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A modified micro-solid phase extraction device for in-port elution and injection into portable liquid chromatography: A proof-of-concept study

C.E. Rodríguez-Palma, R. Herráez-Hernández *, P. Campíns-Falcó

MINTOTA Research Group, Departament de Química Analítica, Facultat de Química. Universitat de València. Dr. Moliner 50, 46100-Burjassot, València, Spain

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ABSTRACT

A micro-solid phase extraction (micro-SPE) device packed with a C18 sorbent (10 mg) has been developed for the enrichment and purification of organic water pollutants prior to their analysis using a portable liquid chromatograph with a dual UV detector. To this end, the sorbent was immobilized at the inlet of a 4 mm syringe filter (0.20 μm), which was modified to reduce its internal volume. The filter was coupled to the needle of the chromatograph. After loading the sample and cleaning the sorbent for analyte purification, the device was installed into the injection port of the chromatograph, and the target compounds were desorbed and transferred directly to the chromatographic column with a small volume of organic solvent. Under optimized conditions, sample volumes as large as 50 mL could be processed with the micro-SPE device, while the analytes were desorbed with only 60 μL of methanol. As a result, efficient preconcentration could be reached, as demonstrated for different water contaminants, namely aclonifen, bifenox, tritosulfuron, triflusulfuron-methyl and caffeine. The proposed micro-SPE device was applied to the analysis of different types of water (river, well, sea, ditch and wastewater). The recoveries of the target compounds in samples ranged from 76 % to 109 %, which allowed their detection at low to sub μg/L levels. All operations were carried out manually, and thus, no additional laboratory instruments such as centrifuges, stirrers or evaporators were required. This proof-of-concept study shows that the proposed micro-SPE approach can be considered a reliable and effective option for the on-site analysis of pollutants in environmental water samples by portable liquid chromatography.

1. Introduction

The increasing demand of chemical information as quickly as possible, preferably at the sampling point, is behind the development of a variety of portable instruments. In this respect and taking into account that liquid chromatography (LC) is a prominent technique in many fields of application, a lot of effort has been devoted to the design and implementation of portable liquid chromatographs. Over the past decade, hand-portable chromatographs operating under different formats and equipped with either UV or MS detectors have been developed, mostly through the miniaturization of the components of conventional LC systems [1–3]. Satisfactory results in terms of resolution, precision and portability have been reported with both home-made and commercially available chromatographs, and improved detection capabilities can be expected in the coming years [4–7]. However, in most of the studies reported so far, the instruments were only tested for standard solutions of the target compounds or for samples of low complexity [4,6,8].

Different studies have demonstrated that portable LC is compatible with simple treatments, mostly aimed at purifying the target compounds or at adjusting their concentrations. For example, Li et al. reported a method for the analysis of glycated hemoglobin in blood using a home-made portable LC instrument equipped with UV detection [9]. Samples (1 μL) were subjected to lysis and diluted before being injected into the chromatograph. Coates et al. utilized a portable capillary gradient LC, also with UV detection, for the analysis of caffeine in soft drinks; the samples were diluted with the mobile-phase before being introduced into the chromatograph [10]. We used a commercially available hand portable LC with UV detection for the analysis of active ingredients of dietary supplements such as caffeine [11] and chlorogenic acids [12]. In both cases, the analytes were extracted from the samples with methanol under ultrasonication; the extracts were further diluted with water to adjust the concentrations of the analytes before injecting them into the chromatograph. Compared with the results obtained with benchtop miniaturized LC (nano-LC and capillary-LC), the portable LC approach was clearly superior in terms of time of analysis, but the sensitivity

* Corresponding author.
E-mail address: rosa.herraez@uv.es (R. Herráez-Hernández).

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attainable was lower because the volume of sample that could be injected was also lower. Thus, depending on the application, a prior sample treatment to enrich the analyte may be mandatory. This was illustrated through the analysis of different methylxantines in environmental water samples [13]. These pollutants could be detected and quantified at suitable concentration levels by portable LC only after a preconcentration step. To this aim, the samples (25 mL) were processed through commercially available SPE cartridges packed with 100 mg of C18 sorbent, and then the analytes were desorbed and collected with 200 μL of methanol. The same cartridges were also used to improve the sensitivity and selectivity in the analysis of the drug scopolamine in different beverages [14].

To fully exploit the advantages of portable LC, especially its capability for operating on-site, simplified sample treatments would be highly desirable. Reducing the work-scale during the sample preparation may be an alternative [15]. However, as explained above, in procedures developed for portable miniaturized LC, only conventional-scale sample treatments have been used so far.

Different miniaturized sample treatment techniques have been proposed for on-site sample preparation such as solid-phase microextraction (SPME) with fibers coated with a polymer or with capillaries coated internally with a polymer (in-tube SPME), stir bar sorptive-extraction (SBSE), as well as miniaturized versions of SPE (micro-SPE). Sample preparation by micro-SPE relies on the same principles that SPE but using a lower amount of sorbent (typically a few mg), and it can be carried out under a variety of formats, for example, by dispersing the sorbent into the liquid sample (dispersive micro-SPE) or by immobilizing it into needles, syringes or pipette tips [13,16-18]. The employment of micro-SPE with sorbents packed into needles, syringes or tips typically involves the same steps than conventional-scale SPE, although the different stages of the extraction are usually carried out by applying successive draw-eject cycles [17,18]. The sample volumes that can be loaded into the extraction device at a time is usually very low (< 1 mL), which limits the enrichment factors that can be reached [19]. Moreover, draw-eject schemes can cause clogging during the analysis of complex samples with miniaturized chromatographic systems due to the small internal diameter of tubing [20].

