1 Introduction

Monolithic polymer columns were first introduced in the late 1980s and early 1990s [1, 2]. Few years later, in 1996, the first monolithic silica column application appeared [3] (but conventional size columns became only commercially available in 2000) and with them new applications of LC [4]. They are made of highly porous rods with two types of pore structure (macropores with a typical size of 2 μm and mesopores about 13 nm) which allows the use of higher flow rates (up to 10 mL/min) thus reducing the analysis time without loss of resolution and with a much lower backpressure than the ones obtained with conventional LC columns. Monolithic columns have been applied to the analysis of drugs, pollutants, and food-relevant compounds, as well as in bioanalytical separations [4]. However, they have not been used as much as it was initially expected and the number of articles dealing with their practical applications is still relatively low.

Triazolopyrimidine sulfoanilide family of pesticides (flumetsulam, florasulam, metosulam, cloransulam-methyl, and diclosulam) are frequently used as pre-emergence and/or postemergence herbicides in different cultivars (peanuts, soybeans, etc.) in different countries around the world. Either diclosulam, metosulam, and cloransulam-methyl are registered by the US Environmental Protection Agency (EPA) (http://www.epa.gov), while florasulam is registered by the European Union (EU) [5] and metosulam is registered and used by several countries around the world. In previous works developed by our group [6, 7], we have studied the CE separation of this group of pesticides using UV detection and their determination in water [6], soil [7], and soy milk samples [8] – in this last case by CE-ESI-MS – by the development of a suitable SPE procedure. These works represent the first simultaneous CE separation and analysis of triazolopyrimidine sulfoanilide family of pesticides in such samples. However, although CE methods have proven to be good enough for pesticide determination, both GC and HPLC
methods are still the methods of choice for pesticide analysis because of their robustness and performance; that is why GC and HPLC methods should be developed for the simultaneous determination of this group of pesticides. In fact, GC or HPLC methods are the ones most highly accepted for the establishment of official methods regarding the analysis of pesticides. Concerning the analysis of this group of herbicides by HPLC, to our knowledge, up to now no HPLC method has been developed for the simultaneous analysis of these pesticides. Furthermore, monolithic columns have not been used for the analysis of any of these compounds.

Regarding the individual HPLC determination of some of these herbicides, Laganá et al. [9] have developed an HPLC-MS/MS method for the analysis of metosulam and flumetsulam, among others, using a conventional silica column. In that work retention times for flumetsulam and metosulam were between 10.5 and 13.5 min for the first of them and between 17.5 and 19.5 min for metosulam. In a second work of the same group [10] also metosulam was analyzed by HPLC-MS/MS and, in this case, retention time was also 17 min. Similar analysis times were obtained by other authors [11]. Because of the high flow rates that can be used with monolithic columns, their use could provide a quick and feasible analysis of these compounds, comparable to the ones previously obtained by CE in terms of resolution and analysis time [6–8]. In addition, the development of methods using conventional detectors like a UV detector (which are generally used by most laboratories) is of great importance, since instruments like a mass spectrometer, which is extremely useful in complex samples like soils or foods, is very expensive and not every laboratory can afford it.

As a result, in this work we describe a rapid method based on SPE-HPLC-UV using a C18 monolithic column to determine five triazolopirimidine sulfoanilide herbicides (flumetsulam, florasulam, metosulam, cloransulam-methyl, and diclosulam), frequently used as pre-emergence and/or postemergence herbicides in different countries, in mineral and tap waters, and soil samples. The analysis time as well as the LODs of soil samples have been improved with respect to our previous works [6–8]. To our knowledge, this work represents the first separation of these compounds and their first simultaneous determination in such samples by HPLC-UV (also using a monolithic column). It is also the first time that an SPE protocol is used for the simultaneous extraction of this group of pesticides from tap waters.

