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DEVELOPMENT OF COST-EFFECTIVE DISPERSIVE LIQUID-LIQUID MICRO-EXTRACTION TECHNIQUE FOR PRECONCENTRATION OF MULTI-RESIDUE HERBICIDES IN ENVIRONMENTAL WATERS PRIOR TO CHROMATOGRAPHIC ANALYSIS

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ABSTRACT. High-density dispersive liquid-liquid microextraction method coupled with high-performance liquid chromatography with a diode array detector (HD-DLLME-HPLC-DAD) was developed for extraction and determination of six commonly used sulfonylurea herbicides in matrices of environmental waters. For simultaneous extraction of the target herbicides, the optimum experimental parameters that 024influence extraction efficiency were investigated. Under optimized conditions, the limit of detection (LOD) and quantification (LOQ) were 0.8–1.5 and 1.9–5.1 ng mL^{-1}, respectively. The precisions in terms of relative standard deviations (% RSDs) of both intra-day and inter-day precisions (n = 6) were found to be 2.92 to 7.08 and 3.01 to 8.13, respectively. Furthermore, applicability of the developed method was investigated by analyzing spiked tap, lake, river and underground water samples and satisfactory recoveries were obtained in the range of 84.3–101.7% with RSDs < 9.8% (n = 6) and the target analytes were not detected in real samples. The proposed method offered several attractive features including fast analysis time, simplicity, sensitivity, and selectivity. Therefore, the trace level enrichment and assessment of sulfonylurea herbicides residues from environmental water matrices using HD-DLLME-HPLC-DAD could be utilized as a reliable alternative in routine laboratory analysis of contaminated environmental waters.

KEY WORDS: Environmental water, High-density dispersive liquid-liquid microextraction, HPLC-DAD, Miniaturization analytical technique, Sulfonylurea herbicides, Trace level enrichment

INTRODUCTION

Sulfonylurea herbicides (SUHs), a large family of herbicides extensively used in agriculture, have gained quite significant attention all around the world due to their broad-spectrum and high herbicidal activity at low dosage application rates (10-40 g ha^{-1}), good crop selectivity, and low mammalian toxicity as a result of their low application rates [1, 2]. Depending on the pH, SUHs, which are susceptible to contraction of the sulfonylurea linkage degrade in water 10 to1000 times faster than the others [3]. Although the application rates are low, due to relatively high solubility in water and moderate to high mobility, these herbicides may result in leaching into deeper soil and potentially entering surface waters [4, 5]. Despite the fact that SUHs rapidly decompose in water and soil due to their thermal and chemical instabilities, they still exist in some matrices at a trace level [6] and are being detected in surface and ground waters [7, 8]. Therefore, their presence in environmental matrices has emerged to pose serious environmental and human health problems [9, 10].

Several analytical techniques developed for determination of SUH residues including gas chromatography coupled with mass spectrometry (GC-MS) [11], high performance liquid chromatography equipped with multi wavelength or ultraviolet detector (HPLC-DAD/UV) [1, 12–15], HPLC coupled to tandem mass spectrometry (HPLC-MS/MS)[1, 3, 7, 16], and HPLC coupled to mass spectrometry (HPLC-MS) [17], etc have been applied. Since most SUHs are unstable at high temperature, a time-consuming procedure of sample derivatization or

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hydroxylation was required prior to analysis with gas chromatography, which limited the application of these detection methods in the determination of SUHs. HPLC-UV/DAD and HPLC-MS can be used directly for analysis without derivatization, despite the volatility or heat stability of these analytes. HPLC-UV/DAD is a fast, simple, easy to use and widely available technique, which is usually chosen in the determination of SUHs.

Since the occurrence of SUHs in the environmental samples is at a trace level, sample pretreatment is needed to extract and enrich them before analysis. Liquid-liquid extraction (LLE) [18, 19] and solid phase extraction (SPE) [7, 8, 15] were the most commonly used techniques in sample pretreatments. LLE can offer high reproducibility and high sample capacity, but requires long processing time utilizing large volume of sample and also use large quantities of toxic organic solvents. SPE can overcome some drawbacks of LLE such as high consumption of both sample and organic solvent, but it also requires longer experimental time because of column conditioning, washing, loading and elution. On the other hand, sorbents ready to use in SPE are relatively expensive, besides the problem associated with the sample carry over effect which causes increased cost of sample handling [20, 21].

Very recently researches [22, 23] have started widely focusing on and committed to the development of efficient, inexpensive, automated, and miniaturized extraction techniques that might substantially reduce the consumption of toxic organic solvents. To this end, single drop microextraction (SDME) [24], hollow-fiber liquid phase microextraction (HF-LPME) [12, 25] and solid phase microextraction (SPME) [26, 27] techniques significantly minimized and worked towards avoiding the use of organic solvents in sample preparation procedures. However, SPME is expensive, fibers are fragile, has a short lifetime and takes long time to condition the sorbent [27]. Despite its ease of use and effectiveness, SDME is merely used for laboratory research due to its drop instability and the main drawbacks of HF-LPME are poor reproducibility and lengthy equilibration times [21].

