally related compounds, cinosulfuron showed 250% cross-reactivity while all other compounds studied showed <3.5%. Quantitative determinations of triasulfuron in soil samples obtained from a field trial were measured by automated immunoassay. They compared favorably with HPLC measurements (correlation coefficient of 0.96). The intra- and interassay coefficients of variation were less than 5 and 11%, respectively.

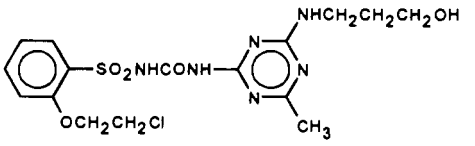
**Keywords:** *Monoclonal antibody; automated immunoassay; triasulfuron; herbicide; soil analysis*

## INTRODUCTION

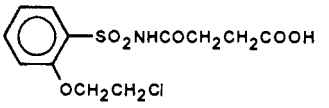
Immunochemical methods for monitoring pesticide residues are recognized as valuable alternatives to conventional methods such as HPLC or GLC, since they provide rapid, sensitive, and cost-effective analyses of soil and water samples (Hammock and Mumma, 1980; Vanderlaan et al., 1988; Van Emon and Lopez-Avila, 1992). The introduction of very potent herbicides such as sulfonylureas, showing bioactivity at very low application rates (10–40 g/ha) (Beyer et al., 1987; Amrein and Gerber, 1985), has necessitated the development of highly sensitive analytical methods. HPLC combined with photoconductivity or UV detection is the method the most commonly used. However, a cumbersome cleanup of the sample is required to reach a detection limit of 0.1 μg/kg of soil (Zahnow, 1982; Iwanzik and Egli, 1989; Bussmann et al., 1990). Other methods include gas chromatography with electron capture detection (Ahmad and Crawford, 1990), capillary electrophoresis (Dinelli et al., 1993), and bioassays which are highly sensitive but lack specificity (Hsiao and Smith, 1983; Iwanzik et al., 1988; Günther et al., 1989; Sunderland et al., 1991). For the monitoring of sulfonylureas, immunoassays may represent the method of choice in meeting regulatory and marketing needs (Peter et al., 1989). A polyclonal antibody-based immunoassay for chlorsulfuron was described by Kelley et al. (1985), with a sensitivity subsequently improved to 0.05 ppb (Sharp et al., 1989). We reported recently on the development of a sensitive competitive ELISA based on monoclonal antibodies (MAbs) selective for triasulfuron, the active ingredient of Logran. The limit of detection of the assay was 0.1 ppb in soil. The MAbs generated with a simple hapten corresponding to the chloroethoxy phenylsulfonamide moiety of triasulfuron with an additional succinic acid spacer (Figure 1, hapten B) showed only limited cross-reactivity with other related sulfonylureas (Schlaeppi et al., 1992). However, despite the convenience of the microtiter plate format, ELISA has some limitations. The passive adsorption




