Desalination and Water Treatment
www.deswater.com
doi: 10.5004/dwt.2021.26905

217 (2021) 329–338
March

A quick and inexpensive method to determine 2,4-dichlorophenoxyacetic acid residues in water samples by HPLC

Naghmeh Orooji[a], Afshin Takdastan[a,b,c], Reza Jalilzadeh Yengejeh[a,*], Sahand Jorfi[b,c], Amir Hossein Davami[d]

[a]*Department of Environmental Engineering, Ahvaz Branch, Islamic Azad University, Ahvaz, Iran, Tel. +986133163430; Fax: +986133163384; emails: r.jalilzadeh@iauahvaz.ac.ir (R.J. Yengejeh), n.oroji2007@gmail.com (N. Orooji)*
[b]*Department of Environmental Health Engineering, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran, emails: afshin_ir@yahoo.com (A. Takdastan), Sahand369@yahoo.com (S. Jorfi)*
[c]*Environmental Technologies Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*
[d]*Department of Environmental Management – HSE, Ahvaz Branch, Islamic Azad University, Ahvaz, Iran, email: Davami.ah1352@gmail.com*

Received 22 May 2020; Accepted 7 December 2020

ABSTRACT

This study aimed to develop and validate salting-out assisted liquid–liquid extraction (SALLE) with high accuracy for measuring 2,4-dichlorophenoxyacetic acid (2,4-D) in water samples. Several parameters affecting the extraction, including the volume of salting-out solvent, type, and the amount of extracting solvent, pH and the volume of sample solution were optimized. Then, to validate the proposed method, a high-performance liquid chromatography equipped with a C18 column with a UV detector at 283 nm was applied. The optimal salting-out parameters were obtained as follows: 1 mL of acetonitrile was added to 4 mL of sample solution with pH = 2 and 5 mL salting-out solvent containing 5%w/v sodium chloride. Under optimal SALLE conditions, the extraction efficiency was obtained 99.69 % in a calibration curve of 0.01–50 μg/L with R^2 = 0.9999, and the limits of detection and quantification were 0.004 and 0.01 μg/L, respectively. The recovery percentage of 2,4-D in real samples via the SALLE method was obtained between 95.98 and 115%, confirming the sample's insignificant effect on extraction efficiency. The method was successfully used for the determination of 2,4-dichlorophenoxyacetic acid in water samples containing incurred residue. The procedure proved to be quick, accurate, precise, sensitive, and selective.

Keywords: 2,4-dichlorophenoxyacetic acid; Salting-out assisted liquid–liquid extraction; Water; Ahvaz

1. Introduction

Phenoxyacetic acid compounds are among the most important pesticides, widely used in agriculture [1,2]. The most important herbicide for the group is 2,4-dichlorophenoxyacetic acid (2,4-D). In 1982, the World Health Organization classified the matter as a dangerous matter (class 3) and declared its standard level in the ppm water supply to 100 μg/L, and later, the classification was changed to class 2 with 70 μg/L of standard level [3,4]. When this material is in contact with humans for the long run, it causes skin and eye stimulation, and the central nervous system is the main body for the fungicide [5,6]. The ability of 2,4-D to cause liver and kidney cancer was proven [7,8]. Environmental Protection Agency has introduced it as the third most applied pesticide in the world

* Corresponding author.