To overcome these limitations, Assadi and coworkers developed dispersive liquid-liquid microextraction (DLLME) technique [28]. It is a modified version of solvent extraction that has the ability to enhance enrichment intensely, contains a very small amount of toxic solvent used and is the technique in which acceptor to donor phase ratio is greatly reduced compared to other methods used for similar purposes [23, 29, 30]. The extraction and dispersive solvents are promptly injected into the aqueous sample to create a cloudy solution, which is the essential component of the trace enrichment principle in DLLME. Due to the high surface interactions between the droplets of the extraction solvent and aqueous sample solution, extraction equilibrium is quickly attained [22, 31]. Furthermore, as a microextraction technique, DLLME provides features such as ease of use, fast, cost-effectiveness, high recovery, the use of inexpensive equipments, and widely accessible laboratory supplies [32]. The technique has commonly been used to extract trace levels of pesticides ever since it was first introduced, mostly from water samples [29, 33–35], foods [36–38], and especially from juices and vegetables [39, 40]. However, there are very few studies on the application of the technique for the determination of SUHs in waters and none on the use of high-density solvent based dispersive liquid-liquid microextraction (HD-DLLME), for simultaneous extraction of these six target SUH residues (metsulfuron-methyl, chlorosulfuron, niclosulfuron, prosulfuron, flazasulfuron and chlorimuron-ethyl) from environmental water samples of different sources. Hence, the objective of this study was to develop, optimize and assess the accuracy and investigate the applicability of the method by employing the simplicity and high efficiency of HD-DLLME for trace extraction and enrichment of selected six sulfonylurea pesticide residues in the matrices of environmental waters (tap, underground, lake, and river) prior to chromatographic analysis.

EXPERIMENTAL

Chemicals and reagents

Analytical standards of metsulfuron-methyl (MSM), chlorosulfuron (CS), niclosulfuron (NS), and prosulfuron (PS) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Flazasulfuron (FS) and chlorimuron-ethyl (CME) were purchased from Chem Service Inc. (West Chester, USA). All the standards were of the highest purity, i.e., > 97 %. The solvents utilized including acetonitrile (ACN) and acetone, acquired from Sigma Aldrich (Steinheim, Germany), methanol (MeOH) received from Carlo Erba (Rodano, Italy) and isopropanol (IPA) and chloroform were the product of Sigma Aldrich (Seelze, Germany) were of HPLC grade reagents. Dichloroethane was the product of Avocado Research chemicals Ltd. (Cheshire, UK). Common chemicals such as NaCl, obtained from Sigma-Aldrich (Steinheim, Germany) and (NaOH) was the product of Merck Chemicals (Darmstadt Germany). Hydrochloric acid (HCl) was purchased from Sigma Aldrich (St. Louis, MO, USA). Ultrapure water was obtained by purifying with, a 8000 Aquatron water Still (Bibby Scientific, Staffordshire, UK) and deionizer (EASY Pure LF, Dubuque).

Instruments and equipment

Chromatographic analyses were carried out using the Agilent 1200 series HPLC system (Agilent Technologies, Waldbronn, Germany) outfitted with a quaternary pump, vacuum degasser, standard and preparative autosampler, thermostat column compartment, autosampler thermostat, and a diode array multiple wavelength detector. LC Chemstation software (B.02, 01-SR1) was used for sample processing, and data acquisition. Chromatographic separation was performed using a ZORBAX ODS-C_{18} (150 x 3 mm, i.d., 3.5 µm particle size) analytical column from Agilent Technologies. The sample solution pH was measured using Adwa pH meter (model 1020, Adwa Hungary Kft, Hungary). For sample preparation, XW-80A vortex (Jing Industrial Co., Ltd. Shanghai, China), a centrifuge (Model 80-2, Jiangsu Zhenji instruments Co., Ltd. Jiangsu, China), a 15 mL centrifuge tube, Corning integrated (Corning, NY, Mexico), and ultrasonic heater, Dacon®, were utilized.

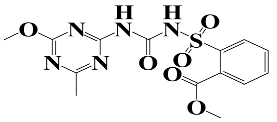
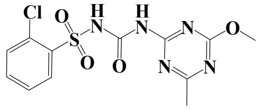
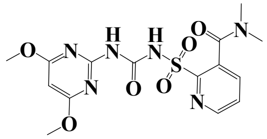
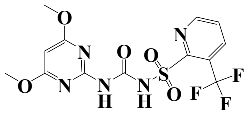
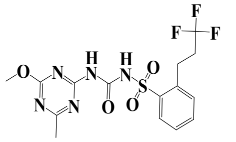
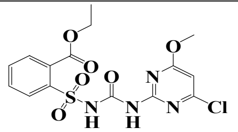
Chromatographic conditions

Chromatographic separations were achieved using isocratic delivery mode with binary mobile phase, both containing 0.01% acetic acid, i.e., solvent A (ultrapure water) and solvent B (acetonitrile) in a 1:1 volume ratio. Before the sample injection, the HPLC column was conditioned with the mobile phase for 15 min. Analysis was performed with the flow rate of 1 mL min^{-1}, column temperature at 30°C, injection volume of 15 µL and UV detection was performed at 230 nm. Peak area was used as instrumental response and for comparison of the responses. Under these chromatographic conditions, baseline separation was maintained for all the target analytes in 8 min run time.