**Triasulfuron**




**Hapten A**




**Hapten B**

**Figure 1.** Structures of triasulfuron, hapten A, and hapten B.

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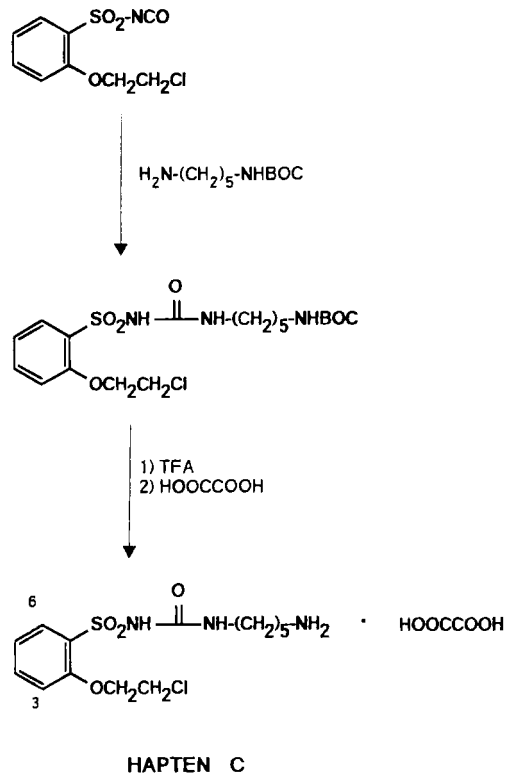
of the antigen or the antibody to the solid phase represents a source of assay variability, mainly due to desorption during incubation periods and washing steps (Lehtonen and Viljanen, 1980). Assay precision was improved significantly by using magnetic particle-based ELISA for residue analysis, where antibodies were covalently bound to the solid phase (Rubio et al., 1991; Lawruk et al., 1993). In addition, the ELISA format is less suited for automation, and thus for high throughput analysis, due to the slow diffusion of reagents to the continuous solid phase (Pesce et al., 1977). Rapid progress in the automation of heterogeneous assays has been made recently in the field of clinical chemistry, and several fully automated instruments are now available. One of them, the Ciba-Corning ACS:180 benchtop immunoassay analyzer, combines the advantages of particulate solid phase, allowing rapid reaction kinetics, and acridinium ester as highly sensitive nonisotopic label, allowing full automation. The sample throughput of the instrument is as much as 180 tests per hour (Boland et al., 1990). Using one of our MAbs specific to triasulfuron, we formated a competitive assay on the ACS:180 based on hapten covalently bound to $NH_2$-coated paramagnetic particles and acridinium ester-labeled MAb. We compared several solid-phase antigens based on homologous and heterologous hapten systems for optimal assay sensitivity. The most appropriate one consisted of the chloroethoxy phenylsulfonylurea moiety of triasulfuron with an extended aminopentyl spacer allowing a single-step coupling of the hapten to the magnetic particles without protein carrier. Both assay sensitivity and precision were improved compared to that of the manual ELISA.

## EXPERIMENTAL PROCEDURES

**Chemicals and Instruments.** Bovine $\gamma$-globulins (BGG) and glutaraldehyde (25%) were purchased from Fluka (Buchs, Switzerland). Primary amine-coated superparamagnetic iron oxide particles of 0.5–1.5 $\mu$m diameter (Groman et al., 1985) and *N*-hydroxysuccinimide-activated dimethyl acridinium ester [2′,6′-dimethyl-4′-(*N*-succinimidyloxycarbonyl)phenyl 10-methylacridinium-9-carboxylate methosulfate] (Law et al., 1989) were obtained from Ciba-Corning Diagnostics (East Walpole, MA). The compound 2-(2-chloroethoxy)benzenesulfonyl isocyanate (Meyer and Föry, 1984) was obtained from Ciba-Geigy Ltd. (Basel, Switzerland). All other reagents were described previously (Schlaeppi et al., 1992), including pure MAb 4147-19-4 to triasulfuron prepared from mouse ascitic fluid and the two haptens, 3-[6-[(3-hydroxypropyl)amino]-4-methyl-1,3,5-triazin-2-yl]-1-[2-(2-chloroethoxy)phenylsulfonyl]-urea (CGA 222335) and 1-[2-(2-chloroethoxy)phenylsulfonyl]-monoamidosuccinic acid (CGA 246131), designated haptens A and B, respectively. Their structures are shown in Figure 1. As described previously (Schlaeppi et al., 1992), we prepared BSA–hapten A by the diazonium method and BSA–hapten B by the active ester method (molar ratio 1/14).

The automated chemiluminescent immunoassay (CLIA) was run on the Ciba Corning ACS:180 benchtop immunoassay analyzer, which fully automates each step of the assay including the acquisition of the chemiluminescent signal (Boland et al., 1990). We used a magnetic separation rack to perform the manual CLIA. It consisted of two parts: an upper rack holding the test tubes and a lower part containing the magnets. The chemiluminescent signal was measured on a Magic Lite Analyzer II. The three instruments were from Ciba-Corning Diagnostics. $^1$H NMR spectra were measured at room temperature with a Bruker AC 300-MHz instrument using tetramethysilane as an internal standard. Mass spectrometry was performed on a Finnigan 4500 instrument with a direct-exposure probe.