In the present study we have developed a micro-SPE device aimed at simplifying preconcentration and purification of organic pollutants from water samples that can be coupled to a portable LC instrument. To this aim, the sorbent (C18) has been immobilized at the inlet of a commercial syringe filter connected to the injection needle. After passing the sample through the device and washing the sorbent, the micro-SPE device is installed into the injection port of the chromatograph, and the analytes are desorbed and transferred to the chromatographic column using a small volume of organic solvent. In such a way, the desorption/injection of the target analytes directly into the chromatograph is possible without any intermediate step. Although not limited, portable LC is highly desirable for the analysis of compounds of low stability. For this reason, in the present work two different diphenyl-ether and phenyl urea herbicides have been selected as target compounds. According to the literature, these types of herbicides degrade relatively rapidly in water [21,22]. The two diphenyl-ether herbicides, aclonifen and binenox, are included in the list of priority substances of the Water Framework Directive of the European Union [23]; the maximum allowable concentrations established for aclonifen and bifenox are 0.12 μg/L and 0.04 μg/L, respectively. Caffeine, which is a pollutant frequently found in water due to its widespread human consumption [24,25], has also been included in the present work for comparative purposes, as the quantification of this compound using off-line SPE with conventional cartridges and portable LC had been previously reported [13]. The utility of the proposed approach has been evaluated by analyzing different types of environmental waters.

2. Materials and methods

2.1. Chemicals and solutions

All reagents used throughout the study were of analytical grade. Bifenox was purchased from Chemservice (West Chester, PA, USA); aclonifen, tritosulfuron, triflusulfuron-methyl and caffeine were acquired from Sigma-Aldrich (Steinheim, Germany). Methanol and acetonitrile, both HPLC grade, were purchased from VWR Chemicals (Randnor, PA, USA).

Stock solutions of the analytes were prepared by dissolving the appropriate amounts of the commercial standards in methanol and kept at 4°C until use. Working solutions of the analytes and their mixtures were prepared by diluting the stock solutions with ultrapure water obtained from an Adrona system (Riga, Latvia).

2.2. Apparatus and chromatographic conditions

The chromatographic assays were carried out in a portable Axcend Focus LC™ LC liquid chromatograph (Axcend Corp, Provo, UT, USA) equipped with a 100 mm × 150 μm i. d. column packed with ODS, 1.7 μm particle size and a dual LED UV absorption detector that provided the chromatograms at 235 nm and 275 nm simultaneously. An Axcend Drive system (Version 2.1.0) was used for data acquisition and calculation. The volume of the injection loop was 40 nL.

The mobile phase was a mixture of water-acetonitrile 97:3 v/v (Solvent A) and water acetonitrile 3:97 v/v (Solvent B) in gradient elution mode. The elution programs were selected according to the hydrophobicity of the analytes, so that the total time required for the chromatographic analysis were similar for all of them (≈ 10 min); although not tested, the analytes could be most probably separated in a single run by optimizing the gradient elution program, although at expense of increasing the chromatographic run time. For the analysis of aclonifen and bifenox, the percentage of B in the mobile phase was increased from 35% at 0 min to 97% at 6.0 min; this composition was kept constant until 9.0 min, and then, decreased to 35% at 9.1 min and kept constant until the end of the run (10.0 min). For the analysis of tritosulfuron and triflusulfuron-methyl the percentage of B was increased from 10% at 0 min to 97% at 9.0 min, then decreased to 10% at 9.1 min and kept constant until the end of the run (10.0 min). For the analysis of caffeine, the percentage of B was increased from 5% at 0 min to 97% at 6.0 min, decreased to 5% at 6.1 min and kept constant until the end of the run (7.0 min). In all instances, the mobile phase flow rate was 1 μL/min.

All the solvents were filtered through 0.22 μm nylon membranes purchased from GVS (Sandfor, ME, USA) before use.

2.3. Micro-SPE

The customized micro-SPE device was built by placing the C18 sorbent (55 μm, 70 Å, Phenomenex, CA, USA) inside the inlet of 4 mm syringe filters equipped with a 0.20 μm pore size cellulose membrane (Corning Incorporated, Germany). The scheme of the device is shown in Fig. 1. The sorbent was immobilized between two polyethylene frits (20 μm, Supelco, Bellefonte, PA, USA). A 4 mm length segment of the outlet tube of the filter was removed with a cutter; the remaining tube was filled with glass beads of 2 mm diameter (Labbox, Barcelona, Spain). The glass beads were maintained inside the tube by attaching the filter to the injection needle.

For conditioning the sorbent, aliquots of 1 mL of methanol followed by 1 mL of nanopure water were passed through the micro-SPE device. After loading the sample, 1 mL of nanopure water was used for washing the sample matrix. The remaining water was flushed out the device with air. To this end, a 1 mL empty syringe with the plunger positioned at the top of the barrel was attached to the micro-SPE device, and then the plunger was moved down. For desorption and injection of the analytes,