Standard solution preparation

The stock standard solution of each target analyte, with a concentration of 500 µg mL^{-1}, was prepared by dissolving 12.5 mg with acetonitrile (ACN) in a 25 mL volumetric flask. Intermediate standard solutions of 20 µg mL^{-1} were obtained by diluting the stock solution with ACN. Other working solutions of lower concentrations were prepared from the intermediate solution in the same solvent. All standard solutions were stored in the refrigerator at 4 °C, when not in use. The chemical structures, common names, abbreviations and the octanol water partition coefficient (log K_{ow}) at pH 7 and 20 °C, and other relevant physicochemical properties of the target pesticides are shown in Table 1.

Table 1. Physicochemical properties of target analytes of the sulfonylurea herbicides [5].

Name	Structure	Solubility, mg/L (25 °C, pH 7)	pK$_a$	log K$_{ow}$ (pH 7)
Metsulfuron-methyl		2790	3.3	1.74
Chlorosulfuron		31800	3.6	-0.99
Niclosulfuron		12000	4.3	-1.8
Flazasulfuron		2100	4.37	-0.06
Prosulfuron		4000	3.76	-0.21
Chlorimuron-ethyl		1200	4.2	0.36

Environmental water samples collection

Various types of environmental water samples were collected from Bishoftu town which is found in the Oromia Regional State, Ethiopia, with geographical location of 8°44'40''N latitude and 38°59'9''E longitude at an altitude of 1925 m, a.s.l. Samples of Hora lake water, tap water, and underground water were collected from this area. River water was also collected from the area located 10 km away from Bishoftu town, Mojo River, where more than 15 floriculture industries established around and effluents of floriculture industries are directly discharged to the river. Before analysis, each water sample was filtered through a 0.45 µm micropore membrane filter and then stored in polyvinyl chloride bottles, in a refrigerator, for a maximum of 24 h at 4 °C without any further sample pretreatment.

High-density dispersive liquid-liquid extraction procedure

A 5 mL portion of each type of waster sample, which was filtered and kept in the refrigerator, was adjusted to pH 2, were measured and transferred into a 15 mL falcon tube with a conical bottom. Afterwards, it was fortified with appropriate amount of mixture of each target analytes and left to stand for about 15 min for equilibration. Then, before using the sample for DLLME, the aqueous solution of 15% (*w/v*) NH_4AC was added and vortex agitated for 1.5 min to dissolve in the water sample. Subsequently, the organic phase consisting of a mixture of 800 µL MeOH and 175 µL dichloroethane was injected into the sample solution with a 2-mL syringe. The mixture was vortexed for 0.5 min at high speed followed by centrifugation of the content at 4,000 rpm for 5 min to enhance sedimentation of the fine organic droplets. The organic phase of the sediment was carefully removed using a 1-mL syringe, transferred to a 1.5-mL glass vial, and allowed to air dry at room temperature. The residue was ultimately dissolved with 300 µL of solvent containing 0.01% acetic acid and 0.01% acetonitrile (1:1, *v/v*) mixture, followed by vortexing for 1 min. The resulting solution was subsequently filtered with a 0.22-µm nylon filter, transferred to a 1.5-mL vial and injected into the HPLC for the determination of the target analytes.

RESULTS AND DISCUSSION

Optimization of parameters

The most important variables that affect extraction efficiency, such as sample pH, ionic strength, and vortex (extraction time), as well as the type and volume of extraction and dispersive solvents, were investigated to determine the optimal conditions using the univariate approach. By adding 200 ng mL^{-1} working standard solutions to 5 mL of ultrapure water, each experiment was carried out at least in triplicate. The average peak areas of the replicate analyses were taken into account to assess the impact of experimental circumstances on the extraction efficiency in all the given procedures. Preliminary experimental results and review of related literatures were used to determine quantitative value ranges of parameters optimization.

Effect of extraction solvent type

To achieve a successful extraction in the DLLME process, the choice of the appropriate extraction solvent is of utmost significance. When injected into the aqueous solution, the chosen solvent that has a density greater than water, forms a cloudy solution in the presence of a dispersive solvent, and demonstrate the extraction of target analytes [37]. In the present study, four organic solvents; namely, chloroform, dichloromethane, dichloroethane and chlorobenzene were tested by injecting a mixture of equal volume of each of these alternative extraction solvents and the same amount of methanol as dispersive solvent. It was observed that for all organic extraction solvents a phase separation was achieved. However, the highest peak area for most target analytes was obtained with dichloroethane, Figure 1. Therefore, dichloroethane was chosen as the extraction solvent for further studies.

Effect of extraction solvent volume

The effect of extraction solvent volume on the extraction efficiency was evaluated by varying the volume in the range of 50–200 µL, while other experimental parameters were kept constant. It was observed that the extraction efficiency of the DLLME procedure was meaningfully affected by the volume of extracting solvent. The peak areas of all the target analytes increased with the volume of dichloroethane up to 175 µL, then decreased for higher value, i.e., 200 µL which may be due to the dilution effect of the sediment phase of extraction solvent [41]. Thus, 175 µL was selected as optimum volume of the extraction solvent.