**Hapten Synthesis.** A third hapten, *N*-[2-(2-chloroethoxy)-benzenesulfonyl]-*N*′-(5-aminopentyl)urea oxalate (CGA 292566), designated hapten C, was specially designed for a single-step covalent coupling of the hapten to the $NH_2$-coated magnetic particles. The pathway leading to hapten C is shown in Figure 2.




HAPTEN C

**Figure 2.** Synthesis of *N*-[2-(2-chloroethoxy)benzenesulfonyl]-*N*′-(5-aminopentyl)urea oxalate (hapten C).

*N-[2-(2-Chloroethoxy)benzenesulfonyl]-N′-{5-[(BOC)-amino]-pentyl}urea* (**1**). To a stirred solution of 2.61 g (10 mmol) of 2-(2-chloroethoxy)benzenesulfonyl isocyanate in 10 mL of chlorobenzene was added 2.42 g (12 mmol) of *N*-BOC-1,5-diaminopentane (Krapcho and Kuell, 1990) in 10 mL of chlorobenzene over 10 min, keeping the exothermic reaction at 20 °C. After 3 h at room temperature, the reaction mixture was poured into 100 mL of petroleum ether and the precipitate was collected on a filter to yield 4.2 g of a semisolid material. This crude product was purified by flash chromatography on silica gel, eluted with methylene chloride (yield of compound **1**, 1.77 g): $^1$H NMR (DMSO-$d_6$) $\delta$ 9.97 (b, 1H, $SO_2NH$), 7.81 (dd, 1H, H-6), 7.61 (m, 1H, H-4), 7.28 (d, 1H, H-3), 7.12 (t, 1H, H-5), 6.72 and 6.42 (t, 1H each, N***H***$CH_2$), 4.40 (t, 2H, $ArOCH_2$), 4.00 (t, 2H, $CH_2Cl$), 2.93 and 2.86 (q, 2H each, $NCH_2$), 1.39 (s, 9H, $CH_3$), 1.1–1.4 [m, 6H, $CH_2(\boldsymbol{CH_2})_3CH_2$].

*N-[2-(2-Chloroethoxy)benzenesulfonyl]-N′-(5-aminopentyl)-urea Oxalate* (**2**) (*CGA 292566*). To a solution of 1.2 g (2.6 mmol) of compound **1** in 10 mL of methylene chloride was added at room temperature 3 mL of trifluoroacetic acid. The mixture was stirred at room temperature for 2 h and then evaporated to dryness. The residue was extracted four times in 10 mL of acetic acid ethyl ester each time, and these combined extracts were evaporated to yield 1.02 g of a white powder. Without further purification, this intermediate was added at room temperature to a saturated solution of 5 g of oxalic acid in 20 mL of acetic acid ethyl ester. After stirring for 5 min, the solid was collected on a filter and washed with 15 mL of acetic acid ethyl ester, yielding 0.25 g of compound **2**, a white solid (mp 163–166 °C). The filtrate was evaporated to dryness and the residue was again treated with 10 mL of the above-mentioned saturated solution of oxalic acid in acetic acid ethyl ester. The mixture was diluted with 50 mL of acetic acid ethyl ester, stirred vigorously for 15 min, and filtered, yielding an additional 0.58 g of compound **2**: $^1$H NMR (DMSO-$d_6$) $\delta$ 7.78 (d, 1H, H-6), 7.77 (bs, remaining exchangeable protons), 7.60 (t, 1H, H-4), 7.25 (d, 1H, H-3), 7.10 (t, 1H, H-5),

**Table 1. Comparison of Four Different Solid-Phase Antigens by Automated CLIA**

| antigen bound to the magnetic particles | $I_{50}$,[a] ng/mL | slope[b] |
|---|---|---|
| BSA–hapten A | 0.10 ± 0.02 | 1.09 |
| BSA–hapten B | 0.68 ± 0.03 | 1.14 |
| hapten B[c] | 0.27 ± 0.02 | 1.16 |
| hapten C | 0.19 ± 0.03 | 1.04 |

[a] Triasulfuron concentration for 50% inhibition in the competitive CLIA (average of 4–15 determinations with standard deviations). [b] Slope of the four-parameter logistic curve fit. [c] Hapten B was bound to BGG-coated magnetic particles (see Experimental Procedures).

