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Salting-out assisted liquid–liquid extraction combined with capillary HPLC for the determination of sulfonylurea herbicides in environmental water and banana juice samples

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ABSTRACT

A salting-out assisted liquid–liquid extraction (SALLE) combined with capillary high performance liquid chromatography with diode array detector (capillary HPLC-DAD) was proposed for extraction and determination of residues of nine sulfonylurea herbicides (SUHs) in environmental water and banana juice samples. Various parameters affecting the extraction process such as the type and volume of the organic solvent, sample volume, type and amount of salt, pH of the sample and vortex time were optimized. Under optimum conditions, matrix matched calibration curves were established using river water and banana juice samples. Good linear relationships as well as low limits of detection, LODs (0.4–1.3 and 3–13 μg/L) and quantification, LOQs (1.3–4.3 and 10–43 μg/L) were obtained in water and banana juice samples, respectively. The precision (intra- and inter-day) of the peak areas expressed as relative standard deviations (%, RSD), at two concentration levels were below 10 % in both matrices. Recoveries obtained from spiked environmental waters (river water and groundwater) and banana juice samples, at two concentration levels, ranged from 72 to 115%. The results of the analysis revealed that the proposed SALLE-capillary HPLC method is simple, rapid, cheap and environmentally friendly, being successfully applicable for the determination of SUH residues in waters and banana juices.

1. Introduction

Sulfonylurea herbicides (SUHs) are one of the most important classes of pesticides which have been used worldwide for the control of many grasses and most broad-leafed weed species in a variety of crops and vegetables [1–3]. Their use has developed rapidly because of their high efficacies at low dosages. The sulfonylurea products are now the second most common kind of herbicides after the glyphosates and more than 30 products have been commercialized [4].

The intensive application of the herbicides has resulted in contamination of the atmosphere, environmental waters, soils and agricultural products (wheat, corn, fruits, vegetables, etc.) [5,6]. As a result, raw fruits and vegetables as well as their processed products, such as juices, could also be contaminated by SUHs and hence could give rise to serious health and safety problems for consumers. Based on these observations, the European Union (EU) has set maximum residue limits (MRLs) of SUHs in various agricultural products [7] and environmental water intended for human consumption [8]. For instance, the MRLs of SUHs in banana fruits are in the range of 10–50 μg/kg [7,9]. Consequently very sensitive analytical methods to detect ultra-trace levels of these compounds are needed.

Different analytical techniques have been used for the determination of SUHs and other classes of urea pesticides (phenylurea herbicides and benzoylurea insecticides) in vegetables, soil and water matrices [10]. Due to their polar characteristics, low volatility and thermal instability, SUHs cannot be directly determined by gas chromatography (GC) without prior derivatization, which is a time consuming process [11]. As a result, high performance liquid chromatography (HPLC) coupled with diode array detector (DAD) [12–17], mass spectrometry (MS) [5,18–21], or tandem mass spectrometry (MS^n) [22] have been widely used for the determination of SUHs. Also, miniaturized techniques such as capillary electrophoresis (CE) [23–25] or capillary liquid chromatography (cHPLC) with DAD [26–29] has been applied, showing several advantages, such as better resolution, lower detection limits and lower solvent consumption, being more environmentally friendly than conventional HPLC.

Various sample treatment methods have been proposed for the determination of SUHs being SPE the most popular one [3,12–14,16,23,30]. Polar pesticides such as SUHs can be successfully

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extracted from water samples using salting-out liquid–liquid extraction (SALLE) [31,32]. This technique is based on liquid–liquid extraction (LLE), in which the addition of an appropriate amount of a salt to a mixture of aqueous sample and water–miscible organic solvent causes a separation of the solvent from the mixture and thus the formation of a two-phase system and simultaneously the target analytes are separated into the organic phase [33,34]. For very high polar compounds the procedure can be modified by including an ion-pair formation step necessary for an efficient extraction [35,36]. The method is simple, fast, cheap, and environmentally safe and the obtained extracts could be directly injected or evaporated and reconstituted into a suitable solvent before to be injected into HPLC, CE or GC instruments [33,37].

In this work, a simple, fast and environmentally friendly SALLE methodology in combination with cHPLC-DAD has been developed and validated for the quantitative determination at trace level of nine SUHs in environmental water and banana juice samples. The target analytes were chosen based on their priority of use and taking into account the EU legislation, water samples and fruit juices were considered relevant matrixes to demonstrate the applicability of the method for monitoring these residues. Important parameters influencing the sample extraction technique and analyte separation were also optimized in order to obtain maximum extraction efficiency and sensitivity. The combination of a simple sample treatment such as SALLE with this miniaturized technique can provide a useful method for the monitoring of these herbicides in routine analysis.