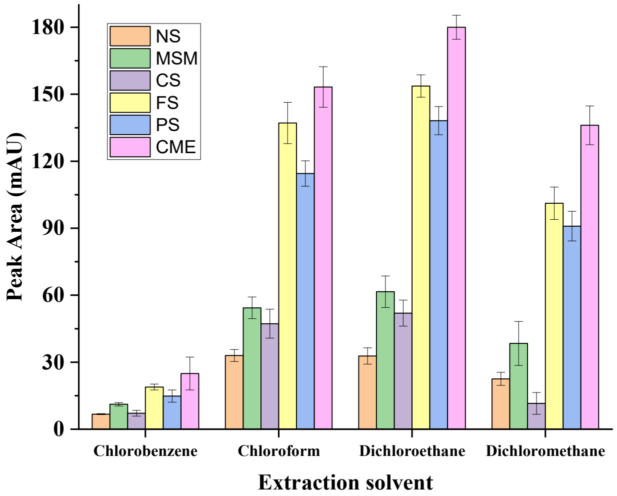


Figure 1. Effect of extraction solvent type. Extraction conditions: sample size, 5 mL; dispersive solvent, methanol; dispersive solvent volume, 800 µL ; salt type and amount added, NH_4AC (15% m/v); pH of solution, 2.0; vortex agitation time, 0.5 min; volume of extraction solvent, 800 µL; centrifugation speed 4000 rpm for 5 min; n = 3.

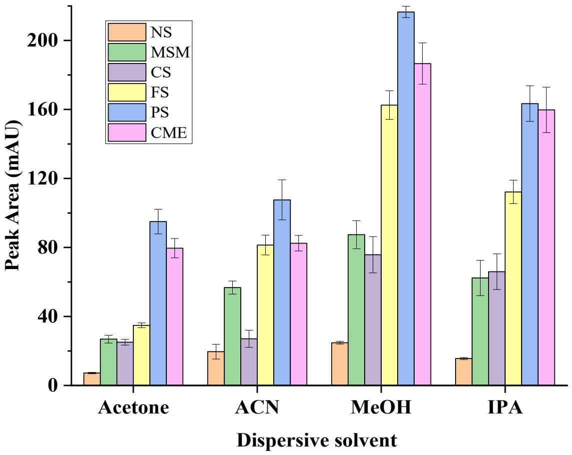


Figure 2. Effect of dispersive solvent type. Extraction conditions: Sample size, 5 mL; extraction solvent, dichloroethane; extraction solvent volume, 175 µL; dispersive solvent volume, 800 µL; salt type and amount added, NH_4AC (15% m/v); pH of solution, 2.0; vortex agitation time, 0.5 min; centrifugation speed, 4000 rpm for 5 min; n = 3.

Selection of dispersive solvent type

The type of dispersive solvent was studied to get the optimum extraction efficiency. Dispersive solvents often need to be miscible with the extraction solvent and the sample solution [35, 37]. In this study, four solvents; namely, methanol (MeOH), isopropanol (IPA), acetone and acetonitrile (ACN) were evaluated. The highest peak areas for all the SUHs were obtained with MeOH followed by IPA (Figure 2). This phenomenon could be due to the high polarity of these solvents caused by their hydroxyl group and the hydrogen bond formed between these solvents with dichloroethane and water which as a result increases their miscibility in the extraction solvent and the sample solution [42]. The same solvent was also reported as dispersive solvent for the extraction of SUHs from wine, water and soil samples [14, 43]. Therefore, MeOH was chosen as dispersive solvent in this study.

Effect of dispersive solvent volume

Dispersive solvent volume is another crucial factor that affects the solubility of the extraction solvent in the aqueous sample and thus, can affect the extraction efficiency of the target analytes [9]. The volume of methanol was varied in the range of 600–1200 µL with 200 µL interval. The extraction efficiency was found to increase with the volume of methanol up to 800 µL and then decreased at higher volumes. The most probable reason could be associated to the solvent volume, i.e., at low dispersive solvent volume, the organic extractant sediment phase might not be formed properly, giving a low peak area. On the other hand, the use of a higher volume of methanol could enhance the solubility of the analytes into the aqueous phase due to the increase of partitioning of the dispersive solvent in the aqueous sample, leading to lower extraction efficiencies [31]. Based on the experimental results, 800 µL methanol was chosen as optimum.