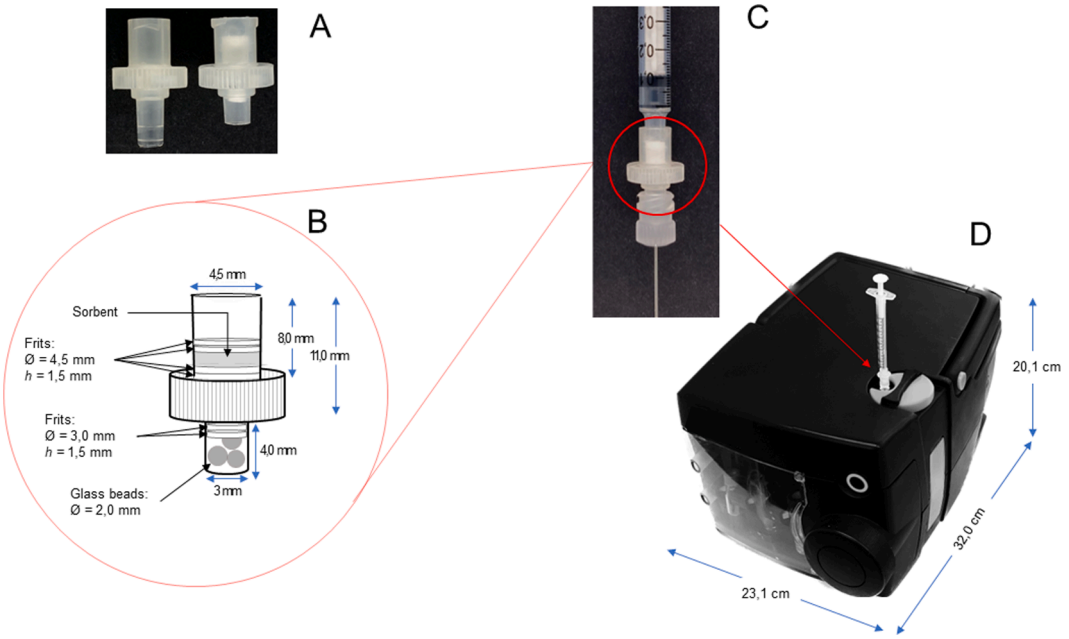

Fig. 1. A) Image of an original and a modified syringe filter; B) schematic representation of the modified syringe filter; C) image of the micro-SPE device; D) image of the micro-SPE device installed in the injection port of the portable LC.

the micro-SPE device was installed in the injection port of the portable LC and 60 μL of methanol were passed through it (unless otherwise, stated). In such a way, the injection loop of the chromatograph was filled with the extract. Finally, the chromatographic run was started with using the corresponding option of the software provided by the supplier of the chromatograph. All liquids were passed through the micro-SPE device manually at an approximately constant flow rate by applying positive pressure with syringes of different sizes (1-10 mL).

All assays were carried out by triplicate at ambient temperature.

2.4. Analysis of water samples

Different water samples were used throughout the study: seawater, well water, ditch water, river water and wastewater collected upstream of a wastewater treatment plant. All samples were collected at different points of the Comunitat Valenciana region (East Spain). Samples were kept at 4 °C and protected from light until analysis. None of the samples used in the study was filtered before analysis.

3. Results and discussion

3.1. Design of the micro-SPE device

The micro-SPE device was built by placing the sorbent at the inlet of a 4 mm syringe filter, as depicted in Fig. 1.

In order to reduce the internal volume, a 4 mm length portion of outlet tube of the syringe filter was removed; this did not affect the fitting of the micro-SPE device to the adaptor of the injection needle (Fig. 1A). Additionally, the remaining inner space was filled with glass beads, as depicted in Fig. 1B. The modified syringe filter was kept attached to the injection needle during the whole sample treatment to maintain the glass beads in place. Keeping the needle connected to the micro-SPE device did not increase significantly the backpressure during the extraction and desorption operations.

3.2. Optimization of the amount of sorbent and eluting volume

We tested different combinations of amount of sorbent and volume of eluting solvent (methanol) in order to optimize the analyte responses (peak areas), using the herbicides bifenox and aclonifen as model compounds. To this end, standard solutions containing 50 μg/L of each herbicide were assayed. Two amounts of sorbent were assayed, 10 mg and 25 mg. To ensure that the injection loop of the chromatograph was filled with the extracts, the eluting volumes (V_e) were selected considering the dead volume of the system, which was about 35 μL for devices packed with 10 mg of sorbent and 55 μL for the devices containing 25 mg of sorbent. These values were estimated from the dimensions of the sorbent compartment, outlet tube of modified syringe filter, the internal volume of the injection needle, and injection circuit of the portable chromatograph (injection loop 40 nL). The V_e assayed were 50, 60 and 100 μL for the device packed with 10 mg of sorbent, and 60 and 100 μL for the device packed with 50 mg of sorbent. Higher volumes were not assayed to avoid excessive dilution of the analytes in the resulting extracts.

The peak area measured for an analyte is proportional to its concentration in the extract obtained after the elution step (C_e):

$$Peakarea = k\,x\,C_e \tag{1}$$

which can be written as

$$Peak\,area = k\,x\frac{m}{v_e} \tag{2}$$

being m the mass of analyte in the extract and V_e the eluting volume, as stated above. If the analyte is completely desorbed from the sorbent m can be calculated from the sample volume (V_s), the concentration of analye in the sample (C_s) and the fraction of analyte recovered (R). Thus, the above equation can be rewritten into

$$Peak\,area = k\,x\frac{v_s x c_s x R}{v_e} \tag{3}$$

Hence

$$Peak\,area\,xV_e = k\,x\,V_s x C_s x R \tag{4}$$

According to Eq.(4), for a given sample (C_s and V_s constant) and under the same working conditions (R constant), the *Peak area x* V_e values remain constant provided that the analyte is completely desorbed:

$$Peak\ area\ x\ V_e = k' \quad (5)$$

If the analyte is desorbed partially *m* decreases, thus lower *Peak area x* V_e values can be expected. In other words, the values obtained for a sample by multiplying the peak areas of the target compound by V_e can be used to check whether such compound is completely desorbed from the sorbent.

In Fig. 2A are depicted the *Peak area x* V_e values calculated from the chromatograms obtained for aclonifen with micro-SPE devices packed with 10 and 25 mg of sorbent, and for the different eluting volumes tested. According to this figure, when using 10 mg of sorbent constant values of *Peak area x* V_e (within experimental variations) were obtained when V_e was $\geq$ 60 µL. However, when using 25 mg of sorbent, the maximum value was reached only when V_e was 100 µL. Similar conclusions were derived from the data obtained for bifenox (Fig. 2B).

The peak areas obtained for the devices packed with 10 mg and 25 mg of sorbent and the different V_e assayed are shown in Fig. 2C and D, respectively. It can be deduced that from the three combinations that led to a complete desorption of the analytes, the best option was the employment of 10 mg of sorbent and 60 µL of methanol for elution. This combination provided the highest peak areas for the two herbicides, which can be explained by the fact that they were less diluted. Consequently, this was the option selected for further experiments.