2. Experimental section

2.1. Chemicals and reagents

Analytical standards of chlorosulfuron (CS), foramsulfuron (FRS), nicosulfuron (NS), oxasulfuron (OS), primisulfuron-methyl (PSM), prosulfuron (PS), triasulfuron (TS) and triflusulfuron-methyl (TSM) were obtained from Sigma Aldrich (St. Louis, MO, USA). Flazasulfuron (FS) was obtained from ChemService Inc (West Chester, USA). Both, 1000 mg/L of individual stock standard solutions and an intermediate working solution containing 20 mg/L for NS and FS; 10 mg/L for FRS, OS, TS, CS, PS and PSM and 5 mg/L for TSM were prepared in acetonitrile. Fresh intermediate working solutions were prepared every five days. All solutions were stored under refrigeration below 4 ºC.

All chemicals and reagents used in this study were of analytical grade, while the solvents were of HPLC grade. Methanol, acetonitrile, acetone and ethyl acetate were supplied by VWR BDH Prolabo (West Chester, PA, USA); sodium hydroxide, magnesium sulfate, acetic acid (HOAc), hydrochloric acid and sodium chloride were purchased from Panreac-Química (Madrid, Spain); 2-propanol and citric acid monohydrate were purchased from Sigma Aldrich. Ultrapure water, purified with a Milli-Q Plus system (Millipore Bedford, MA, USA), was used throughout the work. Mobile phase solvents were filtered under vacuum through nylon 66 membranes, 0.2 μm × 47 mm (Supelco, Bellefonte, PA, USA). Nylon syringe filters, 0.22 μm × 13 mm (Agela technologies, New York) were used for filtration of the sample extracts prior to injection into the cHPLC system.

2.2. Instrumentation

The determination of SUHs was performed using an HP-1200 series capillary HPLC (Agilent Technologies, Germany) equipped with a capillary pump (maximum flow rate: 20 μL/min), online degasser and autosampler (8 μL loop), column thermostat and a diode array detector (DAD). Chromatographic separations were achieved on a Luna C_{18} column (150 mm × 0.3 mm I.D., 5 μm particle size) from Phenomenex (Micron, Madrid, Spain) at a temperature of 25 °C. Data acquisition and processing were accomplished using ChemStation software (Rev. A.10.02) from Agilent Technologies.

A vortex Genie 2 model (Scientific industries, Bohemia, USA), a centrifuge Universal 320R (Hettich, Tuttlingen, Germany), a nitrogen evaporator (System EVA-EC, VLMGmbH, Bielefeld, Germany) were used for sample preparation. A pH-meter with a resolution of ± 0.1 (Crison model pH 2000, Barcelona, Spain) was also employed.

2.3. Chromatographic conditions

The reversed phase separation of the target analytes containing nine SUHs was performed based on a previous paper [28], using a C_{18} column and a binary mobile phase consisting of solvent A (water) and solvent B (acetonitrile), both containing 0.01% HOAc (*v*/*v*), with a gradient program of 25–45% B (10 min), 45–55% B (3 min), 55–75% (7 min), 75–95% B (1 min) and 95% B (13 min). Prior to the subsequent injection, the capillary HPLC column was re-equilibrated with the initial composition of the mobile phase for 10 min. Analysis was performed with a flow rate of 10 μL/min, using a column temperature of 25 °C and an injection volume of 3 μL. The DAD monitoring wavelength of 230 nm was employed [27].

2.4. Sample treatments based on SALLE

Two kinds of samples were analyzed: water and banana juice samples. Different environmental water samples (groundwater and river water) were collected from Castril (Granada, Spain). Banana juice sample was also obtained from a local market in Granada, Spain. Both water and banana juice samples were stored at 4 °C in the dark prior to analysis, without any further sample pretreatment.

2.4.1. Water samples

For applying SALLE, 4 mL of each water sample containing 0.1 mol/L citrate buffer pH 2 was placed into a 15 mL falcon centrifuge tube with conical bottom. The sample was fortified with appropriate concentrations of SUH standards and kept for 30 min for equilibration. Afterwards, 1 mL of acetonitrile was added and the content was then gently shaken with a vortex, at the highest vortexing speed, for about 10 s. Then, 1.6 g of $(NH_4)_2SO_4$ (i.e., 40%, *m*/*v*) was added to the mixture and further vortexed for 3 min. At this stage, the content was separated into two phases and the SUHs were extracted into the upper organic phase (acetonitrile phase). To facilitate the separation of the upper organic phase, the mixture was centrifuged for 5 min at 9000 rpm. The supernatant was then withdrawn using a 1 mL syringe with a sharp needle tip and transferred to a 2 mL glass vial and was then dried under blowing nitrogen at room temperature. The residue was re-dissolved in 200 μL of methanol containing 0.01% acetic acid (1:1, *v*/*v*) followed by vortexing for 2 min. The solution was then filtered with a 0.2 μm nylon filter and transferred into 200 μL insert vial, which was housed in a 1.5 mL amber vial and finally placed on the autosampler of the capillary HPLC equipment for injection.