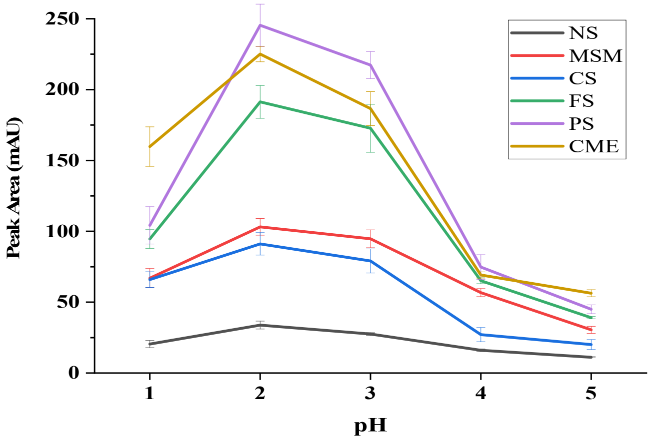


Figure 3. Effect of pH value. Extraction conditions: Sample size, 5 mL; extraction solvent, dichloroethane; extraction solvent volume, 175 µL; dispersive solvent, methanol; dispersive solvent volume, 800 µL; salt type and amount added, NH_4AC (15% m/v); vortex agitation time, 0.5 min; centrifugation speed, 4000 rpm for 5 min; n = 3.

Sample solution pH

The SUHs used during this investigation are slightly acidic compounds, with pK_a values ranging from 3.6 to 5.2 [36]. Therefore, to convert them into their neutral forms and enhance the affinity of the analytes for the extraction solvent, the pH of the aqueous solution must be lower than their pK_a values [1]. Hence, the impact of sample pH was considered and optimized over the range 1–5, keeping the other experimental parameters constant. In a more acidic solution, however, lower peak areas were observed probably because a very acidic pH could accelerate the hydrolysis of the pesticide compounds [2]. The results indicated that the peak area increases when pH raises from 1 to 2 and then decreases by increasing pH from 2 to 5 (Figure 3). At higher pH, the target analytes might not be completely transformed to their neutral forms and thus complete transfer of the analytes from the sample solution to the organic phase may not be achieved. As a consequence, in the subsequent experiments the pH of sample solutions was set at 2.0. Similar findings were also reported by other workers [14, 44].

Effects of the salt type

Solubility of both the analytes as well as the extraction solvent in the aqueous phase could be decreased by salt addition and this in turn enhances the analytes transfer into the organic phase [34]. As different salts could cause to different degrees of phase separation [45], the effect has been evaluated by addition of different salts such as NaCl, $(Na)_2SO_4$, $MgSO_4$, and ammonium acetate (NH_4AC), using 15% (m/v) of each salt, as a potential salting-out agent. It was observed that except for $(Na)_2SO_4$ all other salts induce very clear phase separation, the highest response for all of the analytes was obtained when NH_4AC was used as the salting-out agent, Figure 4.

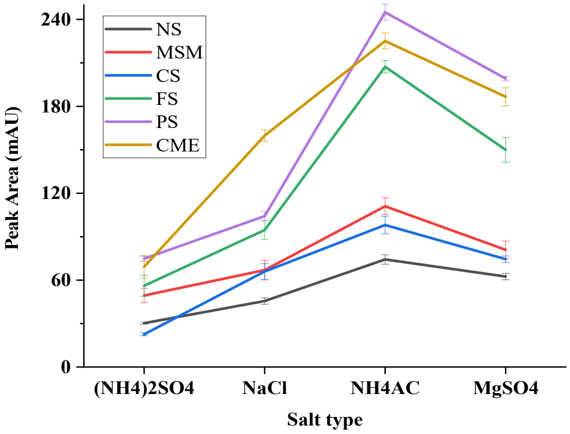


Figure 4. Effect of salt type. Extraction conditions: sample size, 5 mL; extraction solvent, dichloroethane; extraction solvent volume, 175 µL; dispersive solvent, methanol; dispersive solvent volume, 800 µL; pH of solution, 2.0; amount of salt added, 15% (m/v); vortex agitation time, 0.5 min; centrifugation speed, 4000 rpm for 5 min; n = 3.

Effect of salt concentration

Appropriate quantity of inorganic salts can improve extraction efficiency by increasing the polarity of the water phase and reducing the solubility of the target analytes in the aqueous phase [34]. Varying salt concentrations may cause variation in the degrees of phase separation [32]. A salting-out study was carried out by adding different amounts of NH_4AC salt in the range of 0.5–1.5 g (or 10–25%, *m/v*), in the aqueous sample solution. The peak areas of the target analytes were increased as the concentration of the salt increased, from 0.5–0.75 g. However, at higher concentrations, the peaks were observed to decrease slightly for all the target analytes and, thus 15% *m/v* (0.75 g) was chosen to be the optimum for the subsequent experiments. Excessive inorganic salts will increase the viscosity of the solution, resulting in a lower diffusion coefficient and further reducing the extraction efficiency of the target analytes [10].

The impact of vortex time on the extraction efficiency of SUHs

To speed up the formation of turbid dichloroethane solution, vortex agitation is primarily used. This study investigated the extraction efficiency as a function of vortex time, which was varied as follows; i.e., 0.25, 0.5, 1.0, 1.5, and 2.0 min while maintaining a constant vortex rotation speed. The highest extraction efficiency was attained for most analytes after 0.5 min of vortex duration. The high contact surface between the extractant and the aqueous sample may have contributed for attainment of rapid equilibrium [31]. However, as the vortex time was extended, there was little to no noticeable difference in the results of these experiments, Figure 5. Thus, a vortex time of 0.5 min was found to be the optimum extraction time.