7.02 (t, 1H, N***H***$CH_2$), 4.41 (t, 2H, Ar$OCH_2$), 3.98 (t, 2H, $CH_2$-Cl), 2.95 (q, 2H, CONH***$CH_2$***), 2.75 (t, 2H, ***$CH_2$***$NH_3^+$), 1.48, 1.34, and 1.22 [m, 2H each, $CH_2$(***$CH_2$***)$_3CH_2$]. We confirmed the structure of hapten C by mass spectrometry. The positive chemical ionization mass spectrum with methane as reagent gas showed a weak protonated molecular ion $m/e$ 264 [2, $M^+$ + H] and fragments at $m/e$ 328 [1, $M^+$ − 35, Cl], 314 [1, $M^+$ − 49, $CH_2Cl$], 236 [100, 264 − 128, $OCN(CH_2)_5NH_2$], 219 [66, $M^+$ − 144, $HNCONH(CH_2)_5NH_2$], 200 [56, 236 − 36, HCl].

**Preparation of the Solid-Phase Antigens.** As shown in Table 1, four different solid-phase antigens were prepared. The coupling of BSA–hapten A, BSA–hapten B, hapten C , and BGG to magnetic particles was done with glutaraldehyde (Groman et al., 1985). The particles (60 mg) were washed by magnetic separation two times with methanol (6 mL) and three times with 4.8 mL of phosphate-buffered saline (PBS) (0.1 M sodium phosphate, 0.15 M NaCl, pH 5.0). The magnetic particles were resuspended in 0.8 mL of PBS (pH 5.0), and 0.2 mL of glutaraldehyde (25%) was added. The mixture was stirred for 3 h at room temperature. The magnetic particles were washed three times with PBS (pH 5.0) and resuspended in 1 mL of PBS (pH 5.0) containing, respectively, 5 mg of either BSA–hapten A, BSA–hapten B, or BGG or 1 mg of hapten C. After 16 h at room temperature, the particles were magnetically separated and the supernatant was saved for measuring unbound protein conjugates or unbound hapten C. The particles were washed five times with PBS (pH 7.0) containing 1 M NaCl, three times with PBS, and finally three times with PBS containing 0.3% BSA (PBS–BSA). The hapten-bound particles were stored in 5 mL of PBS–BSA at 4 °C. Unbound hapten C or protein conjugates were measured by UV absorbance at 280 nm, which corresponded to the peaks of both hapten C and proteins. We determined that 34% of BSA–hapten A, 98% of BSA–hapten B, 28% of hapten C, and 97% of BGG were covalently bound to the magnetic particles. The coupling of hapten B to BGG-coated magnetic particles was done by adding 0.9 mg of the *N*-hydroxysuccinimide ester of hapten B (Schlaeppi et al., 1992) to 12 mg of the BGG particles (see above) in PBS. After 4 h of incubation at 4 °C, the magnetic particles were washed and stored as described above. The binding capacity of each solid-phase antigen was titrated by manual and automated CLIA with acridinium-labeled MAb 4147-19-4.

**Preparation of Acridinium Ester-Labeled MAb 4147-19-4.** Purified MAb 4147-19-4 (55 μg dissolved in 160 μL of PBS, pH 8.0) was labeled by reacting for 20 min at room temperature with 20 μg of *N*-hydroxysuccinimide-activated dimethyl acridinium ester dissolved in 20 μL of DMSO. The reaction was quenched by adding 20 μL of L-lysine (10 mg/mL of $H_2O$) for 10 min. The labeled MAb was separated from unbound acridinium by gel filtration on a Bio-Gel P6 column equilibrated with PBS–BSA. The specific chemiluminescent activity of the labeled MAb was calculated as $1.4 \times 10^6$ photon counts/ng of MAb, which corresponded to approximately two molecules of acridinium per molecule of MAb.

