

# Online Sequential-Injection Chromatography with Stepwise Gradient Elution: A Tool for Studying the Simultaneous Adsorption of Herbicides on Soil and Soil Components

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**S** Supporting Information

**ABSTRACT:** The adsorption of triazine herbicides simazine (SIM), atrazine (ATR), and propazine (PRO) as well as the metabolites deisopropylatrazine (DIA), deethylatrazine (DEA), and 2-hydroxyatrazine (HAT) on soil, humic acid, and soil modified with humic acid was studied by sequential-injection chromatography with UV detection at 223 nm. An online monitoring system was assembled, which was composed of a tangential filter and a peristaltic pump for the circulation of the soil (25 g L<sup>-1</sup>) or humic acid (2.5 g L<sup>-1</sup>) suspensions. A stepwise gradient elution separated the compounds using three mobile phases whose compositions were 28, 40, and 50% (v v<sup>-1</sup>) methanol in 1.25 mmol L<sup>-1</sup> ammonium acetate buffer, pH 4.7. The sampling throughput was about six analyses per hour; the linear dynamic range was between 100 and 1000 μg L<sup>-1</sup> for all of the studied compounds. The detection limits varied from 9 μg L<sup>-1</sup> for ATR to 36 μg L<sup>-1</sup> for DEA. At contact times <2 h, humic acid was the material with a higher adsorptive capacity (from 1470 ± 43 μg g<sup>-1</sup> for DIA to 2380 ± 51 μg g<sup>-1</sup> for PRO). In soil, HAT exhibited the highest adsorption (23.8 ± 0.2 μg g<sup>-1</sup>). The presence of humic acid in the soil increased the adsorption of ATR (14 ± 1 to 23 ± 2 μg g<sup>-1</sup>) and PRO (21.5 ± 0.5 to 24.0 ± 0.2 μg g<sup>-1</sup>), decreased the adsorption of HAT (23.8 ± 0.2 to 18 ± 2 μg g<sup>-1</sup>), and did not affect DIA and DEA. The adsorption of SIM was negligible in all of the sorbents studied. Simazine is the herbicide with the greatest potential for leaching to water bodies followed by DEA and DIA.

**KEYWORDS:** liquid chromatography, atrazine, simazine, propazine, metabolites, humic acid, soil

## INTRODUCTION

The occurrence of herbicides in surface and ground waters is controlled by several factors such as the intensity of application, the amount and frequency of precipitation, and the irrigation and physicochemical properties of the soil (amount of organic matter and clay minerals, pH, permeability, etc.).<sup>1,2</sup> Once the herbicide is applied to a crop it is susceptible to biotic<sup>3</sup> and abiotic<sup>4</sup> transformations, generating metabolites and degradation products. If the rate of transformation is faster than the rate of transport, then the degradation products and metabolites, instead of the parent herbicides, may be the predominant forms that reach ground waters at detectable concentrations.<sup>2</sup> The transport of parent herbicides and their degradation products depends on the rate, extent, and reversibility of sorption processes (the relative rates at which these compounds migrate through the subsurface are inversely related to their tendency to sorb onto soil and other geological materials).<sup>2</sup> Sorption is affected by the chemical composition of the mineral surfaces, natural organic matter (NOM), and competition with other contaminants.<sup>5</sup> The geochemical mobility and the potential runoff of herbicides to natural waters are evaluated by kinetic and thermodynamics parameters determined under different conditions of pH, ionic strength, and chemical composition of the aqueous phase.<sup>6</sup>

Atrazine (ATR) and simazine (SIM) are among the most frequently detected herbicides in ground and surface water.<sup>7</sup> They are used to stop pre- and postemergence broadleaf and grassy weeds in major crops such as sugar cane, maize, soybean, and citrus fruits.<sup>7</sup> Consistent evidence that ATR acts as an

endocrine disruptor that demasculinizes and feminizes male vertebrates were recently found by Hayes et al.<sup>8</sup> Atrazine and SIM were banned from the European Community since 2004, but they are still used in large areas of Brazil and the United States.<sup>9,10</sup> Microbial-mediated oxidation of one or both of the side chains of triazines produces deethylatrazine (DEA) or deisopropylatrazine (DIA), which are compounds that are less hydrophobic than the parent herbicide and are much less studied with regard to their toxicity.<sup>11</sup> Deethylatrazine is the more frequently detected metabolite in ground waters, which is consistent with works demonstrating that microorganisms oxidize the ethyl group of ATR or SIM more rapidly than the isopropyl group of ATR or propazine (PRO).<sup>2,12</sup>

The adsorption of triazines is characterized by a fast initial step (few minutes) followed by slow intraparticle diffusion that takes from several hours to several days to reach equilibrium.<sup>13–15</sup> Characterization of the initial steps of adsorption would be valuable to evaluate the potential runoff of herbicides during floods or heavy rains. High-performance liquid chromatography separates the herbicides from the soluble soil-matrix components and resolves the total sorption into intraparticle-diffused and labile-sorbed fractions.<sup>16</sup> Sorption kinetics of herbicides and their metabolites on soils are usually investigated for individual compounds to avoid the effects of

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competition by the adsorption sites.<sup>17</sup> This experimental condition may be not representative of field conditions, where mixtures of herbicides are applied. Fast online liquid chromatography would be useful to differentiate among the most leachable compounds in a competition situation.