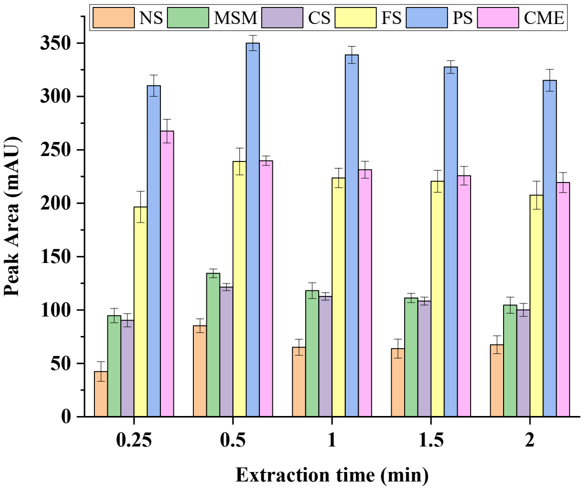


Figure 5. Effect of extraction time. Extraction conditions: sample size, 5 mL; extraction solvent, dichloroethane; extraction solvent volume, 175 μL; dispersive solvent, methanol; dispersive solvent volume, 800 μL; pH of solution, 2.0; salt type and amount, NH_4AC (15% m/v); centrifugation speed 4000 rpm for 5 min; n = 3.

Analytical performance of the proposed method

Before applying the whole procedure to real samples, all the crucial analytical parameters were determined for each pesticide studied such as the linear range (LR), the correlation coefficient (r^2), the limits of detection (LODs), the limits of quantification (LOQs), the inter- and intra-day precisions. The entire procedure was tested for real sample solutions, i.e. lake, river, underground and tap waters. But, precision study of the river water was taken as the model representative of all real water samples after spiking with two concentration levels (Level 1 and Level 2; as shown in Table 2) of the working standard solution. The solutions were separately processed according to the DLLME procedure and HPLC-DAD analysis stated in the experimental section.

Calibration curves and precision study

Under the optimal conditions, analytical figures of merit were used to validate the proposed analytical methodology. The method linearity was validated via linear calibration obtained by fortification of each real sample with standard solutions, and the corresponding curves were generated by plotting analyte extract peak areas against the standard concentration between 2.5 and 1000 ng mL^{-1}. All of the target analytes taken into account in this study have shown good linearity, with correlation coefficients > 0.997 (Table 2).

The repeatability (intra-day precision) and reproducibility (inter-day precision) of the proposed method precision were also assessed. River water was spiked with a mixture of the six SUHs at two concentration levels (µg L^{-1}): Level 1: 65 for CS and MSM; 95 for NS; 125 for FS, PS, and CME; and Level 2: 130 for CS and MSM; 190 for NS; 250 for FS, PS, and CME in order to evaluate the repeatability of the method. Under the optimal experimental conditions, the sample was extracted in triplicate and injected in duplicate the same day. Using the same concentration levels for three consecutive days, the same water sample that was used to evaluate repeatability was used to validate the method reproducibility. As seen in Table 2, the method precisions for intra-day and inter-day precision (n = 6) ranged from 2.92 to 7.08 and 3.01 to 8.13, respectively, in term of relative standard deviations (%RSDs). The experimental findings of precision that were attained for both repeatability and reproducibility (RSDs < 9.8%), demonstrated a low variability of the extraction method.

Sensitivity

The sensitivity of the method guaranteed the detection and confirmation of SUHs residues in water found at levels below or above the limits of detection (LODs). The limits of detection (LOD) and limits of quantification (LOQ) were determined as the lowest concentration yielding a signal-to-noise (S/N) ratio of 3 and 10, respectively. The results are given in Table 2, showing that the LODs ranged from 0.8 to 1.5 ng mL^{-1} while LOQs from 1.9 to 5.1 ng mL^{-1}, respectively. It was also found that the maximum residue limits (MRLs) of most analytes under study were fairly above the LOD/LOQ determined in this study; for instances the MRL for niclosulfuron is 50 mg kg^{-1}, for prosulfuron 20 mg kg^{-1}, etc [44]. Thus, the developed method can satisfactorily be utilized to analyze residues of these compounds in complex matrices with certainty.

Table 2. Analytical figures of merit for the DLLME technique combined with HPLC-DAD for sulfonylurea herbicide residues under study.

Analyte	LR[a]	LOD[a]	LOQ[a]	r^2	Level 1		Level 2	
					RSD[b]	RSD[c]	RSD[b]	RSD[c]
NS	4.5-750	1.1	3.2	0.9986	8.69	4.12	7.86	4.34
MSM	3-500	0.8	2.5	0.9979	9.78	5.31	7.17	7.90
CS	3-500	0.9	2.8	0.9994	5.22	4.7	2.92	5.15
FS	6-1000	1.5	5.1	0.9992	7.35	8.36	6.12	8.13
PS	2.5-500	0.8	1.9	0.9986	9.25	3.01	6.91	3.72
CME	6-750	1.2	3.8	0.9998	9.21	4.15	7.83	4.80

Level 1: 65 µg/L for CS and MSM; 95 µg/L for NS; 125 µg/L for FS, PS, and CME. Level 2: 130 µg/L for CS and MSM; 190 µg/L for NS; 250 µg/L for FS, PS, and CME. LR[a], LOD[a] and LOQ[a] (ng/mL); RSD[b]: intra-day precision (*n* = 6); RSD[c]: inter-day precision (*n* = 9).