2 Experimental

2.1 Chemicals and samples

All chemicals were of analytical reagent grade and used as received. ACN (HPLC-grade) and formic acid (100% pure) were from Merck (Darmstadt, Germany). Cloransulam-methyl [methyl 3-chloro-2-[(5-ethoxy-7-fluoro(1,2,4)-triazolo(1,5-c)pyrimidin-2-yl)sulfanyl] amino]benzoate], diclosulam [N-(2,6-dichlorophenyl)-5-ethoxy-7-fluoro(1,2,4)triazolo(1,5-c)pyrimidine-2-sulfonamide], florasulam [N-(2,6-difluorophenyl)-8-fluoro-5-methoxy(1,2,4)triazolo(1,5-c)pyrimidine-2-sulfonamide], flumetsulam [N-(2,6-difluorophenyl)-5-methyl(1,2,4)triazolo(1,5-a)pyrimidine-2-sulfonamide], and metosulam [N-(2,6-dichloro-3-methylphenyl)-5,7-dimethoxy(1,2,4)triazolo(1,5-a)pyrimidine-2-sulfonamide] obtained from Dr. Ehrenstorfer (CYMIT QUIMICA, Barcelona, Spain) were used without further purification. Standard solutions of each pesticide were prepared in ACN and kept in the dark under refrigeration at 4 °C. Working mixtures of pertinent concentrations were prepared daily by appropriate combination and dilution with water. Distilled water was deionized by using a Milli-Q gradient system A10 (Millipore, Bedford, MA, USA). Mineral water samples were purchased from a local supermarket while soil samples were collected from a rural area of the city of La Laguna, in Tenerife.

2.2 Apparatus

A Waters HPLC system (Waters, Milford, MA, USA) was used consisting of two pumps (Model 510), a Waters automated gradient controller (Model 680), an injector (Rheodyne Model 7125 with a 20 μL loop) and a UV detector (Waters Model 486 tuneable absorbance detector). Detection took place at 205 nm. The Millenium32* software (Waters) and a PC were employed for data storage and evaluation. The analytical column was an Onyx Monolithic C18 column (100 × 4.6 mm) from Phenomenex (Madrid) with mesopores of 13 nm and macropores of 2 μm.

2.3 Chromatographic conditions

After a suitable optimization procedure optimum chromatographic separation was carried out at ambient temperature (25 °C) using a two stage gradient, with an initial composition of mobile phase A of 75 and 25% of mobile phase B. The gradient was programmed to linearly decrease the amount of mobile phase A to 55% in 3 min at 5 mL/min flow rate, and then to increase up to 75% in 3 min more, also at 5 mL/min. Mobile phase A consisted of water with 15 mM formic acid, while mobile phase B consisted of pure ACN containing 15 mM formic acid. At the end of the day the column was cleaned for 15 min with water and 15 min more with ACN at 1 mL/min flow rate. In order to assure the correct identification of the selected compounds, real samples were spiked with increasing amounts of each standard and injected into the HPLC system.
2.4 Water samples

Mineral or tap waters were spiked with the selected herbicides and after 3 h they were submitted to a previously developed SPE protocol [6]. Briefly, the SPE procedure was performed using a Vac-Master manifold from IST (IST, Hengoed, South Wales, UK). A 250 mL volume of this spiked solution with 10 mL of 1 M hydrochloric acid was slowly passed through a C18 SPE cartridge (Sep-Pak Plus C18 cartridge) from Waters, previously activated by flushing with 5 mL ACN followed by 2 mL of 0.01 M hydrochloric acid. After loading the sample into the SPE cartridge, it was dried under a vacuum of –10 mmHg (1 mmHg = 133.322 Pa) for 15 min. The retained herbicides were eluted with 10 mL of ACN. The organic solvent was then evaporated to dryness at 40 °C using a Rotavapor R-200 (from Büchi Labortechnik, Flawil, Switzerland). The residue was dissolved in 1 mL of water/ACN (1:1) and directly injected into the HPLC instrument.

2.5 Soil samples

Eight grams of soil was weighed and spiked with the selected herbicides. After 2 h, they were extracted with 300 mL of water and 1.2 mL of 0.1 M NaOH in an ultrasonic bath for 20 min. Afterwards, the samples were centrifuged at 4000 rpm for 10 min. The supernatant was then separated. 4 mL of 1 M HCl was added and they were centrifuged again at 4000 rpm for 5 min. Then, 200 mL of the supernatant was passed through a C18 SPE cartridge (Sep-Pak Plus C18 Cartridge) from Waters following the same activation and elution protocol described for water samples.