[9,10]. Since the presence of these toxic materials in water has lots of disadvantages to aquatics and humans, rivers need proper management measures to control pollutant resources [11]. The first step in controlling and managing the residual toxic materials in water resources is determining their concentrations with high accuracy and comparing them with the existing standards' values. The most applied method in monitoring organic compounds (pesticides), especially in the low content, is chromatography [12–14]. To date, several methods have been developed for determining 2,4-dichlorophenoxyacetic acid (2,4-D), including liquid chromatography (LC), LC-MS, gas chromatography (GC), GC-MS, high-performance liquid chromatography (HPLC), electrochemical sensor method, and capillary electrophoresis. Most of these methods are often complicated and time spending. The analyte needs to be derivatized before GC analysis, which will affect the accuracy of the results. Additionally, the reagents used are normally toxic. These compounds' high polarity makes it impracticable to analyze them directly by GC, and they must first be derivatized to stable and more volatile compounds. LC and LC-MS do not require a derivatization step and can provide better sensitivity and selectivity. However, these methods have some disadvantages, such as expensiveness and difficulty in the application of the method. In this study, HPLC was used for analysis [15]. HPLC is the most common method used for separating and determining these compounds because most pesticides are non-volatile. HPLC procedures involve assay of nonderivatized acids but have quite high detection limits [16]. However, the concentration of 2,4-D in samples is very low in environmental water. The matrix of some samples is complex, therefore a sample preparation and pre-concentration step are necessary before analysis [17]. Sample preparation is one of the important steps in the degradation process. Several procedures were reported for the pre-concentration of pesticides from water matrices, including solid-phase extraction (SPE) [18–20], solid-phase microextraction (SPME) [21–23], liquid–liquid microextraction (LLME), liquid–liquid extraction (LLE) [24], hollow-fiber liquid-phase micro-extraction (HFLPME) and dispersive liquid–liquid micro-extraction (DLLME) and molecularly imprinted solid-phase microextraction (MISPE), have been used. However, these methods have some disadvantages such as emulsion formation, using the large volume of organic solvents, expensiveness and difficulty in the application of the method, long-term extraction time, instability of micro drop, reduction of solid-phase performance with time, and in some cases the lower accuracy [16,25,26]. For example, the conventional LLE procedures are time-consuming, generally labor-intensive, and require large quantities of expensive, toxic and environmentally unfriendly organic solvents. SPE often suffered from the plugging of cartridges and consumption of an appreciable amount of toxic solvents at the elution step. Much less solvent is used in the SPE method compared to the LLE method, but the SPE method needs the column to be prepared and is considered a relatively expensive method. SPME has problems such as high cost, fragility and reduced solid-phase efficiency over time. In solid-phase cartridge methods, soxhlet and dispersive LLME, chlorine solvents and heavier than water methods have been used. The LPME method has problems such as long extraction time, micro-drop instability and in some cases low accuracy [27]. Due to the mentioned problems, more compatible and environmental-friendly techniques like salting-out assisted liquid–liquid extraction (SALLE) are introduced [28,29]. In this method, the water sample is mixed with an appropriate volume of organic solvents, and the migration of organic compounds takes place from the aqueous to organic phases [30]. Most sodium chloride or the other suitable salt is added to the mixture to prevent foaming during extraction and increase the extraction process's efficiency. Finally, the organic solvent containing analyst is decreased under pressure which becomes concentrated [25]. The most important factors affecting extraction efficiency are the volume of extracted solvent, ionic force, pH and sample volume. The advantages of SALLE is including, ease of operation, rapid extraction time, cost-effectiveness, high recovery, and high enrichment factors. SALLE is a single-stem extraction method in which the high ratio of volume sample to the solvent increases analyst concentration. Acetonitrile was found as a promising extracting solvent owing to its compatibility with reversed-phase HPLC [25].

Regarding the widespread application of 2,4-D as an integral component in agriculture in Khuzestan province, no studies were conducted to identify this pesticide's residuals by SALLE in Ahvaz water treatment plants. Therefore, considering the importance of water quality monitoring in the provision of safe and healthy drinking water resources, this study aimed to launch, develop and validate a SALLE technique as a new method with high accuracy to measure the partial amounts of 2,4-D in water samples using HPLC. The reason for selecting this pesticide was its high consumption in Khuzestan province, its longer stability, and more water solubility. In this study, the optimization of 2,4-D extraction agents from water samples was performed to achieve the maximum extraction enrichment factor and pre-concentration of negligible amounts of 2,4-D in water samples by the SALLE method. Then, to find the related pesticide in the water sample, precision, accuracy, and sensitivity of the method were analyzed as the first report for analyzing low concentrations of 2,4-D in water samples.

2. Materials and methods

2.1. Chemicals

All chemicals applied in this research were of analytical grade. 2,4-Dichlorophenoxyacetic acid ($C_8H_6C_{l2}O_3$) (CAS number 94–75–7) with high purity (99% w/w) was obtained from Merck, Co., (Germany). All organic solvents, including acetonitrile, methanol, ethyl acetate, ethanol, acetone, acetic acid were obtained from Merck Co., (Germany) distilled water (with HPLC grade) was obtained from Merck, Co., (Germany). Sodium chloride 5% w/v, 0.02 M sodium hydroxide (NaOH), 0.02 M hydrochloric acid (HCl) and sodium thiosulfate 10% w/v were purchased from Merck Co., (Germany). Furthermore, three pH buffer solutions (4 ± 0.02, 7 ± 0.02, and 9 ± 0.02) were obtained from Merck Co., (Germany). The stock solution of 2,4-D (1000 mg/L) was prepared by dissolving a specific amount of 2,4-D in distilled water, and then diluted to the desired concentrations.