Applications of the DLLME method to the environmental water samples

Four environmental water samples, including underground, river, lake, and tape water, were used to validate the accuracy of the proposed method. The unspiked real sample was analyzed to check the presence of the target pesticides and none of the tested water samples exhibited signals corresponding to values above the LODs. The results that were obtained may indicate that the tested samples were either free of pesticide residues or contained levels that were below the detectable limits. The accuracy of the presented method was assessed using the average relative recovery (%RR) of each sample spiked at two concentration levels and extracted in triplicate (Table 3). To evaluate the accuracy of the methods, relative recovery was determined using peak area ratio of each analyte after extraction with the proposed method to the peak area of the standard solution for similar two concentration levels (Table 3). For the analyzed water samples,

%RR at two concentration levels were varied from 84.3 to 101.7, with %RSD < 9.8. The results obtained for recovery were indicated that the matrices of water samples have no significant effect on the performance of the proposed method.

Table 3. Relative recovery (RR) values of the proposed method in environmental water samples.

Water sample	Spiked level	Analytes					
		NS	MSM	CS	FS	PS	CME
		%RR (%RSD, n = 3)					
Underground water	Level 1	87.9(7.4)	89.2(9.3)	86.6(9.2)	89.6(8.9)	86.4(5.2)	88.9(9.8)
	Level 2	92.5(8.4)	87.2(3.1)	95.0(4.2)	97.1(4.3)	91.6(4.7)	91.1(5.3)
River water	Level 1	89.0(6.5)	86.3(4.9)	89.9(6.6)	90.2(5.0)	89.1(4.7)	85.9(8.8)
	Level 2	84.3(5.4)	88.7(3.5)	90.8(1.6)	89.3(2.15)	84.4 (8.3)	93.1(4.2)
Tap water	Level 1	96.0(3.1)	91.9(2.2)	94.6(4.2)	94.8(5.3)	95.1(2.7)	96.1(2.6)
	Level 2	96.2(2.5)	93.7(5.5)	92.5(6.7)	87.5(6.4)	97.9(1.2)	97.5(7.4)
Lake water	Level 1	93.49(4.9)	89.3(3.5)	90.9(6.4)	101.7(3.6)	93.7(4.7)	92.4(2.9)
	Level 2	89.1(2.4)	92.4(3.1)	94.8(2.8)	99.1(6.6)	92.3(5.6)	93.1(4.1)

Level 1: 65 µg/L for CS and MSM; 95 µg/L for NS; 125 µg/L for FS, PS, and CME. Level 2: 130 µg/L for CS and MSM; 190 µg/L for NS; 250 µg/L for FS, PS, and CME.

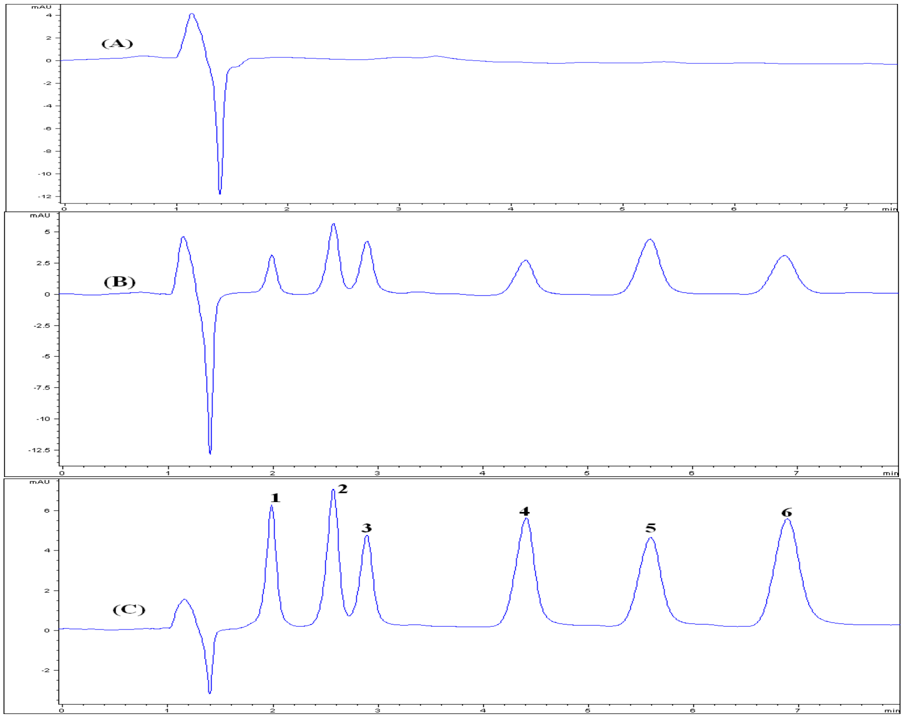


Figure 6. Typical chromatograms of blank (A) and spiked (B) river water sample, standard (C) at concentrations 130 µg/L for CS and MSM; 190 µg/L for NS; 250 µg/L for FS, PS, and CME; Extraction conditions: the same as those indicated in Figure 5; Peaks identification: 1- niclosulfuron, 2- metsulfuron-methyl, 3- chiorsulfuron, 4- flazasulfuron, 5-prosulfuron, 6-chlorimuron-ethyl.