Sequential-injection analysis (SIA) was conceived for online process monitoring using robust instrumentation capable of feeding the process controllers with high time-resolution data obtained by fully automated methods.<sup>18,19</sup> Coupling SIA instruments to short monolithic columns enabled the development of sequential-injection chromatography (SIC), which added the capability of performing the separation of simple mixtures by sequential-injection analysers.<sup>20,21</sup> The potential of this relatively new separation technique for online process monitoring was first proven by Klimundova et al.<sup>22</sup> who determined lidocaine and prilocaine in *in vitro* release tests from semisolid dosage forms. The separation of compounds with very different polarities in reversed-phase liquid chromatography has been achieved by modulation of the elution force of the mobile phases using the strategies of multisyringe pumping,<sup>23</sup> two-column,<sup>24</sup> automated intube generated gradient,<sup>25</sup> and stepwise gradient elution.<sup>26</sup>

The determination of ATR, SIM, and PRO in spiked river waters by isocratic SIC was previously described.<sup>27,28</sup> The present Article describes the separation of these three compounds as well as the metabolites deisopropylatrazine (DIA), deethylatrazine (DEA), and hydroxyatrazine (HAT) by the stepwise gradient approach. The method was applied for the online determination of the six compounds in suspensions of soil, humic acid, and soil in the presence of humic acid.

## EXPERIMENTAL SECTION

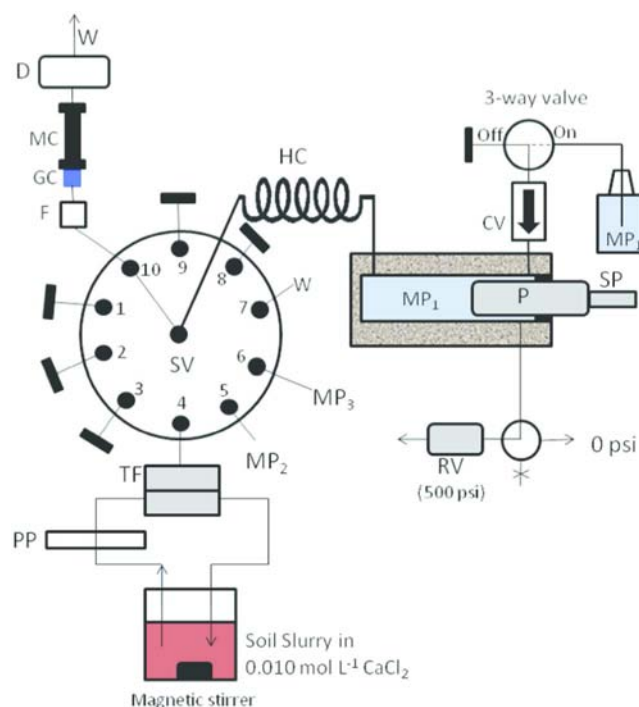
### Apparatus and Reagents.

The SICrom accelerated liquid-chromatography system was provided by FIALab Instruments (Bellevue, WA, USA). The FIALab 5.1 software synchronized the movements of the syringe pump and the selection valve (Figure 1) as well as acquired data from the UV detector. Details on the instrument configuration and mode of operation are described elsewhere.<sup>28,29</sup>

An Alitea C4 V peristaltic pump (PP) from FIALab Instruments fitted to 2.79 mm i.d. Tygon pump tubing pumped the soil suspension through an A-SEP tangential filter (Applikon, Analytical, Schiedam, The Netherlands) at a flow rate of 6.7 mL min<sup>-1</sup>. Filtration was made using a 47 mm diameter cellulose acetate membrane with a 0.22 μm pore size. The outlet of the tangential filter was connected to port four of the selection valve (Figure 1).