2.4.2. Banana juice sample

To apply the SALLE procedure, 2 mL of the juice samples were placed into a 15 mL falcon centrifuge tube with conical bottom, then fortified with an appropriate concentration of SUHs and left for 30 min for equilibration. Before extraction, juice sample was diluted 1:1 with 0.2 mol/L citrate buffer pH 2 in order to reduce the matrix effect and to adjust the pH at the optimum value. Then, after adding 1 mL of acetonitrile, the mixture was vortexed at the

highest speed, for about 30 s to homogenize the content. Thereafter, the same procedure for water samples described above was followed (Section 2.4.1).

For both kinds of samples, a sample throughput of approximately 12 samples per hour with a preconcentration factor of 20 could be obtained.

3. Results and discussion

3.1. Optimization of HPLC conditions

Different binary mobile phases were tested, including acetonitrile or acetonitrile/methanol (1:1, *v/v*), (pure or containing 0.01 % HOAc) as solvent B and water containing 0.01% HOAc as solvent A. As a compromise between adequate retention times for the target analytes and a better sensitivity, acetonitrile (solvent B) and water (solvent A) both containing 0.01% HOAc were chosen as the mobile phase [28].

The effect of the injection volume was investigated over the range from 1 to 6 μL. It was observed that the peak area of all SUHs increased with the injection volume but above 3 μL some peaks, including FRS, OS, TSM and PSM were relatively broad and their resolutions were not satisfactory. Thus, an injection volume of 3 μL was chosen as a compromise between high sensitivity and adequate peak resolution. The effect of the mobile phase flow rate was evaluated in the range of 8–15 μL/min. In general, both retention time and peak width of all SUHs were reduced with the increase of the flow rate but resolution between TSM and PSM decreased for high flow rates. As a compromise, a flow rate of 10 μL/min was chosen for further work. The column temperature was set at 25 °C throughout this work.

3.2. Optimization of the SALLE procedure

The main factors affecting the extraction efficiency in a SALLE procedure, such as the type and volume of the organic solvent, sample volume, type and amount of salt, pH of the sample and vortex time, were evaluated by mean of recovery studies. Efficiency was evaluated by means of the recoveries of the extraction process, estimated as the peak area ratio of the analytes fortified before and after the application of the SALLE procedure. All the experiments for the optimization process were performed in triplicate ($n=3$) by spiking ultrapure water with 100 μg/L of a mixed standard solution containing nine SUHs.

3.2.1. Selection of the organic solvent

The selection of an appropriate extraction solvent is critical in a SALLE procedure. The organic solvent must be highly polar, miscible in water and induce phase separation up on addition of the appropriate salt. In this work, the following solvents were tested (polarity index are indicated in parentheses): acetonitrile (5.8), acetone (5.1), methanol (5.1), ethyl acetate (4.4) and isopropanol (3.9) [33,34]. A mixture of acetone/acetonitrile (1:1, *v/v*) was also investigated. A series of experiments were performed using 3 mL water sample containing 0.1 mol/L citrate buffer (pH 2) and 25% NaCl (*m/v*) and 2 mL of each organic solvent and with exception of methanol, in which the two phase system was not observed, the results for the rest of solvents are shown in Fig. 1. As can be seen, the highest extraction recoveries were obtained for almost all SUHs when acetonitrile was used as an extraction solvent. This might be attributed to its closer polarity to that of water [38]. Thus, acetonitrile was selected throughout the work.

3.2.2. Study of sample volume

Different sample volumes, in the range of 2–5 mL, were investigated in order to evaluate this effect in SALLE. It was found that in all cases the recoveries obtained for all target analytes were in the range of 79–97%. Although recoveries obtained were in the acceptable range, i.e., 70–120%, following the requirements set by the European Commission [39] best results were obtained for all SUHs (i.e., in the range of 83–97%), when 4 mL aqueous sample were used. As a result, 4 mL was selected as the optimum volume for the subsequent experiments.

3.2.3. Effects of the salt type and concentration

Different salts can cause different degrees of phase separation [37,40]. Therefore, in this study the effect of three different salts; NaCl, $MgSO_4$ and $(NH_4)_2SO_4$ were evaluated, using 25% (*m/v*) of each salt, as a potential salting-out agent. It was observed that all salts could induce phase separation, but better and reliable results in terms of reproducibility and recoveries were obtained when $(NH_4)_2SO_4$ was used as a salting-out reagent.