The chromatograms of the target SUH residues in the river water sample before and after spiking at the concentration (level 2) utilized for precision study using the developed methods are displayed in Figure 6. By comparing the peaks of the fortified and blank samples, selectivity was evaluated. These chromatograms clearly show the absence of the chromatographic peak from co-extracted components and that they are well resolved for all analytes, indicating a high level of selectivity for the target pesticides at their retention time. As a result, the given chromatogram confirms the developed DLLME techniques selectivity. Similar characteristics were present in other water samples tested for this study.

Comparison of HD-DLLME with other reported extraction methods

To evaluate the performance of the present method, i.e., DLLME–HPLC–DAD for extraction, enrichment, and determination of SUH residues, it was compared with other methods reported in the literature for extraction of the same herbicide class [14, 30, 36, 43, 46–49] and the results are shown in Table 4. As can be seen, in terms of the LODs, precisions, and accuracy of the proposed method were better than or comparable to most of the other methods applied for extraction of SUH residues from different and the same type of matrices, i.e., water sample for the current method. Although the LOD of magnetic solid phase extraction (MSPE) method [48] using magnetic multi-walled carbon nanotubes (mag-MWCNTs) as adsorbents were lower, the synthesis steps of mag-MWCNTs were very complicated, expensive and a large number of organic solvents were consumed for elution and preparation. There may also be the problem of sample carryover effects which leads to false postive results [36, 48, 49]. The proposed DLLME is simple and unlike the SPE method, it does not require multi-steps conditioning, washing, loading and elution [20, 21, 48]. In addition, the proposed method is found to use simpler equipment, exhibits a wider linear range, integrated pretreatment and preconcentration in the single step, and utilizes micro level amount of organic solvents; which would make the procedure easier, more efficient, quicker, and promising for carrying out analysis of contaminated matrices in the environmental water samples.

Table 4. Comparison of the proposed method with other methods applied for the extraction and determination of sulfonylurea herbicides.

Extraction method	Determination method	Matrix	Linear range (ng/mL)	LOD (ng/mL)	RSD (%)	Recovery (%)	Ref.
SD-SFO-DLLME	HPLC-UV	Water and soil	5-1000	0.24-2.7	2.73-12.4	76-107	[14]
IL-DLLME-DSPE	HPLC-DAD	Soymilk	7.8-500	1.53-2.32	1.12-6.48	82-119	[36]
VA-IL-DLLME	HPLC-DAD	Wine	11-450	3.2-6.6	1-6.9	79-106	[43]
CPE	HPLC-UV	Water, soil and rice	4-2000	0.8-1.2	0.4-7.8	82-95	[46]
QuEChERS	HPLC-DAD	Soymilk	200-5000	20-40	<15	61-108	[47]
MSPE	HPLC-DAD	Water	0.05-5	0.01-0.04	2-12.9	77–107	[48]
MMF-SPME	HPLC-DAD	Water	0.1-200	1.8-18.0	1.2-9.9	71-119	[49]
DLLME	HPLC-DAD	Water	2.5-1000	0.8-1.5	2.9-9.8	84-102	This work

CPE; cloud point extraction. VA-IL-DLLME; vortex-assisted ionic liquid DLLME. QuEChERS; Quick, easy, cheap, effective, rugged and safe. MSPE; magnetic solid-phase extraction. SD-SFO-DLLME; solvent-based demulsifications surface floating organic droplet DLLME. MMF-SPME; multiple monolithic fiber solid phase microextraction. IL-DLLME-DSPE; ionic-liquid based DLLME followed by dispersive solid phase extraction.

CONCLUSION

The analytical sample preparation technique developed and optimized in this study, i.e., HD-DLLME was successfully applied in the extraction and quantitative determination of the most commonly utilized six SUHs at trace level in environmental water samples. All the experimental attributes taken into account in the study were assessed and optimized. No matrix interferences were co-extracted during the procedure used to extract trace level herbicides from water samples including tap, river, lake, and underground waters, and were not seen in the analysis at their respective retention times. It was found that the analytes could be extracted from contaminated samples using the optimized experimental approach with only little amount of extraction and dispersive solvents, and a short equilibration time. The proposed method combines the benefits of a short analysis time, simplicity, low consumption of organic solvent, sensitivity, and cost effectiveness as well as a high level of linearity over a broad range of analyte concentrations. As a result, the HD-DLLME analytical technique might be thought of as a good and reliable option for selective and sensitive extraction and experimental determination of the SUH residues in environmental water samples in routine laboratory analysis.

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