The analytical standards (Pestanal grade) of DEA (6-chloro-*N*-(propan-2-yl)-1,3,5-triazine-2,4-diamine), DIA (6-chloro-*N*-ethyl-1,3,5-triazine-2,4-diamine), HAT (4-(ethylamino)-6-(isopropylamino)-1,3,5-triazin-2-ol), SIM (6-chloro-*N,N'*-diethyl-1,3,5-triazine-2,4-diamine), ATR (6-chloro-*N*-ethyl-*N'*-(propan-2-yl)-1,3,5-triazine-2,4-diamine), and PRO (6-chloro-*N,N'*-di-(propan-2-yl)-1,3,5-triazine-2,4-diamine) were purchased from Sigma-Aldrich (Sigma-Aldrich Brazil, São Paulo). Stock solutions of these compounds were prepared at concentration of 500.0 mg L<sup>-1</sup> in methanol. These standards, solids, and solutions were stored in a freezer at -18 °C. Methanol (MeOH) of HPLC grade was supplied by J.T. Baker (Phillipsburg, NJ, USA). The sodium salt of humic acid was purchased from Sigma-Aldrich (lot STBB 1688) and characterized as described previously.<sup>30</sup> Ammonium acetate (NH<sub>4</sub>Ac) and acetic acid were purchased from Merck (Rio de Janeiro, Brazil). The water used in all experiments was distilled in all-glass equipment and deionized using the Simplicity 185 system from Millipore (Billerica, MA, USA) coupled to an UV lamp. All other reagents used in this work were of analytical grade from Merck, Sigma, or Aldrich.

Chromatographic separations employed three mobile phases (MP): MP<sub>1</sub> = methanol in 1.25 mmol L<sup>-1</sup> NH<sub>4</sub>Ac buffer at pH 4.7 (28:72, v



**Figure 1.** Sequential-injection chromatograph used for the online monitoring of the adsorption of triazines and metabolites in a soil suspension. SP = syringe pump, P = piston, RV = relief valve, CV = check valve, HC = holding coil (4 m × 0.8 mm i.d.), SV = selection valve, TF = tangential filter, PP = peristaltic pump, F = 10 μm pore inline filter, GC = 5 × 4.6 mm monolithic guard column, MC = 25 × 4.6 mm monolithic column, D = UV detector (223 nm) with a 4 cm light-path length and 10 μL flow cell, W = waste, MP<sub>1</sub> = mobile phase 1: 28% (v v<sup>-1</sup>) MeOH in 1.25 mmol L<sup>-1</sup> NH<sub>4</sub>Ac (pH 4.7), MP<sub>2</sub> = mobile phase 2: 40% (v v<sup>-1</sup>) MeOH in 1.25 mmol L<sup>-1</sup> NH<sub>4</sub>Ac (pH 4.7), and MP<sub>3</sub> = Mobile phase 3: 50% (v v<sup>-1</sup>) MeOH in 1.25 mmol L<sup>-1</sup> NH<sub>4</sub>Ac (pH 4.7).

v<sup>-1</sup>) and MP<sub>2</sub> and MP<sub>3</sub> were composed of methanol (HPLC grade) and NH<sub>4</sub>Ac buffer (1.25 mmol L<sup>-1</sup>, pH 4.7) at the volumetric ratios of 40:60 and 50:50, respectively. All mobile phases were filtered through 0.45 μm regenerated cellulose acetate membranes, sonicated for 30 min, and purged with high-purity He for 20 min prior to use.

**Sequential-Injection Procedure.** Separation was achieved in a 25 × 4.6 mm reversed-phase C<sub>18</sub> monolithic column (MC) coupled to a 5 × 4.6 mm guard column (GC) from Phenomenex (Torrance, CA, USA). A total of 500 μL of the suspension was used per experimental point. From this volume, 100 μL cleaned the sampling line (the piece of tubing connecting the outlet of the tangential filter and port four of the selection valve, Figure 1), and 400 μL was used for the chromatographic analysis. The mobile phase composed of 28:72 (v v<sup>-1</sup>) methanol in 1.25 mmol L<sup>-1</sup> NH<sub>4</sub>Ac buffer at pH 4.7 (MP<sub>1</sub>) conditioned the C<sub>18</sub> column and enabled the elution and separation of DIA, DEA, and HAT (the first elution step used 3600 μL of MP<sub>1</sub>). The elution of SIM required 1500 μL of MP<sub>2</sub>, whereas the separation of ATR and PRO required two sequential-elution steps with 1500 μL of MP<sub>3</sub>. Reconditioning of the chromatographic column was achieved by elution with 1500 μL of MP<sub>1</sub>. For a detailed description of the SIC procedure, please see the Supporting Information.