3 Results and discussion

3.1 HPLC separation

In previous works developed by our group [6–8] we have studied the CE separation of this group of pesticides and their determination in water [6], soil [7], and soy milk samples [8] by the development of a suitable SPE procedure. However, to our knowledge there does not exist an HPLC method capable of separating the same family of compounds.

In order to separate these pesticides and to achieve very low analysis times as the ones obtained by CE, a silica monolithic column with mesopores of 13 nm and macropores of 2 μm was used. Different gradient programs were tested, at several flow rates, with mobile phases composed of different percentages of ACN, water, and formic acid. Figure 1A shows the optimum separation of this group of compounds using the optimum elution gradient program (described in Section 2) using 205 nm as detection wavelength (in this figure the compounds injected were dissolved in water/ACN, 1:1). Peak efficiencies ranged between 35 700 number of theoretical plates per meter (NTP/m) for flumetsulam and 70 400 NTP/m for cloransulam-methyl (see Table 1). As it can be seen in the figure, a good separation of the five herbicides was obtained in less than 2.3 min. The high flow rate used in this case (5 mL/min) allowed a considerable reduction of HPLC analysis times [9, 10] down to a few minutes. Furthermore, this analysis time is lower than the ones obtained in our previous works dealing with the CE separation of these compounds [6–8]. In the best of these approaches, the lowest analysis time was 6.5 min [7], while for [6] and [8] the analysis time was around 9 and 23 min, respectively.

It was also observed that the solvent of the injected standard had a very high effect on the peak efficiencies of the analytes as it has already been indicated in the literature [12, 13]. Figure 1B shows the chromatogram of the injection of the same group of compounds dissolved in pure ACN. As it can be seen in the figure, the peak efficiencies had become worse with ACN alone. The improvement in the peak efficiencies when the sample is dissolved in water/ACN (1:1) or water alone may be caused by the “on-column focusing” effect (widely described in the literature) which occurs when solutes are concentrated or “focused” onto the top of the analytical column by injecting the sample in a solvent of lower eluting strength than that of the mobile phase. In our case, because the use of water/ACN (1:1) or water alone provided good peak efficiencies, subsequent experiments were carried out by dilution of the analytes in this solvent or mixture of solvents.

The use of water alone or the mixture of water/ACN (1:1) provided LODs, calculated as three times the S/N, in the low μg/L level, between 60 μg/L for flumetsulam and 90 μg/L for florasulam. These LODs are comparable, in some cases lower than the ones obtained in our previous work by CE-MS [8], which were between 74 and 150 μg/L, and higher than the ones obtained by CE-UV using online preconcentration strategies [6, 7]. In these two works [6, 7] the LODs were between 6.54 and 31.5 μg/L. Despite this fact, the LODs of both CE and HPLC methods are in the same range.

It should also be mentioned that, as it can be seen in Fig. 1, the baseline of the chromatogram is not constant. This change is caused by the low wavelength used to determine these compounds (205 nm). At this low wavelength and with the changes in the composition of the mobile phase in such a small time, such a change occurs. When working at higher wavelength values at which these compounds absorb less (up to 260 nm), such a change does not take place. Despite this change, as it will be demonstrated below, the repeatability of the method in terms of retention time and peak areas is good enough and also calibration can be feasibly developed.
3.2 Method validation

Once HPLC conditions were optimized, the performance of the method was examined by carrying out a repeatability study at three levels of concentration (0.5, 0.75, and 1.5 mg/L) with three consecutive injections during the same day (n = 3) on three different days (n = 9). The results of this repeatability study are shown in Table 1. Intraday precision RSD values were lower than 0.18% for retention times and between 0.68 and 2.25% for peak areas.

Table 1. Calibration parameters and results of the repeatability study obtained for the selected compounds.