Afterwards, the effect of $(NH_4)_2SO_4$ concentration on the recoveries was evaluated by adding different amounts in the range of 0.6–2.0 g (or 15–50%, *m/v*) in the aqueous sample solution. Fig. 2 shows that the recoveries of the SUHs were improved as the amount of salt increased from 15–40%; *m/v* (0.6–1.6 g) and remained approximately constant upon addition of higher amounts of salt. Similar phenomenon was also reported for analysis of other acidic compounds [36]. Thus, 40%; *m/v* (1.60 g) of $(NH_4)_2SO_4$ was chosen as the optimum quantity for the subsequent studies.

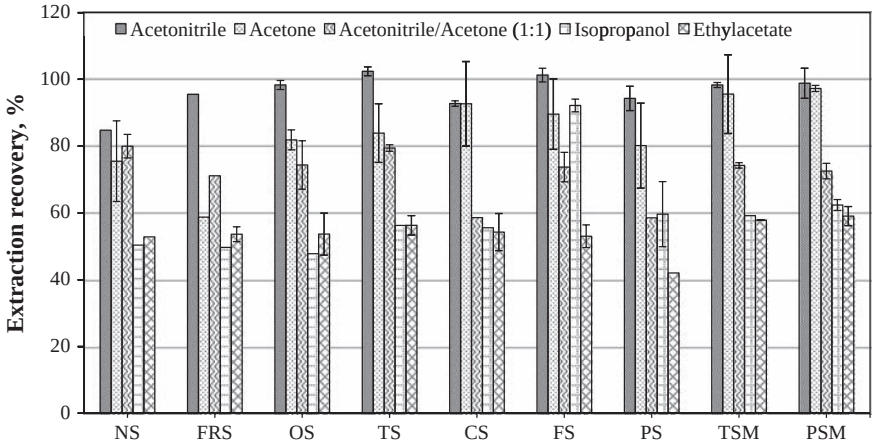

Fig. 1. Effect of extraction solvent type. Extraction conditions: 3 mL water sample containing 0.1 mol/L citrate buffer (pH 2) and 25% NaCl (*m/v*); vortex agitation time, 5 min; centrifugation speed and time, 9000 rpm and 5 min.

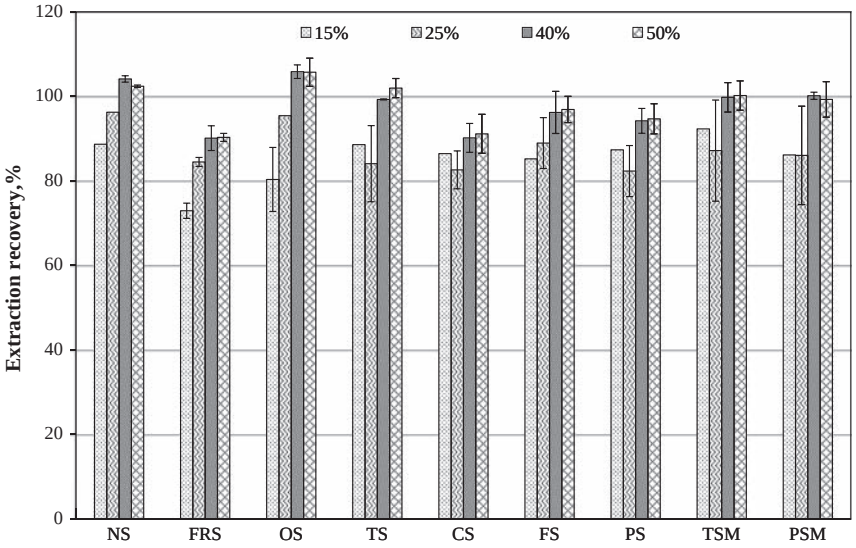

Fig. 2. Effect of the amount of $(NH_4)_2SO_4$. Extraction conditions: 4 mL water sample containing 0.1 mol/L citrate buffer (pH 2); volume of acetonitrile, 2 mL; other conditions in Fig. 2.