**Sample and Adsorption Experiments.** A soil sample was collected at the experimental farm of the Escola Superior de Agricultura Luiz de Queiroz da Universidade de São Paulo (ESALQ-USP) in the Piracicaba municipality, São Paulo, Brazil, in an area with no history of the application of herbicides. The sampling, soil treatment, and major characteristics are described elsewhere.<sup>31</sup> In brief, this is a clay-rich soil (53% clay, 18% silt, and 29% sand) with a total organic carbon content of 1.58% (m m<sup>-1</sup>). This is a sample that was

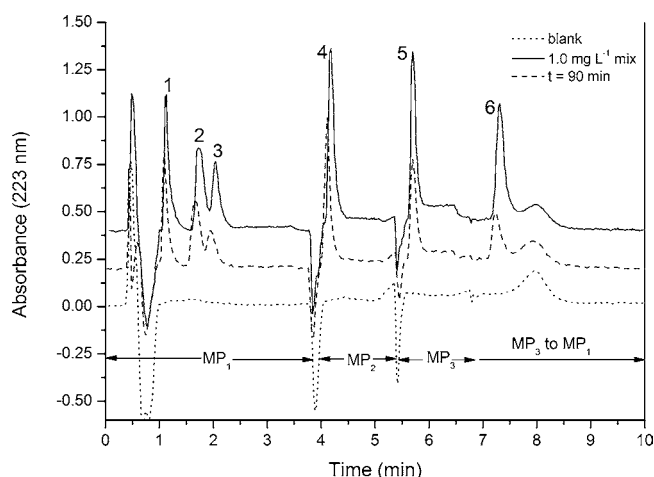
dried at 35 °C under vacuum and has been kept in a desiccator. Under these conditions, the physicochemical properties have not significantly changed over time.

A 1.0 mg L<sup>-1</sup> mix of DIA, DEA, HAT, SIM, ATR, and PRO (100 mL) prepared in 0.010 mol L<sup>-1</sup> CaCl<sub>2</sub>, whose pH was set to 5.3 (the pH of the soil solution), was added to a beaker containing a stir bar and the tubing connecting the solution to the tangential filter and the SIC system (Figure 1). The peristaltic pump was turned on to fill the tubing and the filtration system with the mix solution. After all of the tubing was filled with the solution, an aliquot was sampled and analyzed by the SIC system, registering the signal in the absence of sorbents. Next, a mass of approximately 2.5 g (weighed with a precision of ±0.1 mg) of soil acid was added to the beaker (under vigorous stirring), and the sampling was started to perform a sequence of nine measurements. The suspension was stirred and pumped through the loop and filtration system (Figure 1) throughout the experiments. In the case of the adsorption on humic acid, the mass of the adsorbent was 0.025 g (±0.1 mg). To evaluate the influence of humic acid, a binary adsorption system containing 2.5 g of soil and 0.025 g of humic acid was studied. All of the experiments were performed in triplicate.

## RESULTS AND DISCUSSION

**Triazine Separation.** The separation of SIM, ATR, and PRO has been achieved by isocratic reversed-phase liquid chromatography automated by SIC using a mobile phase composed of 44:56 (v v<sup>-1</sup>) MeOH in 1.25 mmol L<sup>-1</sup> NH<sub>4</sub>Ac buffer (pH 4.7).<sup>27,28</sup> Under these conditions, metabolites DIA, DEA, and HAT elute at the dead volume of the column. To separate these compounds, it is necessary to enhance their retention in the hydrophobic stationary phase by reducing the concentration of organic modifier in the mobile phase. Several compositions of the mobile phase were empirically tested, which were aimed at the separation of the three metabolites. A concentration of methanol of 28% (v v<sup>-1</sup>) enabled baseline separation (resolution >1.5) between DIA and DEA as well as a reasonable separation between DEA and HAT (resolution = 0.90). However, under these conditions, SIM, ATR, and PRO either did not elute from the column or did elute as broad ill-defined peaks after refilling the syringe pump several times. To overcome this difficulty, two additional elution steps with discrete increments of the concentration of organic modifier were implemented (40 and 50% v v<sup>-1</sup>).<sup>26</sup> A typical chromatogram under these conditions (described in the Supporting Information) enabled the baseline separation of HAT, SIM, ATR, and PRO. Simazine eluted in a single elution step with the 40% MeOH (MP<sub>2</sub>), whereas ATR and PRO eluted in two elution steps using 50% MeOH (MP<sub>3</sub>), as shown in Figure 2.

Separation was achieved in less than 8 min, but an additional 2 min were required for column reconditioning, so about six samples could be analyzed per hour, which is suitable to study the adsorption of herbicides in soil or soil components with high temporal resolution. An improvement of the resolution between DEA and HAT was possible by decreasing the concentration of MeOH on MP<sub>1</sub> to 15% (v v<sup>-1</sup>) (Figure 1S). This change in composition required modifications in the SIC procedure so that a baseline separation of DIA, DEA, and HAT was achieved in two elution steps of MP<sub>1</sub> (emptying the syringe, refilling it with MP<sub>1</sub>, and emptying it again), with DIA being eluted in the first step and DEA and HAT being eluted in the second step. Simazine eluted with MP<sub>2</sub>, and ATR and PRO eluted with MP<sub>3</sub> (compositions not modified). Despite the improved resolution between DEA and HAT, this procedure was not explored further because the total time of the analysis increased to 15 min.