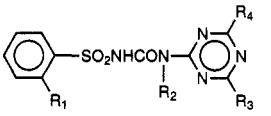
**Competitive CLIA for Triasulfuron Determination.** The format of the CLIA run on the ACS:180 instrument was the following: 200 μL of triasulfuron standard solutions or soil samples diluted in PBS supplemented with 0.1% Tween 20 (PBS–Tween) was pipetted automatically in the cuvette followed by 50 μL of acridinium labeled MAb 4147-19-4 (450 pg). After 2.5 min of incubation at 37 °C, 100 μL of hapten-bound particles (corresponding to 200 ng of BSA–hapten A or BSA–hapten B or 30 ng of hapten C) was added and the incubation was continued for 5 min at 37 °C (total incubation time of 7.5 min). The particles were washed two times by magnetic separation with 500 μL of water before light emission was measured. The solid-phase antigen consisted of a mixture of hapten-bound and BGG-bound particles, the latter being added to maintain a constant amount of magnetic particles of 40 μg per cuvette to avoid any loss of solid phase during the two washing cycles. Chemiluminescence was measured by injecting a dilute alkaline hydrogen peroxide to initiate the reaction. Light emission was integrated over 5 s and expressed as photon counts. Data were processed using a four-parameter logistic curve fitting program as described previously for the ELISA (Schlaeppi et al., 1989). The nonspecific binding of acridinium-labeled MAb 4147-19-4 to BGG-bound particles was subtracted. It represented between 1.9% and 3% of the total photon counts bound to the solid-phase antigens (hapten-bound plus BGG-bound particles). The manual CLIA was carried out essentially as the automated one, except that the incubation periods were 30 min at room temperature. The washing was done manually three times with 500 μL of PBS–Tween.

**Soil Sample Preparation and HPLC Analysis.** The soil samples were obtained from a triasulfuron field trial done in one location in Switzerland. Triasulfuron was applied at 15 g of ai/ha. The chemical and physical properties of the sandy loam soil were the following: pH 7.0, sand 57.4%, silt 29.1%, clay 13.5%, humus 1.5%. Soil samples were taken at three different depths and extracted for immunoassay measurements as described previously (Schlaeppi et al., 1992). A few minor modifications were introduced to reduce the sample cleanup to a minimum. Briefly, 10 g of soil was extracted for 2 h with 40 mL of methanol/aqueous phosphate buffer (0.07 M, pH 7.0, 80:20). After filtration and acidification to pH 2.5–3.0 with 85% phosphoric acid, the samples were transferred on a ChemElut 1020 (Analytichem International) cartridge and the samples eluted with dichloromethane/*n*-hexane (80:20). The organic phase was evaporated and the residue dissolved in 20 mL of PBS–Tween before the CLIA measurement (see above). The same soil samples were extracted and analyzed by HPLC according to the procedure described previously (Iwanski and Egli, 1989; Bussmann et al., 1990).

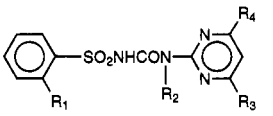
## RESULTS AND DISCUSSION

**Comparison of Different Haptens and Assay Optimization.** The optimal amounts of the various solid-phase antigens and labeled MAb were determined by checkerboard titrations. They corresponded to 450 pg of MAb and 10–30 ng of hapten per tube. As shown in Table 1, significant differences in assay performances were observed between the four solid-phase antigens tested. The BSA–hapten B conjugate, which corresponded to the homologous protein–hapten conjugate used for preparing MAb 4147-19-3 (Schlaeppi et al., 1992), produced the least sensitive automated CLIA, with an $I_{50}$ of only 0.68 ng/mL) compared to 0.09 ng/mL obtained by the previously reported ELISA (Schlaeppi et al., 1992), although the latter assay was performed with the same conjugate. The coupling of the *N*-hydroxysuccinimide ester of hapten B to magnetic particles previously coated with BGG improved the $I_{50}$ of the assay by 2.5-fold (0.27 ng/mL), but it remained less sensitive than the ELISA. We tried to improve the assay sensitivity by performing selected modifications of the solid-phase antigen. Extensive studies done by Hammock and collaborators have shown that the sensitivity of both monoclonal and polyclonal immunoassays was significantly improved by modifying either the length, the structure, or the position of attachment of the spacer arm used to link the target molecule to the