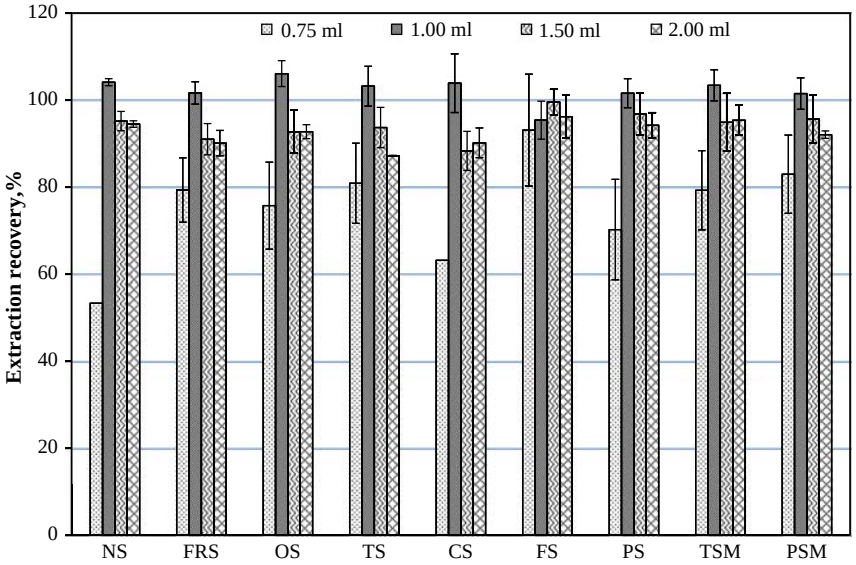

Fig. 3. Effect of the acetonitrile volume. Extraction conditions: 4 mL water sample containing 0.1 mol/L citrate buffer (pH 2) and 40% $(NH_4)_2SO_4$ (m/v); other conditions in Fig. 2.

3.2.4. *Effect of acetonitrile volume*

The volume of acetonitrile is also one of the important parameters that could influence the extraction performance of SALLE. The influence of the acetonitrile volume on the extraction recovery is shown in Fig. 3. It can be seen that the recoveries of all SUHs increased with the volume of acetonitrile from 0.75 to 1.0 mL and then decreased, except for FS, upon further increase in the volume of the acetonitrile. With low volumes, the interface between the acetonitrile and the aqueous phases was not clear and the collection of the organic layer was difficult. On the other hand, with volumes upper to 1 mL, a relatively high volume of the organic phase was separated and dried under N_2 stream, taking long time and producing some losses of analytes. Based on the experimental results, 1 mL acetonitrile was selected as the optimum volume in all the subsequent experiments.

3.2.5. *Effect of sample pH*

In SALLE, the sample pH is a parameter that plays a significant role as it affects the extent of the ionization as well as the solubility of the ionizable organic compounds. For acidic SUHs, the sample solution should be rather acidic in order to facilitate the extraction of the neutral molecular forms with the organic solvent [2]. The effect of sample pH was evaluated by varying its value from 1.5 to 3.5 using 0.1 mol/L citrate buffer whose pH was adjusted using HCl and NaOH. The obtained results showed that satisfactory recoveries were obtained for all SUHs at pH 2. As has also been reported earlier [1,28], the increase in the acidity of the sample solution (i.e., pH <2), could accelerate the acid catalyzed hydrolysis of SUHs. Furthermore, at higher pH, the SUHs might not be completely neutral and thus, complete transfer of the analytes to the acetonitrile phase could not be satisfactory. Therefore, a pH 2 was selected as the optimum value for the subsequent studies.

3.2.6. *Effect of vortex agitation time*

Agitation of the sample solution also plays a key role, influencing the kinetics of the extraction. Vortex shaking enhances the contact between the acetonitrile and the aqueous solution and thus, the formation of a two-phase system. Besides, in the present

study, vortex-shaking was also employed to enhance the dissolution of the salting-out salt (i.e., $(NH_4)_2SO_4$). Therefore, a vortex time was evaluated in the range of 1–7 min, at the maximum vortex speed and thus, reliable extraction recoveries were obtained at the vortex time of 3 min. As a result, 3 min was chosen as the optimum vortex time in further experiments.

3.3. Evaluation of the proposed method

3.3.1. Calibration curves and analytical performance characteristics

The proposed SALLE combined with the capillary HPLC-DAD method was evaluated using matrix-matched calibration curves established for each kind of matrix, water and banana juice samples. River water was chosen as representative matrix in the case of water samples. The calibration curves were constructed by spiking the water samples with a mixture of nine SUHs at five concentration levels: 5, 10, 25, 50 and 100 μg/L for NS and FS; 2.5, 5, 12.5, 25 and 50 μg/L for FRS, OS, TS, CS, PS and PSM and 1.25, 2.5, 6.25, 12.5 and 25 μg/L for TSM as well as for banana juice samples, with a mixture of eight SUHs at five concentration levels: 50, 100, 150, 300, and 500 μg/L for NS and FS; 25, 50, 75, 150 and 250 for FRS, OS, TS, PS and PSM and 12.5, 25, 37.5, 75, and 125 μg/L for TSM. CS could not be measured in this sample because of the presence of an interfering peak from the matrix. In both cases, each level was extracted in duplicate (experimental replicates) at the optimum conditions. Each extract was then injected in duplicate (instrumental replicates). Calibration curves were obtained by using the peak areas as instrumental responses versus SUH concentrations. The coefficients of determination (R^2) for all the analytes were higher than 0.990, which indicated a good linearity over the concentration range studied. The limits of detection (LOD) and quantification (LOQ) were considered as the minimum analytes concentrations yielding 3 and 10 times the signal-to-noise (S/N) ratio, respectively. The performance characteristics of the proposed SALLE method in both river water and banana juice samples are shown in Table 1. For water samples the obtained LODs were of the same order than the maximum content recommended by EU for water samples and comparable or better than those reported in other methods (see Table 4). In the case of banana juice, the obtained LODs were always below the MRLs set by EU for raw bananas and only in two cases (FRS and FS), the LOQs were higher. In this sense, the methodology proposed in this work could be useful for the monitoring of SUHs in environmental water and fruit juice samples.