3.3. Optimization of the sample flow rate

The effect of the sample flow rate on the extraction efficiency was evaluated by passing through the micro-SPE device 50 mL of standard solutions of aclonifen and bifenox at different flow rates within the 5 - 40 mL/min range. Flow rates lower than 5 mL/min were not used to avoid long times of analysis. The flow rates were estimated by measuring the time required to deliver the whole sample through the micro-SPE device. It has to be noted that, since the samples were loaded into the micro-SPE devices manually, the flow rates given along the study are approximate values. The results obtained are depicted in Fig. 3. According to this figure, the analytical responses did not change significantly when the flow rate was increased from 5 to 10 mL/min. However, a slight decrement in the peak areas was observed for both analytes at flow rates $\geq$ 20 µL/min. Consequently, all solutions were passed through the micro-SPE at flow rates below 10 mL/min.

3.4. Optimization of the sample volume

The effect of the sample volume was evaluated by processing volumes of standard solutions of the target analytes within the 10-50 mL interval. The concentrations assayed were 50 µg/L for aclonifen and bifenox, and 100 µg/L for tritosulfuron, triflusulfuron-methyl and caffeine. After loading the samples, the micro-SPE devices were flushed with 1 mL of nanopure water and dried with air. Then, analytes were desorbed/injected into the chromatograph with 60 µL of methanol, as

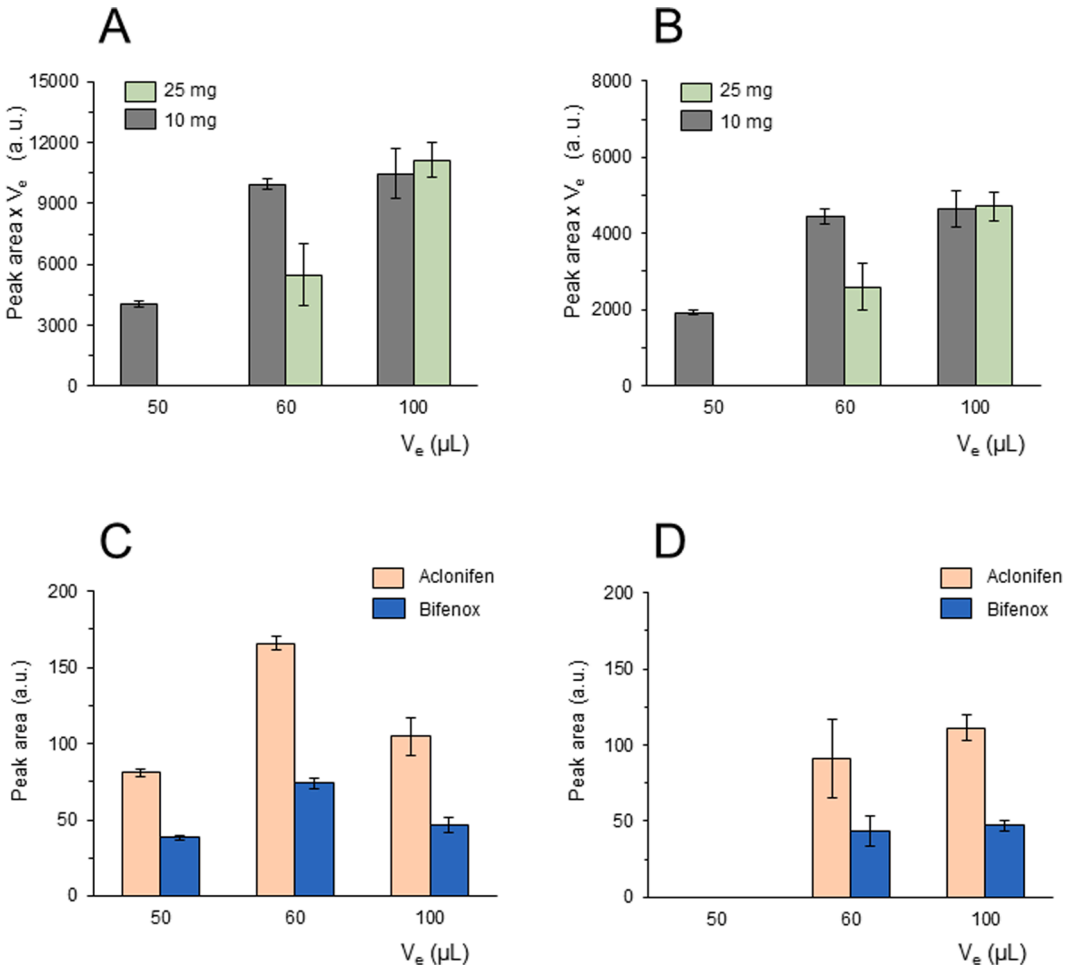

Fig. 2. Results obtained in the study carried out for optimizing the amount of sorbent and elution volume (V_e). Results obtained by plotting the *peak area x* V_e against V_e for A) aclonifen, and B) bifenox; results obtained plotting the *peak area* against V_e for a micro-SPE device packed with C) 10 mg of sorbent, and D) 25 mg of sorbent. Sample volume, 4 mL; concentration of the analytes 1000 µg/L. For other experimental details, see text.

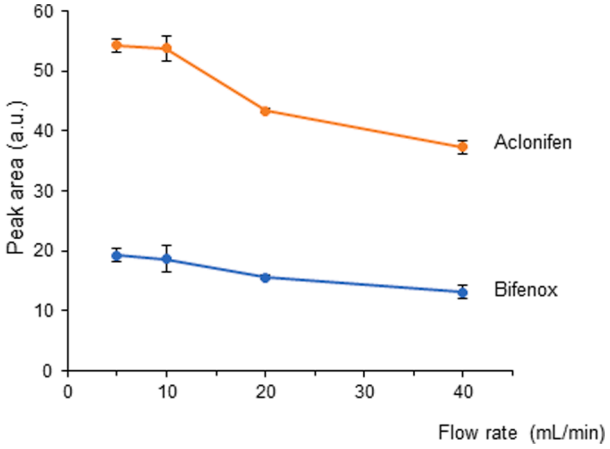

Fig. 3. Effect of the sample flow rate on the extraction of aclonifen and bifenox. Concentrations of the analytes, 50 μg/L; sample volume, 50 mL. For other experimental details, see text.