**Table 2. Cross-Reactivity of Sulfonylurea Analogues in the Automated CLIA**

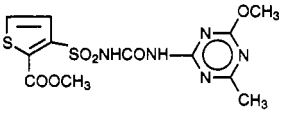


| compound | BSA–hapten A[a] cross-reactivity, %[b] | hapten C cross-reactivity, % | $R_1$ | $R_2$ | $R_3$ | $R_4$ |
|---|---|---|---|---|---|---|
| triasulfuron | **100** | **100** | $OCH_2CH_2Cl$ | H | $OCH_3$ | $CH_3$ |
| metsulfuron-methyl | 1.6 | 1.4 | $COOCH_3$ | H | $OCH_3$ | $CH_3$ |
| chlorsulfuron | 3.8 | 3.3 | Cl | H | $OCH_3$ | $CH_3$ |
| tribenuron-methyl | 0.02 | 0.02 | $COOCH_3$ | $CH_3$ | $OCH_3$ | $CH_3$ |
| cinosulfuron | 267 | 250 | $OCH_2CH_2OCH_3$ | H | $OCH_3$ | $OCH_3$ |

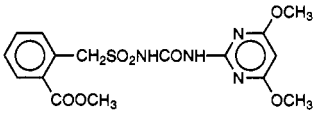


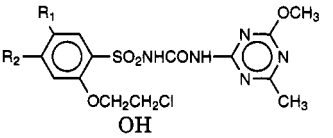
| | | | | | | |
|---|---|---|---|---|---|---|
| sulfometuron-methyl | 0.02 | 0.02 | $COOCH_3$ | H | $CH_3$ | $CH_3$ |
| primisulfuron | 0.06 | 0.06 | $COOCH_3$ | H | $OCHF_2$ | $OCHF_2$ |

| | | | | | | |
|---|---|---|---|---|---|---|
| thiameturon-methyl | 3.2 | 3.2 | | | | |



| | | | | | | |
|---|---|---|---|---|---|---|
| bensulfuron-methyl | <0.02 | <0.02 | | | | |





| | | | | | | |
|---|---|---|---|---|---|---|
| 4-hydroxytriasulfuron | 0.5 | 0.4 | H | OH | | |
| 5-hydroxytriasulfuron | 1.5 | 2.3 | OH | H | | |

[a] Antigen bound to the magnetic particles. [b] Cross-reactivity defined as (trisulfuron concentration for 50% inhibition/triasulfuron analogue concentration for 50% inhibition) × 100.

carrier protein (Goodrow et al., 1990; Harrison et al., 1991; Schneider and Hammock, 1992). Indeed, the sensitivity of a competitive assay is enhanced when the affinity of the antibody for the free analyte is higher than for the solid-phase antigen, which is usually not the case with a homologous hapten system. Therefore, we tested two heterologous solid-phase antigens in our assay configuration. One corresponded to a modification of the spacer location (hapten A) and the other to an increase of the spacer length (hapten C). Hapten A consisted of the triasulfuron molecule with an aminoalkyl spacer on the triazine ring (Figure 1), coupled to BSA by the diazonium method as described previously (Schlaeppi et al., 1992). When the conjugate was covalently bound to the magnetic particles with glutaraldehyde, the $I_{50}$ of the assay was improved by 7-fold compared to the homologous system (Table 1). In the case of hapten C, the length of the spacer (Figure 2) was designed according to our previous specificity study of MAb 4147-19-4, indicating a weak cross-reactivity between triasulfuron and structures corresponding to chloroethoxy phenylsulfonylurea with long aliphatic chains (Schlaeppi et al., 1992). Indeed, as shown in Table 1, the $I_{50}$ of the assay with hapten C was improved by 3.5-fold compared to the homologous BSA–hapten B assay. Moreover, the extended spacer provided the possibility for a single-step coupling of hapten C to the $NH_2$-coated magnetic particles, without prior conjugation to carrier proteins such as BSA or BGG. The simple determination of the hapten density on the magnetic particles allowed a well-controlled preparation of the solid-phase antigen.