3.3.2. Precision study

The precision of the method was evaluated in terms of repeatability (intra-day precision) and reproducibility (inter-day precision) for river water and banana juice samples. Each sample was spiked with a mixture of SUHs at two concentration levels. For water samples: level 1a: 6.25 μg/L for TSM, 12.5 μg/L for FRS, OS, TS, CS, PS and PSM and 25 μg/L for NS and FS; level 2a: 12.5 μg/L for TSM, 25 μg/L for FRS, OS, TS, CS, PS and PSM and 50 μg/L for NS and FS; For banana juice samples: level 1b: 25 μg/L for TSM, 50 μg/L for FRS, OS, TS, CS and PS and 100 μg/L for NS, FS and PSM; level 2b: 75 μg/L for TSM, 150 μg/L for FRS, OS, TS, CS and PS and 300 μg/L for NS, FS and PSM. Repeatability was performed by extracting each sample in duplicate (experimental replicates) and then injected in triplicate (instrumental replicates) on the same day, under the same experimental conditions. Similarly, reproducibility (intermediate precision) was evaluated by extracting each kind of matrix (i.e., water and banana juice samples) at both concentration levels, indicated above, for four consecutive days and each concentration level was injected in triplicate. The results, expressed as relative standard deviation (RSD%) of the peak areas, are shown in Table 2. As can be seen, satisfactory precisions (RSD less than 10%) were obtained in all cases [39].

3.3.3. Trueness study

The applicability of the method was evaluated by performing recovery studies in two different kinds of environmental waters and a commercial banana juice sample in order to check the trueness in these matrixes. Each kind of these samples was spiked at the two concentration levels previously used for the precision study (See Section 3.3.2). Recoveries were then calculated by comparing the average peak area for the analytes in blank samples (water or juice, free of analytes) spiked before the application of the SALLE procedure with the peak area of the corresponding sample spiked after the application of the SALLE procedure. Two samples were summited to the procedure and then the extracts were injected in triplicate. In all cases, the blank samples were analyzed, but, none of these target analytes were detected in any of these samples. However, in banana juice sample, CS was not measured due to its poor resolution with the peak appearing from the matrix. Recoveries and the corresponding RSD (%) of each target SUH in river water, groundwater and banana juice samples are shown in Table 3. The recoveries obtained with the current method were in the range of 72–115%, in both matrices. Thus, the results obtained with the proposed method could be considered in agreement with the current EU legislation [39].

Table 1
Statistics and performance characteristics of the proposed SALLE- capillary HPLC method for the determination of SUHs in water and banana juice samples.

	NS	FRS	OS	TS	CS	FS	PS	TSM	PSM
River water									
R^2	0.998	0.999	0.999	0.999	0.996	0.999	0.999	0.998	0.999
LOD (μg/L)	1.1	1.0	0.9	0.9	0.7	1.3	0.7	0.4	0.8
LOQ (μg/L)	3.6	3.3	3.0	3.0	2.3	4.3	2.3	1.3	2.7
LDR* (μg/L)	3.6–100	3.3–50	3.0–50	3.0–50	2.3–50	4.3–100	2.3–50	1.3–25	2.7–100
Banana juice									
R^2	0.998	0.999	0.999	0.999	***	0.999	0.998	0.999	0.999
LOD (μg/L)	13	5.3	5.4	7.1	***	7.7	5.5	3	4.3
LOQ (μg/L)	43	18	18	24	***	26	18	10	14
LDR* (μg/L)	43–500	18–250	18–250	24–250	***	26–500	18–250	10–125	14–500
MRL**(μg/kg)	50	10	50	50	***	10	20	20	NA

***Not measured in banana juice sample due to the presence of an interfering peak; NA, non-available in the EU pesticide database.

* LDR, linear dynamic range.

** MRL, maximum residue limit.

Table 2
Repeatability (intra-day) and reproducibility (inter-day) of the proposed method (% RSD) in spiked river water and banana juice samples.