**Figure 2.** Chromatogram of a 0.010 mol L<sup>-1</sup> CaCl<sub>2</sub> blank solution (dotted line) superimposed to the chromatogram of a 1.0 mg L<sup>-1</sup> mix (solid line) of the six triazines ((1) DIA, (2) DEA, (3) HAT, (4) SIM, (5) ATR, and (6) PRO) in CaCl<sub>2</sub> and to the chromatogram of a filtered solution sampled from the adsorption medium (dashed line) after 90 min of contact time. The arrows indicate the time intervals at which the column is eluted with each of the mobile phases. The flow rate during the elution steps was 30 μL s<sup>-1</sup>, and the sample volume was 400 μL.

A linear variation in the peak areas was found for concentrations of triazines between 100 and 1000 μg L<sup>-1</sup> (Table 1). The limits of detection (LOD) and quantification

**Table 1. Retention Time, Slope, Linear Correlation Coefficient, Limits of Detection, and Quantification Computed for the Triazines and Metabolites<sup>a</sup>**

compound	t <sub>R</sub> (min)	slope × 10 <sup>3</sup> (L μg <sup>-1</sup> )	r <sup>2</sup>	LOD (μg L <sup>-1</sup> )	LOQ (μg L <sup>-1</sup> )
DIA	1.10 ± 0.01	4.38 ± 0.13	0.9986	19	63
DEA	1.72 ± 0.02	3.95 ± 0.12	0.9985	36	119
HAT	2.05 ± 0.02	2.23 ± 0.08	0.9979	27	91
SIM	4.14 ± 0.02	6.88 ± 0.18	0.9990	25	76
ATR	5.68 ± 0.02	5.38 ± 0.16	0.9986	9	28
PRO	7.27 ± 0.03	5.97 ± 0.05	0.9999	16	52

<sup>a</sup>The results were obtained from the analytical curves constructed for concentrations between 100 and 1000 μg L<sup>-1</sup> for DIA, DEA, HAT, SIM, ATR, and PRO.

(LOQ) were computed as LOD = 3SD/*m* and LOQ = 10SD/*m*, where *m* is the slope of the calibration curves (peak heights) and SD is the standard deviation of the noise in the baseline measured around the t<sub>R</sub> (±10 s) value of each compound. The LOD values varied between 9 μg L<sup>-1</sup> for ATR and 36 μg L<sup>-1</sup> for DEA (Table 1). Although these values are too high for monitoring residual concentrations in environmental waters, they are low enough to characterize the time-dependent uptake of herbicide by soil particles. Despite the baseline deformation as a consequence of the gradient of the refraction index observed after the change of the mobile phase composition (Figure 2), the peak areas and retention times were easily and manually computed using Origin 8 SR2 software (Northampton, MA, USA). Additionally, the baseline drift did not affect the signal-to-noise ratio at the retention times. The relative standard deviation of the retention times computed from a sequence of five to 10 injections (as in the construction

of the calibration curve) was always lower than 1% (Table 1). However, some significant variations in  $t_R$  were observed in interday experiments, so that a daily construction of the calibration curves and spiking of the sample matrix with the triazines is recommended to confirm the peak assignments.

To evaluate the accuracy of the results, a matrix-matched  $\text{CaCl}_2$  solution was prepared in the absence of herbicides and metabolites. This solution was prepared by equilibrating a mixture of  $0.010 \text{ mol L}^{-1}$   $\text{CaCl}_2$  and the studied soil sample at the ratio of 1:40 ( $\text{m v}^{-1}$ ) for 24 h under constant agitation in an orbital shaker ( $25.0 \pm 0.5 \text{ }^\circ\text{C}$ ). The soil particles were removed by centrifugation followed by filtration through a  $0.22 \text{ }\mu\text{m}$  membrane, and the resulting solution was spiked with a mix of triazines to generate a  $1.00 \text{ mg L}^{-1}$  concentration of each compound. The same concentration was also prepared in pure  $0.010 \text{ mol L}^{-1}$   $\text{CaCl}_2$ . These solutions were analyzed by the proposed method and the concentration of triazines was computed by the external calibration method in which the standards were prepared in deionized water. The recoveries were between 97.3 (PRO) and 105.5% (SIM) in pure  $\text{CaCl}_2$  solution and between 93.4 (DEA) and 108.1% (HAT) in the  $\text{CaCl}_2$  soil extract (Table 2). These recoveries suggest that

**Table 2. Recovery Rates Obtained for a  $1000 \text{ }\mu\text{g L}^{-1}$  Mixture of Triazines and Metabolites Prepared in Pure  $0.01 \text{ mol L}^{-1}$   $\text{CaCl}_2$  and a Soil Extract Obtained from Shaking the Soil with  $0.01 \text{ mol L}^{-1}$   $\text{CaCl}_2$**