**Dose–Response Curve and Sensitivity.** The standard curves of the automated assays with BSA–hapten A and hapten C as solid-phase antigens are shown in Figure 3. The minimum detectable amounts of triasulfuron defined as the concentration required to produce a decrease in the percentage of MAb bound equal to 2 times the standard deviation of the blank response were estimated to be 10 and 20 pg/mL with BSA–hapten A and hapten C, respectively. In soil samples, the limit of detection was 0.05 ppb with both assays. Despite the high sensitivity of the automated assay, we determined that no sample-to-sample carry-over was detected for any specimen containing up to 100 μg/mL of triasulfuron. Although high amounts of magnetic particles were added to the tube, the nonspecific binding remained low, representing between 1.9% and 3.0% of the total photon counts bound to the solid-phase antigens. Both the labeled MAb and the solid-phase antigens remained stable at 4 °C for more than 1 year. The performances of the CLIA remained constant over this period of time, and the regular UV-monitoring of the buffer containing the magnetic particles showed no evidence of leakage of the antigen from the particles.

**Specificity.** Table 2 summarizes the cross-reactivity between triasulfuron and other sulfonylurea analogues, including two triasulfuron metabolites. Only cinosul-

**Table 3. Recovery of Triasulfuron from Fortified Soil Samples Measured by Automated CLIA**

| | | triasulfuron recovered | | | | | |
|---|---|---|---|---|---|---|---|
| | | BSA–hapten A[a] | | | hapten C[a] | | |
| sample | triasulfuron added, ppb | ppb[b] (%) | SD[c] | CV,[d] % | ppb[b] (%) | SD | CV, % |
| 101 | 0.2 | 0.20 (100) | 0.03 | 14.5 | 0.22 (110) | 0.04 | 16.4 |
| 206 | 0.2 | 0.21 (105) | 0.02 | 8.1 | 0.22 (110) | 0.02 | 7.3 |
| 101 | 1 | 1.13 (113) | 0.06 | 5.2 | 1.07 (107) | 0.14 | 12.8 |
| 206 | 1 | 1.15 (115) | 0.12 | 10.6 | 1.10 (110) | 0.14 | 12.5 |

[a] Antigen bound to the magnetic particles. [b] Calculated according to standards made of triasulfuron in PBS–Tween (average of four determinations done in triplicates). [c] SD, standard deviation. [d] CV, coefficient of variation.

**Table 4. Concentration of Triasulfuron in Soil Samples Measured by HPLC and by Automated CLIA[a]**

| | | | BSA–hapten A[b] | | | hapten C[b] | | |
|---|---|---|---|---|---|---|---|---|
| sample | sampling depth, cm | HPLC ppb | ppb[c] | SD | CV, % | ppb[c] | SD | CV, % |
| 112 | 0–10 | 0.75 | 0.66 | 0.05 | 7.6 | 0.63 | 0.09 | 14.0 |
| 112 | 10–20 | 0.12 | <0.1 | | | <0.1 | | |
| 112 | 20–30 | 0.13 | <0.1 | | | <0.1 | | |
| 202 | 0–10 | 0.41 | 0.59 | 0.03 | 4.7 | 0.59 | 0.02 | 3.7 |
| 202 | 10–20 | 0.25 | 0.11 | 0.02 | 18.2 | 0.14 | 0.02 | 12.1 |
| 202 | 20–30 | <0.1 | <0.1 | | | <0.1 | | |
| 306 | 0–10 | 1.02 | 0.99 | 0.14 | 14.5 | 1.06 | 0.12 | 10.9 |
| 306 | 10–20 | 0.23 | 0.24 | 0.01 | 3.3 | 0.27 | 0.02 | 7.0 |
| 306 | 20–30 | <0.1 | <0.1 | | | <0.1 | | |

[a] Soil samples were collected from a field treated with triasulfuron. [b] Antigen bound to the magnetic particles. [c] Calculated according to standards made of triasulfuron in PBS–Tween (average of four determinations done in triplicates).