	NS	FRS	OS	TS	CS	FS	PS	TSM	PSM
River water									
Intra-day(% RSD, $n=6$)									
Level 1a	4.3	4.3	3.6	3.1	7.5	3.8	7.5	8.9	3.8
Level 2a	0.6	0.6	1.9	1.9	3.7	3.7	3.5	1.5	1.5
Inter-day(% RSD, $n=12$)									
Level 1a	8.2	7.6	3.6	8.0	8.3	6.7	9.9	4.6	7.6
Level 2a	8.0	0.6	8.7	5.5	7.2	8.7	8.3	4.2	8.1
Banana juice									
Intra-day (% RSD, $n=6$)									
Level 1b	5.3	8.1	8.1	8.5	***	3.4	1.2	7.1	7.0
Level 2b	3.7	4.6	3.2	6.9	***	4.6	8.0	7.9	8.9
Inter-day (% RSD, $n=12$)									
Level 1b	8.2	7.6	8.0	8.3	***	6.7	9.9	4.6	7.6
Level 2b	8.1	8.7	5.5	7.2	***	8.7	8.3	4.2	8.1

*** Not measured in banana juice sample due to the presence of an interfering peak.
Level 1a: 6.25 μg/L for TSM, 12.5 μg/L for FRS, OS, TS, CS, PS and PSM and 25 μg/L for NS and FS.
Level 2a: 12.5 μg/L for TSM, 25 μg/L for FRS, OS, TS, CS, PS and PSM and 50 μg/L for NS and FS.
Level 1b: 25 μg/L for TSM, 50 μg/L for FRS, OS, TS and PS and 100 μg/L for NS, FS and PSM.
Level 2b: 75 μg/L for TSM, 150 μg/L for FRS, OS, TS and PS and 300 μg/L for NS, FS and PSM.

Table 3
Average recoveries (%, $n=6$) for each SUH in river water, groundwater and commercial banana juice.

Analyte	River water (% R (RSD))		Groundwater (% R (RSD))		Banana juice (% R (RSD))	
	Level 1a	Level 2a	Level 1a	Level 2a	Level 1b	Level 2b
NS	105 (4.3)	94 (0.6)	88 (7.3)	108 (8.4)	79 (5.3)	90 (3.7)
FRS	102 (4.3)	91 (0.6)	88 (6.3)	95 (6.1)	108 (8.1)	89 (4.6)
OS	103 (3.6)	94 (1.9)	97 (2.4)	104 (2.8)	82 (8.1)	87 (3.29
TS	105 (3.1)	107 (1.9)	96 (8.2)	94 (4.0)	72 (2.7)	90 (6.9)
CS	93 (7.5)	96 (3.7)	98 (2.4)	92 (2.1)	****	****
FS	109 (3.8)	97 (3.7)	77 (2.6)	107 (6.4)	115 (3.7)	89 (4.6)
PS	107 (7.5)	100 (3.5)	86 (9.2)	85 (6.9)	99 (1.2)	88 (8.0)
TSM	98 (8.9)	109 (1.5)	75 (5.9)	88 (1.6)	78 (7.1)	87 (7.9)
PSM	94 (3.8)	99 (1.5)	79 (7.7)	105 (3.9)	79 (7.0)	92 (8.9)

***Not measured in banana juice sample due to the presence of an interfering peak
Level 1a: 6.25 μg/L for TSM, 12.5 μg/L for FRS, OS, TS, CS, PS and PSM and 25 μg/L for NS and FS.
Level 2a: 12.5 μg/L for TSM, 25 μg/L for FRS, OS, TS, CS, PS and PSM and 50 μg/L for NS and FS.
Level 1b: 25 μg/L for TSM, 50 μg/L for FRS, OS, TS and PS and 100 μg/L for NS, FS and PSM.
Level 2b: 75 μg/L for TSM, 150 μg/L for FRS, OS, TS and PS and 300 μg/L for NS, FS and PSM.

Chromatograms of environmental water and banana juice samples spiked with SUHs and analyzed by using the proposed SALLE-capillary HPLC methodology are shown in Fig. 4.

3.3.4. *Comparison with other methods*

The proposed SALLE procedure in combination with capillary HPLC has been compared with other recently reported methods including hollow-fiber liquid-phase microextraction (HF-LPME) [28], cloud point extraction (CPE) [17] and SPE with various sorbent types such as ionic liquids supported on magnetic nanoparticles (IL-MNPs) [27]; silica supported gold nanoparticles (Au-TEOS or Au-NPs) [26]; silica supported gold nanoparticles functionalized ionic liquids (Au-NP-IL-Silica) [26] and C_{18} [22,24,26], as well as dispersive liquid–liquid microextraction (DLLME) [29]. As can be seen in Table 4, the proposed method needs a shorter extraction time, smaller sample volumes and lower consumption of organic solvents. It also provides similar or lower LODs and wider linear ranges than other reported methods using also liquid chromatography or capillary electrophoresis.