<table>
<thead>
<tr>
<th>Peak</th>
<th>Compound</th>
<th>Efficiency (NTP/m)</th>
<th>Retention time</th>
<th>Intraday precisiona) (n = 3) RSD (%)</th>
<th>Day-to-day precisionb) (n = 9) RSD (%)</th>
<th>Linear range [µg/L]</th>
<th>Calibration curve (n = 7)</th>
<th>R²</th>
<th>S_yx (µg/L)</th>
<th>LODc) (µg/L)</th>
<th>LOQd) (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Flumetsulam</td>
<td>35.700</td>
<td>0.87</td>
<td>0.18</td>
<td>4.31</td>
<td>250 – 2000</td>
<td>y = (3446 ± 2838)x + (3435 ± 3672)</td>
<td>0.9949</td>
<td>1793</td>
<td>60</td>
<td>200</td>
</tr>
<tr>
<td>2</td>
<td>Florasulam</td>
<td>50.400</td>
<td>1.60</td>
<td>0.10</td>
<td>2.25</td>
<td>300 – 2000</td>
<td>y = (25309 ± 1318)x + (852 ± 1550)</td>
<td>0.9980</td>
<td>757</td>
<td>90</td>
<td>300</td>
</tr>
<tr>
<td>3</td>
<td>Metosulam</td>
<td>68.850</td>
<td>1.89</td>
<td>0.11</td>
<td>0.68</td>
<td>250 – 2000</td>
<td>y = (31075 ± 2098)x – (906 ± 2745)</td>
<td>0.9979</td>
<td>1086</td>
<td>83</td>
<td>277</td>
</tr>
<tr>
<td>4</td>
<td>Cloransulam-methyl</td>
<td>70.400</td>
<td>2.11</td>
<td>0.09</td>
<td>1.48</td>
<td>250 – 2000</td>
<td>y = (28233 ± 1562)x + (1581 ± 1328)</td>
<td>0.9983</td>
<td>763</td>
<td>75</td>
<td>250</td>
</tr>
<tr>
<td>5</td>
<td>Diclosulam</td>
<td>55.950</td>
<td>2.24</td>
<td>0.11</td>
<td>1.77</td>
<td>250 – 2000</td>
<td>y = (35195 ± 1751)x + (1391 ± 1488)</td>
<td>0.9986</td>
<td>855</td>
<td>65</td>
<td>217</td>
</tr>
</tbody>
</table>

a) Data given for 0.75 mg/L.
b) Calculated as three times the S/N.
c) Calculated as ten times the S/N ratio.
S_yx: SD of residuals.

Figure 1. Chromatogram of (A) a mixture of the five herbicides dissolved in water/ACN (1:1) and (B) a mixture of the five herbicides dissolved in ACN. Peak identification: (1) flumetsulam, (2) florasulam, (3) metosulam, (4) cloransulam-methyl, and (5) diclosulam. See Section 2 for other conditions. Concentration: 1.0 mg/L for all the compounds except for flumetsulam (1.1 mg/L).
areas while day-to-day precision RSD values were lower than 0.96% for retention times and between 1.75 and 4.31% for peak areas, showing that the precision of the HPLC procedure is high despite the change in the baseline previously mentioned. These RSD values are much lower than those values previously obtained by CE [6–8]. As an example, with the CE methods, RSD values for peak areas were between 2.08 and 6.71% for the intra-day precision and between 6.47 and 9.60% for the day-to-day precision which shows, as it is well known, that the repeatability of the HPLC procedure is higher than that of CE.

Once the repeatability of the method was studied, calibration curves (based on the peak areas) were obtained by injecting each standard \((n = 7)\) three times. Table 1 also shows the calibration parameters as, for instance, calibration equation, correlation coefficients \((R^2)\), \(S_{y/x}\) (SD of residuals), LODs, and the LOQ calculated as ten times the \(S/N\) ratio. As it can be seen, a good linearity, with correlation coefficients \((R^2)\) higher than 0.9949 was observed.