described in the previous section. The results obtained are depicted in Fig. 4. As observed, for aclonifen and bifenox the increment of analyte responses with the volume of sample were nearly linear up to 50 mL. Although not tested, the responses for these compounds could be most probably increased by using larger sample volumes. Increments were also observed for caffeine and triflusulfuron-methyl, although for sample volumes larger than 30 mL the improvement was moderate. Finally, a slight decrement on analyte responses was observed for tritosulfuron for sample volumes larger than 20 mL, suggesting losses due to breakthrough. The above results are in agreement with the octanol/water partition coefficients (K_{ow}) of the tested compounds (Table S1), taking into account that a C18 sorbent was used as extractive material. Based on the results obtained, 50 mL of the samples were used for aclonifen, bifenox and caffeine, whereas for tritosulfuron and triflusulfuron-methyl the sample volumes were 30 mL and 20 mL, respectively.

A schematic representation of overall sample treatment procedure (steps and conditions) is shown in Fig. S1.

In order to study the enrichment achieved with the proposed micro-SPE approach, solutions containing the analytes were injected directly into the portable LC, and the results were compared with those obtained after processing the same solutions with the micro-SPE device under the optimized conditions. The concentrations tested were 50 μg/L for aclonifen and bifenox, and 100 μg/L for caffeine, tritosulfuron and triflusulfuron-methyl.

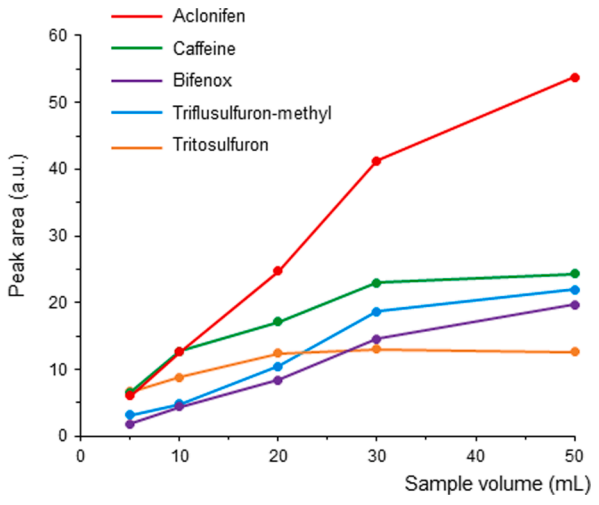

Fig. 4. Effect of the sample volume on analyte responses. Concentrations of the analytes: aclonifen and bifenox, 50 μg/L; tritosulfuron, triflusulfuron-methyl and caffeine, 100 μg/L. For other experimental details, see text.

In Fig. 5 are depicted illustrative examples of the chromatograms obtained throughout the study. As expected, a significant increment of the peak areas was found for all the analytes with respect to those obtained by injecting the same solution into the chromatograph. It has to be remarked that at the tested concentrations triflusulfuron-methyl and tritosulfuron could not be detected when the samples were directly injected into the chromatograph (Fig. 5C and D, respectively), whereas the peak of caffeine was very low, so that its area could not be measured properly (Fig. 5B). Under the conditions used for the analysis of aclonifen and bifenox, an additional peak was observed at a retention time of 7.9 min (Fig. 5A). The intensity of such peak was approximately constant, and it was also observed for a blank (nanopure water directly injected into the chromatograph). Thus, it was concluded that such peak was not directly related to the micro-SPE. This peak was most probably due to the solvents/materials used for the chromatographic separation.

3.5. Analytical performance

The potential utility of the proposed micro-SPE approach for the quantitative analysis of the tested analytes was evaluated by processing solutions containing different concentrations of the individual compounds. The concentration intervals and sample volumes used in each case are listed in Table 1. Since the portable LC was equipped with dual UV detection, the two chromatograms were registered simultaneously for each solution. The results of Table 1 are those obtained at the best working wavelength for each compound, which was 275 nm for caffeine and 235 nm for the rest of compounds. As observed in Table 1, linear responses were obtained for all the compounds tested. The limits of detection (LODs), established as the concentration that resulted in signal-to-noise ratio of 3, were in the 0.25-2.5 μg/L range.

The precision was evaluated by processing successively three aliquots of the working solutions. The results expressed as relative standard deviations RSD were $< 3\%$.

Additionally, the peak height ratios measured at the two working wavelengths ($PHR_{235/275}$) were calculated and further used for qualitative purposes. For triflusulfuron-methyl the $PHR_{235/275}$ value could not be calculated because at the working concentrations this compound was not detected in the chromatograms registered at 275 nm. The results obtained for the rest of compounds are listed in Table 2.

3.6. Application to the analysis of environmental waters

The proposed device was applied to the analysis of real water samples of different origin, namely sea, well, waste, ditch and river waters. Representative examples of the chromatograms obtained for the samples and the same samples after being spiked with some of the analytes are shown in Fig. 6.