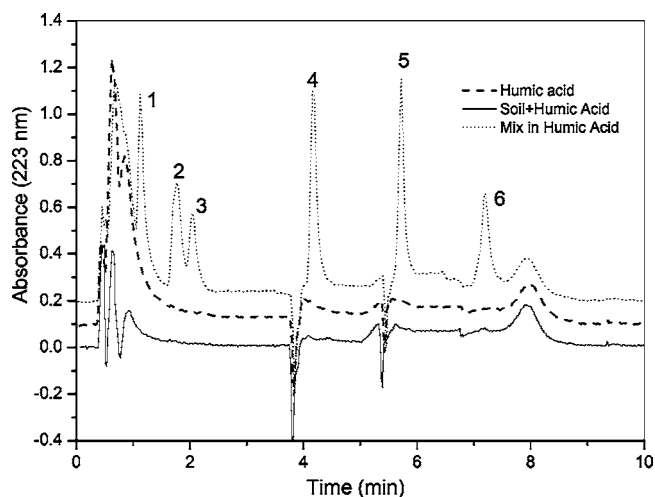
compound	recovery (%)	
	pure $0.01 \text{ mol L}^{-1}$ $\text{CaCl}_2$	$0.01 \text{ mol L}^{-1}$ $\text{CaCl}_2$ soil extract
DIA	$97 \pm 2$	$98 \pm 2$
DEA	$100 \pm 8$	$93 \pm 3$
HAT	$97 \pm 3$	$108 \pm 7$
SIM	$105 \pm 2$	$100.3 \pm 0.9$
ATR	$105 \pm 4$	$100 \pm 2$
PRO	$97.3 \pm 0.4$	$101.3 \pm 0.8$

there is no significant matrix interference coming from either the  $\text{CaCl}_2$  solution or from the  $\text{CaCl}_2$  soil extract. High recoveries were also found in the  $1.0 \text{ mg L}^{-1}$  solutions prepared after the equilibration of humic acid with  $0.010 \text{ mol L}^{-1}$   $\text{CaCl}_2$  at the 1:4000 ( $\text{m/v}^{-1}$ ) ratio. The filtered solution of humic acid shows a broad peak at around 1 min (Figure 3), which can be assigned to soluble organic matter components. This peak decreases in the presence of soil as a consequence of the adsorption of the organic components on the soil particles (Figure 3). Although the DIA peak appears on the tail of this organic matter peak, its integration was easy and did not affect the accuracy of the DIA quantification.

**Adsorption Experiments.** Experimental adsorption data are shown in Figures 4a–c for soil, humic acid, and soil in the presence of humic acid, respectively. The removal of organic substances from aqueous solutions by adsorption onto solid particles can be considered a reversible process that reaches equilibrium with time. In the present work, pseudo-first- and pseudo-second-order kinetic models<sup>32</sup> were tested to describe the experimental data using eqs 1 and 2, respectively:

$$\ln(q_e - q) = \ln(q_e) - k_1 t \quad (1)$$

$$\frac{t}{q} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \quad (2)$$

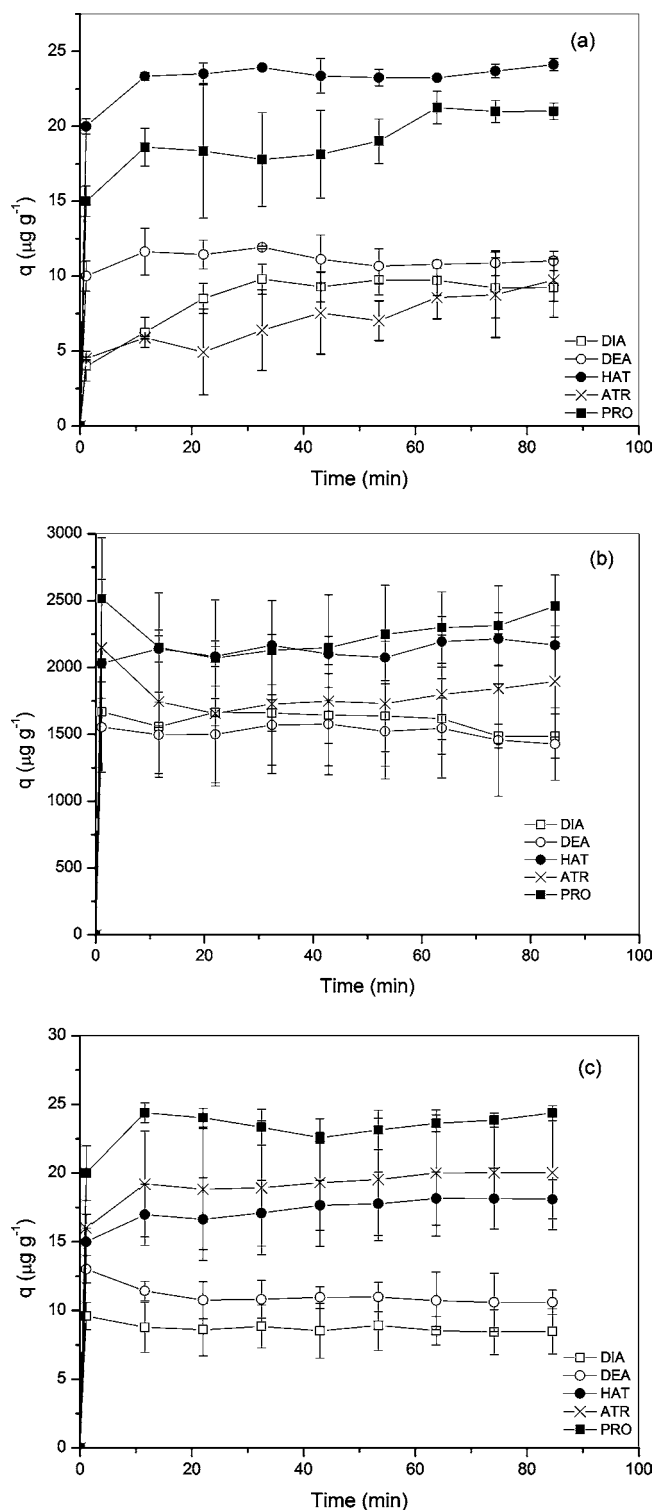


**Figure 3.** Chromatograms of the filtered blanks of humic acid and soil plus humic acid superimposed to the chromatogram of a filtered mix of triazines in the presence of humic acid. The peak assignments are as described in Figure 2.

where  $t$  is the contact time,  $k_1$  and  $k_2$  are the pseudo-first- and pseudo-second-order kinetics constants, and  $q$  and  $q_e$  are the amount of adsorbed triazine ( $\mu\text{g g}^{-1}$ ) at time  $t$  and at the equilibrium, respectively. Because both models are represented by linear equations, the quality of the fitting was evaluated by the linear regression coefficient ( $r^2$ ).<sup>33</sup> The pseudo-second-order kinetic model provided much better  $r^2$  values (Table 3) and fitted the entire range of experimental data (Figure 2S), which is contrary to the pseudo-first-order equation. This is an interesting feature of the model because it enables the estimation of both the equilibrium capacity ( $q_e$ ) and the initial rate of adsorption.<sup>34</sup>

The initial adsorption point was measured at 1.1 min of contact time, which is the time needed for filling the syringe with the mobile phase and cleaning the sampling line with the representative solution (steps one and two of the SIC separation procedure described in the Supporting Information). The initial adsorption rate was very fast, and most of the adsorption occurred in this short time interval (Figure 4). Slower intraparticle diffusion governs the kinetics after initial fast step, as can be seen in the almost flat variation of  $q$  as a function of  $t$  (Figure 4).

Simazine did not interact with any of the sorbents under the studied conditions, so no kinetic parameters could be calculated from the experimental data. The high mobility of SIM in soils has been reported by other authors.<sup>35</sup> The initial concentrations and soil-to-solution ratio play an important role in the adsorption capacities. Zheng et al.<sup>36</sup> studied the adsorption of SIM and ATR in biochar, verifying that the sorption affinity of both herbicides increased with the decrease in the soil-to-solution ratio. The sorbed amount of ATR increased from 451 to 1158  $\text{mg kg}^{-1}$  and that of SIM, from 243 to 1066  $\text{mg kg}^{-1}$  as the soil-to-solution ratio decreased from 1:50 to 1:1000 ( $\text{g mL}^{-1}$ ). However, if both herbicides coexisted in solution, then the competition effects decreased the Freundlich capacity parameter by factors of 1.5 and 2.4 for ATR and SIM, respectively. This fact suggests that ATR has a preference for sorption sites, which is consistent with the results obtained in the present work, especially considering the additional presence of PRO and HAT, which were the compounds with higher affinity for soil and humic acid.



**Figure 4.** Adsorption of DIA, DEA, HAT, ATR, and PRO on (a) 25 g L<sup>-1</sup> of soil, (b) 2.5 g L<sup>-1</sup> humic acid, and (c) 25 g L<sup>-1</sup> soil in the presence of 2.5 g L<sup>-1</sup> humic acid suspensions in 0.010 mol L<sup>-1</sup> CaCl<sub>2</sub>.