### 3.3 Water and soil samples analysis

As it has already been mentioned, in a previous work developed by our group [6] an SPE procedure (using \(C_{18}\) cartridges) was developed for the extraction of these pesticides from water samples. The sample protocol was also applied to the extraction of these pesticides from soil extracts [7] and soy milk samples [8]. In this case and, in order to extract these pesticides from tap and mineral waters, the same protocol was applied to both types of samples. However, in this work, we have tried to improve our previous SPE method by increasing the amount of sample loaded into the cartridge while maintaining a high recovery percentage and trying to avoid interferences from the sample matrix. In our case, the water samples, either tap or mineral water, loaded into the cartridge could be increased up to 250 mL. A higher volume provided the appearance of important interferences in the chromatogram. Figure 2 shows the electropherogram of a spiked tap water sample containing 4 \(\mu\)g/L of each pesticide. As it can be seen in the figure, all the pesticides could be detected at the spiked level. When a non-spiked sample (tap or mineral water) was also submitted to the whole procedure, no interfering peaks were found in any of the samples. Table 2 shows the mean recovery values \((n = 4)\) of the selected pesticides in both types of water. As it can be seen, recovery percentages ranged between 35 and 110% for tap water and between 83 and 104% for mineral water. In both cases, the LODs achieved were in the low \(\mu\)g/L range. The differences observed between both types of samples, especially in the recovery percentages of metosulam, which also has the highest RSD percentage values, can be caused by the sample matrix itself (mineral water samples are usually cleaner than tap water). It should also be mentioned that the mean recovery values obtained for mineral waters are very similar to the ones previously obtained [6] except for metosulam, which was extracted with a recovery of 59–64% in our previous work (it has to be considered that both mineral waters were not the same). Besides, the final LODs obtained in this work for mineral water samples are higher, although in the same range, than the ones obtained in our previous work (which were between 131–328 \(\mu\)g/L) in which SPE-CE-UV was used using online preconcentration strategies [6]. Concerning tap water, this is the first time that the SPE protocol is used for the simultaneous extraction of this group of pesticides from such matrix.

In order to demonstrate the applicability of the method to other type of samples, the proposed SPE procedure was also applied to the analysis of soil samples. In this case we have also tried to increase the amount of sample extracted; which is why 8 g of spiked sample were extracted with 300 mL of water. After suitable centrifugation, acidification, etc. described in Section 2, 200 mL of the extract was submitted to the previously developed SPE procedure. Figure 3 shows the chromatogram of a soil sample spiked at the 93.8 \(\mu\)g/kg level. As it can clearly be seen in the figure, all the pesticides could...
be detected. In the chromatogram, other peaks can clearly be observed but they do not interfere in the correct detection and quantitation of these compounds. Furthermore, when a nonspiked sample was submitted to the whole SPE procedure, no interferences were found, but when a higher amount of soil was used, important interferences appeared (the soil samples belong to a place where these pesticides are not used). Table 2 also shows the mean recovery values ($n = 4$) of the selected pesticides. In this case, mean recoveries ranged between 77 and 92% for all the pesticides. Concerning the high organic matter content and the complexity of soil samples, these recovery percentages are relatively high. It should also be mentioned that the mean recovery values obtained for soil samples are higher than the ones previously obtained [7] which can be caused by the improvement of the SPE procedure. According to the recovery values obtained in this work, LODs of the SPE-HPLC-UV method range between 9.38 µg/kg for flumetsulam and 14.1 µg/kg for florasulam. These LODs values are lower than the ones obtained in our previous work [7] (which were between 18 and 35 µg/kg) in which SPE-CE-UV was used using an on-line preconcentration technique, and very similar to those reported in the literature for other pesticides in soils by either GC or HPLC techniques [14].

### 4 Concluding remarks

In this work, the combination of SPE and HPLC-UV using a monolithic column for the rapid analysis of five triazolopyrimidine sulfonilide herbicides (flumetsulam, florasulam, metosulam, cloransulam-methyl, and diclosulam) in tap and mineral waters and soils is presented. The method allows the separation of these pesticides in less than 2.3 min. The whole procedure provided recovery percentages between 35 and 110% (water samples) or between 77 and 92% (soil samples) with LODs in the low ng/L level for water samples and in the low µg/kg level for soil samples.

J.H.B. wishes to thank the Ministerio de Educación y Ciencia de España for the FPU grant. This work has been supported by the Spanish Ministry of Education and Science (Project AGL2005-02924/AI).

### 5 References