No peaks were observed in the chromatograms of the water samples at the retention times of the analytes except for the river water sample in the conditions used for aclonifen and bifenox (section 2.2). The chromatogram obtained for this sample showed two peaks at 7.6 and 7.9 min, respectively (see Fig. 6A). As explained above, the later peak can be attributed to an impurity of the solvents or materials used along the chromatographic separation. However, the peak at 7.6 min overlapped with aclonifen, as observed in the chromatogram corresponding to the spiked sample (Fig. 6A). In order to check whether this peak could be due to the presence of aclonifen in the river sample tested, the $PHR_{235/275}$ coefficient was calculated within a time window of ± 0.01 min around its retention time, and the results were compared with those obtained for standard solutions of the herbicide. The results are also depicted in Fig. 6A. As observed, the $PHR_{235/275}$ obtained for the unknown peak of the sample was about 18, thus much higher than the value measured for the standard solutions of aclonifen (Table 2). It was, therefore, concluded that the peak observed at 7.6 min in the river

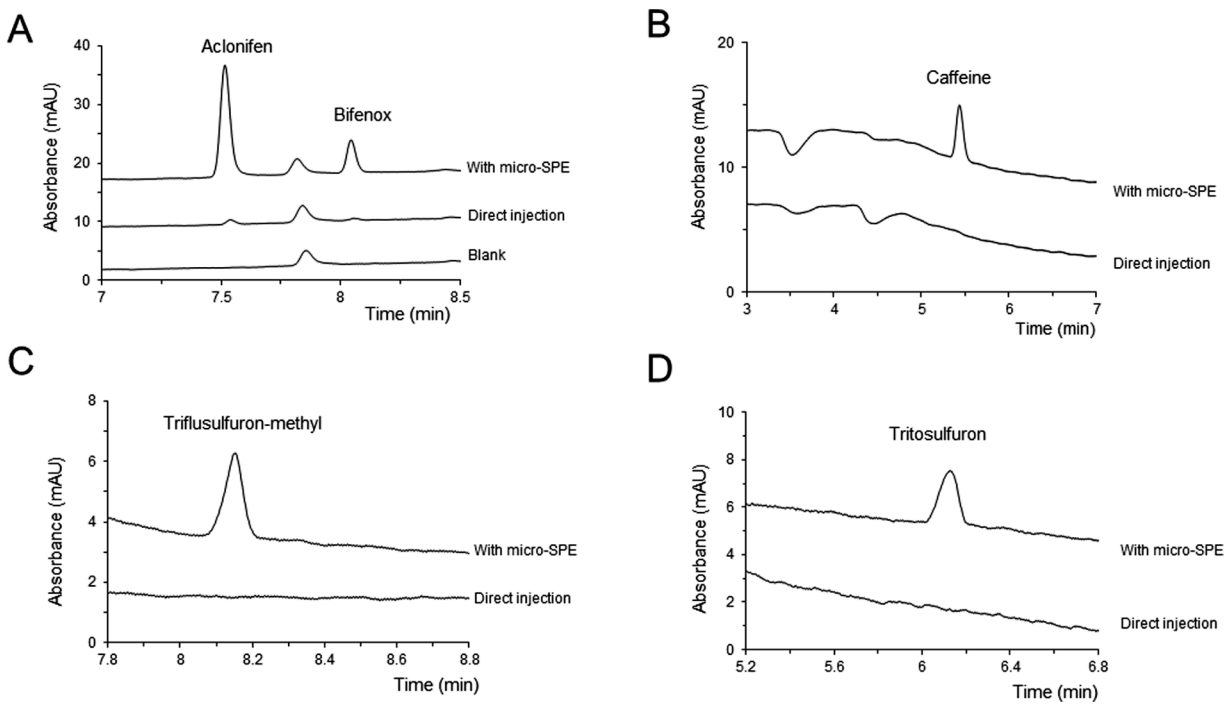

Fig. 5. Chromatograms obtained for the tested compounds by injecting directly the working solutions into the portable LC instrument and after processing them with the proposed micro-SPE device: A) aclonifen and bifenox (the chromatogram of a blank injected directly into the portable LC is also shown); B) caffeine; C) triflusulfuron-methyl; D) tritosulfuron. Concentrations of the analytes: aclonifen and bifenox, 50 μg/L; tritosulfuron, triflusulfuron-methyl and caffeine, 100 μg/L. For other experimental details, see text.

sample was not due to aclonifen. In Fig. 6 are also depicted the $PHR_{235/275}$ values calculated for bifenox, caffeine and tritosulfuron in the spiked samples. The results obtained showed a good concordance with those measured for standard solutions. A good concordance was also observed for all the spiked samples (see Tables 2 and 3). Therefore, the $PHR_{235/275}$ coefficients can be considered a useful tool for the positive identification of the analytes in real samples. This is an advantage of portable LC with dual UV detectors over instruments with single UV detection. Obviously, the $PHR_{235/275}$ coefficients would not allow the unambiguous identification of the analyte in the presence of matrix compounds eluting at retention time similar to that of the analyte.

The efficiency of the extraction was tested by processing different spiked samples. The effect of the sample matrix was evaluated by processing five different types of water using aclonifen and bifenox as model compounds. For the other pollutants two or three different types of waters were tested. All analytes were assayed in wastewater as this sample was expected to have the most complex matrix. In addition, some of the samples were assayed at two concentration levels (intermediate and low). The peak areas of the analytes in the spiked samples were compared to those obtained for standard solutions containing the same concentrations of the analytes in order to calculate the recoveries. The recoveries obtained are summarized in Table 3. As it can be seen in this table, the values obtained were suitable for all the analytes and matrices tested, and no significant effect of the concentration of the analyte on the extraction recovery were observed.

3.7. Utility

During the past years significant improvements towards miniaturization and portability of LC have been made, although most attention has been devoted to technical aspects of the instruments. However, to make portable LC a real option for on-site (preferably real-time) analysis, sample treatments that can be easily implemented in the field are necessary, especially if the analysis are present at trace levels. Several miniaturized sample treatments are now available under a variety of formats, but they have been mainly used for conventional LC. Moreover, in most applications the devices used are too sophisticated to be operated out of the lab and/or involve additional instruments. In the present work, we have developed a micro-SPE device that allows the enrichment of the analytes and their direct introduction into a portable LC instrument. To this aim, the sorbent has been immobilized at the inlet of a syringe filter attached to the injection needle. The device allows to process volumes of environmental waters as large as 50 mL. After the sample loading and cleaning, the analytes can be desorbed and directly introduced into the chromatograph with only 60 μL of methanol due to the reduced internal volume of the micro-SPE device.

All operations can be done manually with suitable precision, and no additional equipment is involved. Moreover, the micro-SPE device can be used with syringes (barrels) of different sizes, so that even when the analysis involves a large sample volume, the sample loading stage can be carried out easily and, in a few min.