By comparing the  $q_e$  values, HAT was the compound with larger adsorption on soil (Table 3), which is in agreement with previous results described in the literature,<sup>31,37</sup> followed by PRO. The adsorption rates of DEA, DIA, and ATR were between  $9.8 \pm 0.7$  (DIA) and  $14 \pm 1 \mu\text{g g}^{-1}$  (ATR). Previous works usually report an adsorption order of  $\text{DEA} < \text{DIA} <$

**Table 3.** Pseudo-Second-Order Kinetic Parameters  $q_e$  ( $\mu\text{g g}^{-1}$ ) and  $k_2$  ( $\text{g } \mu\text{g}^{-1} \text{min}^{-1}$ ) for the Adsorption of Triazine Compounds in Soil, Humic Acid, and the Mixture of Humic Acid and Soil

triazine	parameter	sample		
		soil	humic acid	soil + humic acid
DIA	$q_e$	$9.8 \pm 0.7$	$1470 \pm 43$	$8 \pm 1$
	$k_2$	$0.04 \pm 0.02$	<sup>a</sup>	
	$r^2$	0.96	>0.992	>0.995
DEA	$q_e$	$10.8 \pm 0.8$	$1563 \pm 25$	$10 \pm 2$
	$k_2$			
	$r^2$	>0.99	>0.998	>0.990
HAT	$q_e$	$23.8 \pm 0.2$	$2203 \pm 45$	$18 \pm 2$
	$k_2$	$0.09 \pm 0.03$	$(6 \pm 3) \times 10^{-4}$	$0.040 \pm 0.01$
	$r^2$	>0.999	>0.998	>0.992
ATR	$q_e$	$14 \pm 1$	$1924 \pm 44$	$23 \pm 2$
	$k_2$	$(1.8 \pm 0.7) \times 10^{-3}$	$(3 \pm 1) \times 10^{-4}$	$0.04 \pm 0.01$
	$r^2$	>0.93	>0.995	>0.992
PRO	$q_e$	$21.5 \pm 0.5$	$2380 \pm 51$	$24.0 \pm 0.2$
	$k_2$	$0.013 \pm 0.009$	$(1.6 \pm 0.5) \times 10^{-4}$	$0.06 \pm 0.03$
	$r^2$	>0.995	>0.997	>0.995

<sup>a</sup>The linear regression resulted negative values of  $k_2$

ATR,<sup>17</sup> but at longer contact times and higher soil to solution ratios.<sup>31,38</sup>

Humic acid was the sorbent with a much higher adsorption capacity than the soil, which is a fact that may be explained by several interaction mechanisms such as charge transfer involving aromatic moieties in the humic acid and the aromatic triazines, hydrogen bonding, ionic interaction, and hydrophobic interaction.<sup>37,39</sup> The adsorption order on the basis of the fitted  $q_e$  values in humic acid was  $\text{PRO} > \text{HAT} > \text{ATR} > \text{DEA} > \text{DIA} \gg \text{SIM}$ . The addition of humic acid to the soil increased the adsorption of PRO and ATR, decreased the adsorption of HAT, and did not affect DIA and DEA (Table 3). The adsorption order was  $\text{PRO} > \text{ATR} > \text{HAT} > \text{DEA} \sim \text{DIA} \gg \text{SIM}$ . A previous work on the same soil using a contact time of 24 h, batch approach, and soil-to-solution ratio of 1:4 showed that the incorporation of humic acid increased the adsorption of ATR, DEA, DIA, and HAT (SIM and PRO were not studied).<sup>31</sup> Propazine is the most hydrophobic compound, as verified by its chromatographic behavior and its strong affinity to organophilic clays.<sup>40</sup> As a consequence, PRO may compete with HAT for adsorption in the hydrophobic moieties of humic acid.

The proposed sequential-injection liquid-chromatography method enabled the simultaneous determination of triazines and metabolites in soil and humic acid suspensions after online filtration. The method employs low-cost instrumentation and can be useful for studying nonequilibrium adsorption with high temporal resolution (computer-controlled sampling times), eliminating the manual operations of centrifugation and filtration. Additionally, it can be extended for the online monitoring of biotic transformation, reductive reactions in the presence of sulfides, and advanced oxidative processes. The results obtained from this study show that simazine is the triazine herbicide with a greater potential to be leached to surface or groundwater because its adsorption onto soil particles and humic acid was negligible. Propazine and HAT were the compounds more retained by the particles, but even

so their adsorption was never higher than 60% of the initial concentration after a contact time of 2 h, indicating that these compounds are also potentially leached to natural waters<sup>17</sup> as well as ATR, DEA, and DIA, which showed intermediate adsorption rates.

## ■ ASSOCIATED CONTENT

### 📄 Supporting Information

Detailed description of the SIC procedure; chromatogram of a 1.0 mg L<sup>-1</sup> mix of the six triazines in 0.010 mol L<sup>-1</sup> CaCl<sub>2</sub>; and pseudo-second-order kinetic plots obtained for soil, humic acid, and soil in the presence of humic acid. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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### Notes

The authors declare no competing financial interest.

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