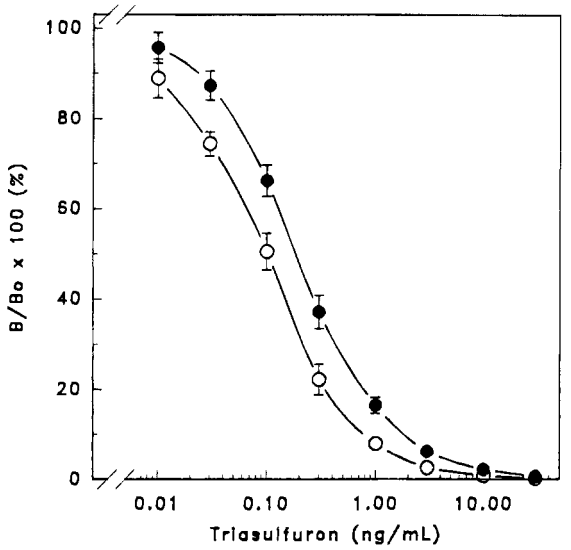



**Figure 3.** Inhibition of binding of MAb 4147-19-4 by triasulfuron in the competitive automated CLIA. The solid-phase antigens correspond to BSA–hapten A (○) and hapten C (●) bound to magnetic particles. $B/B_0 \times 100$ (%) represents the percentage of MAb bound to the solid phase. Vertical bars represent the standard deviations between assays.

furon with a methoxy replacing the chlorine showed high cross-reactivity in the automated CLIA. The remaining sulfonylureas and the two triasulfuron metabolites with a hydroxyl group on the phenyl ring showed low cross-reactivities. These results confirmed our previous extensive specificity study of MAb 4147-19-4 by competitive ELISA (Schlaeppi et al., 1992).

**Analysis of Soil Samples.** The performances of the automated CLIA with either BSA–hapten A or hapten C as solid-phase antigens, were tested on various soil samples coming from a triasulfuron field trial. All untreated soil samples (blank samples) gave values below the detection limit of the CLIA, which corresponded to 0.05 ppb. The photon counts of the blank soil extracts represented 84–93% of those of the buffer control with BSA–hapten A and 93–101% with hapten C, indicating that the rapid cleanup of the soil samples before immunoassays removed most of the possible interfering soil coextracted materials. These results confirmed our previous data obtained by ELISA (Schlaeppi et al., 1992). Untreated soil samples freshly spiked with two different concentrations of triasulfuron gave recoveries between 100% and 115% (Table 3). Measurements by automated CLIA of triasulfuron in treated soil samples compared favorably to those made by HPLC (Table 4). The amounts of triasulfuron measured by both methods were consistent with the depth of sampling, decreasing with increasing sampling depth. Using a positive cutoff of 0.1 ppb, the coefficient of correlation between HPLC and CLIA was 0.96 with either BSA–hapten A or hapten C as solid-phase antigens. The coefficient of correlation between both immunoassays was 0.99. We determined that the average intra- and interassay coefficients of variation were 4.1% and 9.6% with BSA–hapten A and 3.9% and 10.7% with hapten C. These results suggested an improvement of the precision of the CLIA compared to the ELISA (6% and 13%, respectively) (Schlaeppi et al., 1992).

**Conclusion.** We have developed a fully automated immunoassay with high sample throughput for the determination of triasulfuron. Despite the very short incubation of the reagents (7.5 min), sensitivity and precision of the assay were improved compared to the manual ELISA. The automated immunoassay should be a valuable method for environmental monitoring of triasulfuron. Since it is not fully specific for the target analyte, the confirmation of positive results by conventional methods is still required, unless the sample history excludes the presence of cross-reacting compounds. The present instrument can accommodate any combination of up to 13 different assays for each sample. Therefore, it opens the possibility of performing automated multiresidue analysis by immunoassays.

ABBREVIATIONS USED

BSA, bovine serum albumin; BGG, bovine γ-globulin; CLIA, chemiluminescent immunoassay; ELISA, enzyme-

linked immunosorbent assay; $I_{50}$, concentration of triasulfuron reducing the CLIA signal to 50% of the control; MAb, monoclonal antibody; PBS, phosphate-buffered saline.

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