Unlike other micro-SPE based methodologies such as those using pipette tips, microsyringes or injection needles packed with the sorbent [17–19], the proposed device does not involve successive aspiration/inejection cycles to deliver the sample and the solvents through the sorbent. In extraction methodologies based on draw/eject schemes, the solid particles of the sample may accumulate at the inlet of the sorbent compartment during the aspiration part of the cycle and pass to the extract during the desorption step (ejection). In the proposed micro-SPE methodology the sample is passed through the sorbent without reversing the flow direction. In such a way, possible particulate matter of the sample is retained in the upper frit of the micro-SPE device, thus minimizing the risk of clogging, which is one of the main drawbacks of miniaturized-liquid chromatography due to the low internal diameters of tubing and columns [15,16]. From a practical point of view, this is very important considering that with the proposed micro-SPE device samples do not need any prior treatment (not even a filtration).

In the course of our study every micro-SPE device could be used several times. More than 1 L of standard solutions of the analytes could be processed with the same device. When analyzing real water samples,

Table 1
Parameters obtained by processing standard solutions of the analytes with the proposed micro-SPE approach.

Analyte	Sample volume (mL)	Concentration range (μg/L)	Linearity[a], [b] $y = a + bx$ (n =5)	LOD (μg/L)	RSD[c], (n=3) (%)
Aclonifen	50	10-150	a ± S_a: 5 ±3b ±S b: 1.07 ± 0.04 $R^2 = 0.995$	0.25	3
Bifenox	50	10-150	a ± S_a: 0.8 ± 0.6 b ±S_b: 0.361 ± 0.008 $R^2 = 0.9992$	0.5	2
Caffeine	50	50-500	a ± S_a: 4 ± 2 b ±S_b: 0.167± 0.007 $R^2 = 0.998$	2.5	2
Tritosulfuron	20	25-250	a ± S_a: -2 ± 1 b ±S_b: 0.196 ± 0.008 $R^2 = 0.996$	1	1
Triflusulfuron-methyl	30	25-200	a ± S_a: 2 ± 1 b ±S_b: 0.157 ± 0.009 $R^2 = 0.998$	1	1

a: working wavelength: 275 nm for caffeine and 235 nm for the rest of compounds.
b: lines not forced to pass through zero.
c: established at a concentration of 50 μg/L for aclonifen, bifenox and tritosulfuron, 100 μg/L for caffeine, and 150 μg/L for triflusulfuron-methyl.

Table 2
Values of the $PHR_{235/275}$ coefficients obtained for standard solutions of the analytes (n=5).

Analyte	$PHR_{235/275}$
Aclonifen	6.6 ± 0.5
Bifenox	2.1 ± 0.1
Caffeine	0.9 ± 0.1
Tritosulfuron	4.7 ± 0.4
Triflusulfuron-methyl	n. c.*

* n. c.: not calculated because no peak was detected at 275 nm.

the number of times that each device can be used depends on the particulate matter content of the sample. For example, in the present study about 1 L of the seawater sample could be processed without detecting neither backpressure during the sample loading nor particulate matter on the frit. Each micro-SPE device was reused up to 20 times when processing 50 mL of the samples; for a sample volume of 20 mL the same device could be used up to 50 times. In contrast, for the ditch water sample, which contained a significant amount of solid matter, each micro-SPE device could be used to process only ≈ 250 mL of the samples (equivalent to a maximum of 5 and 12 uses for sample volumes of 50 and 20 mL, respectively). The rest of water samples tested had an intermediate content of solid particles, and each micro-SPE device was discarded after processing about 500 mL of sample (equivalent to a maximum number of uses of 10-25, depending on the sample volume). Nevertheless, the cost of each micro-SPE device is very low (< 1.5 €) as only a 4 mm syringe filter, 10 mg of C18 sorbent and four polyethylene frits are necessary (the glass beads and the injection needle are reusable). In addition, with the conditioning step applied before analyzing a sample no carryover effects were observed. Therefore, no additional regeneration of the sorbent was necessary.

As demonstrated for the tested compounds, the proposed micro-SPE device provides substantial enrichment of the analytes, making possible their detection at low to sub μg/L levels, depending on their hydrophobicity and UV absorption features. The LODs reached for the herbicides included in the study were about the same order than those attained with benchtop chromatographs and other miniaturized extraction techniques such as SPME [26,27] or dispersive-micro-SPE [28]. The LOD obtained for caffeine was comparable to the value previously reported by portable LC with conventional off-line SPE using 1 mL cartridges packed with 100 mg of sorbent [13]. The main advantages of the proposed micro-SPE approach are that the amount of sorbent and eluting solvent used for the extraction is substantially reduced, and the analyte can be directly desorbed/transferred to the portable chromatograph in a simpler and faster way.

In principle, the proposed micro-SPE approach can benefit from the fact that SPE is a mature technique, and several sorbents have been developed to enhance both the extraction efficiency of specific targets and/or the selectivity [29]. Therefore, this technique could be extended to other analytes and matrices.

4. Conclusions

In this study, a new micro-SPE device has been developed that allows the desorption and injection of the analytes into a portable LC in one step. The device has been applied to the analysis of organic pollutants in environmental waters. Different herbicides and caffeine have been selected as model compounds. The design of the device and the working conditions have been selected in order to achieve an efficient enrichment of the analytes with a minimum sample treatment. As a result, the analytes could be satisfactory detected and quantified, being the LODs in the 0.25-2.5 μg/L range and the RSD < 3%. The proposed method has been applied to the analysis of different environmental waters, proving recoveries in the 76 -109 % range. The peak height ratios at the two working wavelengths ($PHR_{235/275}$) allowed a more reliable identification of the analytes in the samples.