4. Conclusions

In this study, a simple analytical method has been proposed for sample preparation and quantitative determination of nine SUHs in water and banana juice samples, using SALLE in combination with capillary HPLC-DAD. Various parameters affecting the extraction efficiency of the method including type and volume of the organic solvent, sample volume, types and amount of salt, pH of the sample and vortex time were investigated and optimized. Compared with other recently reported methods, the proposed technique offers advantages such as simplicity, shorter analysis time and lower consumption of organic solvents and sample sizes.

The applicability of the method was evaluated in environmental water samples (river and groundwater) and a commercial banana juice sample and, in all cases acceptable figures of merit

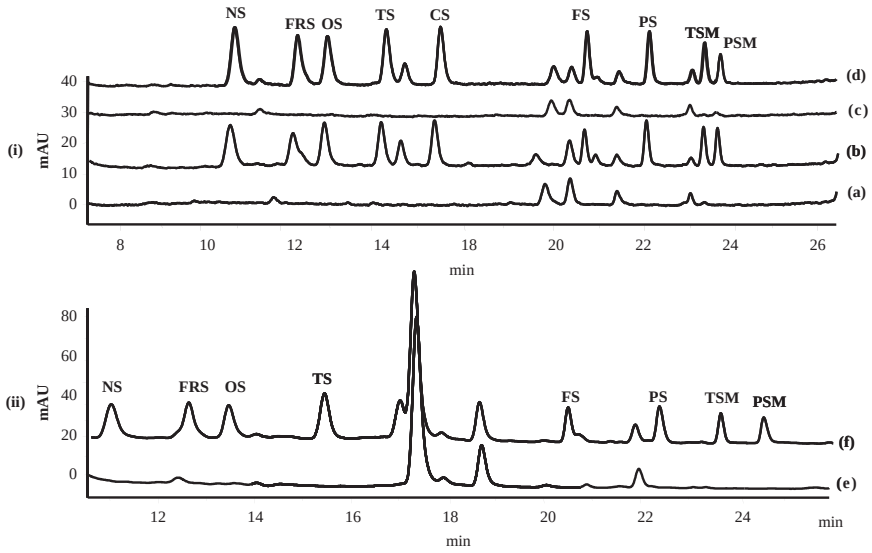

Fig. 4. (i) Chromatograms (a) and (c) show blanks of groundwater and river water samples, respectively. Chromatograms (b) and (d) correspond to groundwater and river water samples, respectively, spiked with 12.5 μg/L for TSM; 25 μg/L for FRS, OS, TS, CS, PS and PSM and 50 μg/L for NS and FS. (ii) Chromatograms (e) and (f) correspond to a blank and a sample of banana juice spiked with 75 μg/L for TSM; 150 μg/L for FRS, OS, TS and PS and 300 μg/L for NS, FS and PSM, respectively.

Table 4
Comparison of the proposed method with others reported methods for extraction and determination of SUHs in water and food samples.

Method		Sample volume (mL)	Linear range (μg/L)	LOD (μg/L)	% RSD	Extraction time (min)	Ref.
IL-MNPs based SPE-cHPLC-DAD		Water (50)	5–100	1.1–2.9	2.3–4.9	–	[27]
HF-LPME-cHPLC-DAD		Water (12)	0.3–40	0.1–1.7	0.9–8.4	60	[28]
Au-TEOS based SPE				2.0–9.0	2.1–4.5	–	
Au-NP-IL-silica based SPE	–cHPLC-DAD	Water (10)	50–1000	2.0–6.0	2.8–4.0	–	[26]
C_{18} based SPE				3.0–6.0	2.6–4.1	–	
SPE-HPLC-UV		Water (1000)	15–150	9.4–14.5	–	> 60	[22]
SPE-HPLC-MS^n			15–150	5.0–8.1			
CPE-HPLC-DAD		Water (18)	4–2000	0.8–1.2	0.4–5.9	17	[17]
SPE-CE-UV		Grape (250)	0.97–200*	0.97–8.3*	5.2–21.4	> 60	[24]
DLLME-cHPLC-DAD		Grape juice (5)	8.0–200	2.0–9.0	1.0–9.8	< 10	[29]
SALLE-cHPLC-DAD		Water (4)	1.3–100	0.4–1.3	0.6–9.9	< 10	This work
		Banana juice (2.5)	10–500	3–13	1.2–9.9		

* μg/kg.

were obtained, except for CS in banana juice sample, which was not measured because of its poor resolution with the peak appearing from the matrix. Based on these results, the developed method could be used as a valuable alternative for the determination of SUHs in environmental water and juice samples.

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