The proposed micro-SPE device offers additional advantages such as simplicity and speed, which are key factors in on-site tests. All operations involved in the extraction were effected manually in a few min, and the manipulation of the sample was reduced to a minimum. For example, the time required to process 50 mL of sample was about 8 min. The prior filtration of the sample is not necessary. In addition, the cost of the micro-SPE device is very low, and it can be reused. The number of uses depends on the particulate matter of the sample, and the volume of sample used in the analysis. Therefore, the proposed approach can be considered a valid alternative for the on-site real-time analysis of organic pollutants in environmental water samples.

Credit author statement

C.E. Rodríguez-Palma: Investigation, methodology, data curation, original draft. R. Herráez-Hernández: data curation, methodology, supervision, original draft, funding acquisition, review & editing. P. Campíns-Falcó: data curation, methodology, supervision, original draft, funding acquisition, review & editing, project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

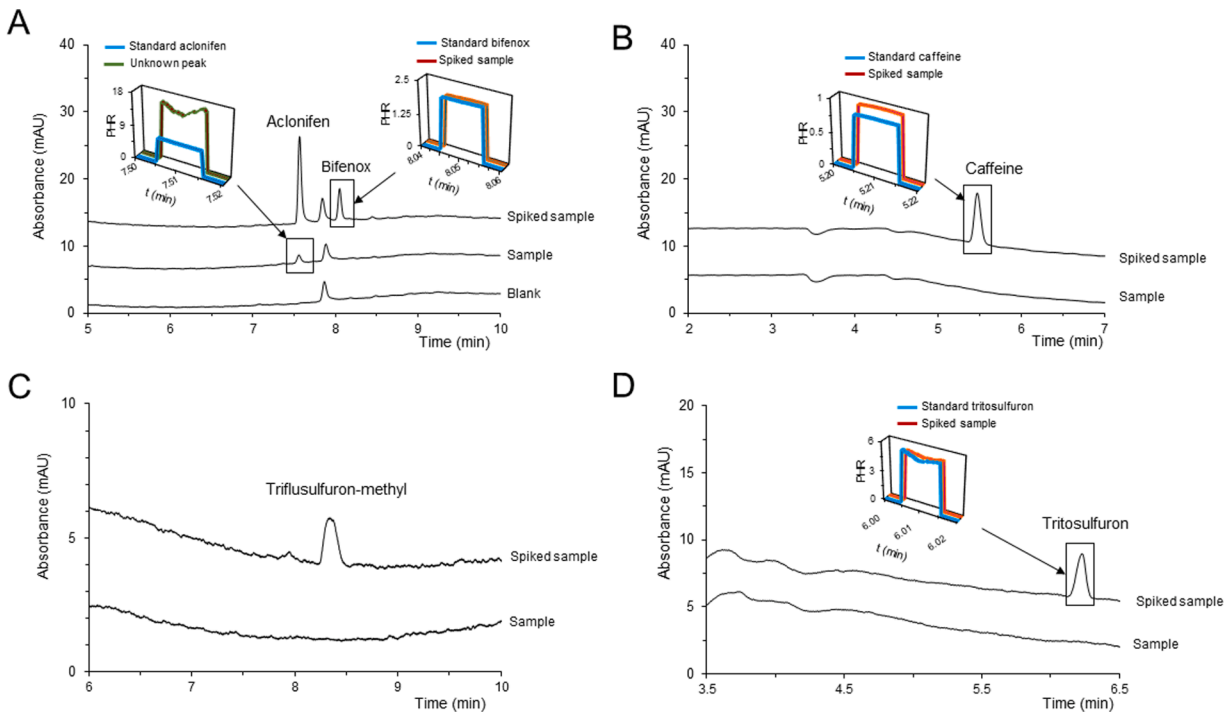

Fig. 6. Chromatograms obtained for real water samples and the same samples spiked with the analytes by the proposed micro-SPE method: A) river water spiked with aclonifen and bifenox (the chromatogram of nanopure water is also shown); B) wastewater spiked with caffeine; C) wastewater spiked with triflusulfuron-methyl; D) sea water spiked with tritosulfuron. Concentration of the analytes in the spiked samples: aclonifen and bifenox, 50 μg/L each; caffeine, 250 μg/L; 100 μg/L for triflusulfuron-methyl and tritosulfuron. In A), B) and D) are also depicted the $PHR_{235/275}$ values calculated at the maximum (retention times ± 0.01 min) of the marked peaks and the values obtained for standard solutions of the analytes. For other experimental details, see text.

Table 3
Recoveries and $PHR_{235/275}$ values obtained for the real samples spiked with the analytes.

Analyte	Type of water	Extraction Concentration tested (μg/L)	Recovery, (n =3) (%)	$PHR_{235/275}$ (n=3)
Aclonifen	Well	50	100 ± 6	6.9 ± 0.4
	Sea	50	100 ± 2	7.0 ± 0.1
		2	93 ± 11	
	Ditch	50	104 ± 3	7.1 ± 0.2
	River	50	76 ± 5	6.9 ± 0.2
	Waste	50	81 ± 1	7.0 ± 0.2
		2	97 ± 9	
Bifenox	Well	50	105 ± 9	2.0 ± 0.1
	Sea	50	89 ± 3	2.0 ± 0.1
		2	94 ± 10	
	Ditch	50	105 ± 9	2.1
	River	50	79 ± 9	2.0 ± 0.1
	Waste	50	84 ± 2	2.0 ± 0.1
		2	93 ± 9	
Caffeine	Sea	10	95 ± 6	0.9
	River	250	109 ± 4	0.9
	Waste	250	93 ± 6	0.9
		10	94 ± 4	
Tritosulfuron	Sea	100	92 ± 3	4.8 ± 0.5
		5	93 ± 11	
	Waste	100	85 ± 1	4.3 ± 0.3
		5	87 ± 8	
Triflusulfuron-methyl	Sea	100	104 ± 7	n.c.*
		5	98 ± 5	
	Waste	100	82 ± 5	n. c.
		5	86 ± 5	

* n. c.: not calculated because no peak was detected at 275 nm.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2023.464216.

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