2.2. Instrumentals

To separate and measure the concentration of 2,4-D, HPLC instrument (Knauer, Germany) and EZ-chrome software equipped with a four-channel pump (model k-1001) and UV-vis detector (model degasser, k-2600) was applied. The column was 100-5-C18 column (250 mm × 4.6 mm) and mobile phase composed of acetonitrile, deionized water, and acetic acid with the volumetric ratio of 80:19.5:0.5. The flow rate of the mobile phase was 1 mL/min. The column's temperature was constantly kept at 40°C. The wavelength of the UV detector was set at 283 nm. The maximum absorption wavelength (λ_{max}) for measuring 2,4-D concentration was 283 nm, which was obtained by a UV-Vis spectrophotometer (DR5000, HACH, Germany). All samples were injected into HPLC by using a 100 μL syringe. An ultrasonic bath (SonoSwiss SW 6H) was applied to sonicate the samples. The samples were shaken by vortex shaker was obtained from Hastaranteb, Co., (Iran).

2.3. Sampling

All of the sampling practices followed the standard method for the examination of water and wastewater and the guidelines for sample protection [31,32]. The sample analysis was performed in the central laboratory of water and wastewater of Ahvaz City, Iran and the laboratory of the Health Department of Ahvaz Jundishapur University of Medical Sciences. In this study, the grape method was applied for sampling. Furthermore, the sodium thiosulfate solution (0.05 mL per 100 mL of the sample) was added to the samples.

2.4. Preparation of standard solutions

The applied method was based on the method 6640B issued by the United States Environmental Protection Agency for chlorophenoxy acid pesticides [31–33]. Then, the stock solution of 2,4-D (1,000 mg/L) was diluted to different concentrations (0.01, 0.5, 1, 5, 10, 25, and 50 μg/L), and 20 μL of samples were injected into the instrument using a syringe. In addition, to evaluate the applied method, the control sample was analyzed, which did not show any peak. The recovery rate and accuracy were analyzed in synthetically spiked samples in three concentrations with seven replications. The recovery percentages and relative standard deviation (RSD) values showed the suitability of the method.

2.5. Sample preparation and extraction procedure

All the water samples were kept under refrigeration at 4°C in brown glass bottles before sample preparation, for a maximum of 24 h. On the extraction day, the sample temperature was adjusted to room temperature. The pH of the solution was adjusted to 2 using 0.02 M HCl and NaOH solution and filter through a 0.45 μm of syringe filter. 5 mL of filtered solution was added with 1 mol of acetonitrile and 5 mL 5% w/v of NaCl, followed by shaking using vortex shaker at 3,000 rpm for 3 min to separate organic and liquid phases [34]. After extraction, the residual solution (organic phase) was injected into a specific vial using a Hamilton syringe and dehydrated using a sodium thiosulfate solution. The final volume decreased to 0.8 mL, and finally, 20 μL of the sample was injected into the HPLC. Afterward, to obtain the maximum extraction efficiency, the effective factors on the extraction efficiency, such as the types and volume of extraction solvent, sample volume, pH of sample and volume of salt were studied and optimized. Finally, to confirm the method, standard solutions were extracted and compared with the obtained results. It is worth mentioning that for performing the accuracy of the method, the recovery experiments were done. In this regard, a specific volume of standard solution was added to the solutions containing 2,4-D concentration less than the instrument's limit, followed by extraction and finding recovery percentage. The experiments were carried out with three replications.

2.6. Calculations

The obtained results were analyzed using Microsoft Excel and Minitab software (version 16). Furthermore, the *T*-paired test and *F*-test were applied for measuring the accuracy of experiments.

3. Results and discussion

3.1. Optimization of extraction conditions

To simplify the optimization procedure, the single variable method was used in which all variables except the studied variable constant. All optimization steps were carried out in a water sample containing 1 μg/L of 2,4-D with three replications.

3.1.1. Selection of extraction solvent

The first step in optimization is selecting the appropriate extraction solvent. Solutions were selected based on lower density than water and the ability to the extraction of target compounds. Apart from the mentioned features, the chromatography instrument's low toxicity and appropriate behavior are the other positive characteristics of extract solvents [16,26]. In this study, extraction performances of the following solvents were evaluated based on the dielectric constant given in parenthesis: methanol (32.7), ethyl acetate (6.02), acetone (20.7), acetonitrile (37.5), and ethanol (24.55) which acetonitrile was selected as a best one, due to the higher repeatability and extraction. According to the results, the extraction efficiency of 2,4-D using acetonitrile was 98.05, which was highest, compared to the other applied solvents (Fig. 1a). This phenomenon leads to the higher distribution fraction and high solubility of 2,4-D in acetonitrile. In general, a higher solvent dielectric constant causes the higher polarity of the solvent. Also, the polarity constant of acetonitrile (5.8) is higher than other solvents [6]. The other solvents did not show a sharper peak in the chromatogram because they could not extract the analyte completely due to their lower polarity than acetonitrile. The polarity index of the solvents given in parenthesis: methanol (5.1), ethyl acetate (4.4), acetone (5.1), acetonitrile (5.8), and ethanol (5.2). Furthermore, acetonitrile is less harmful than other organic solvents that are commonly used in conventional

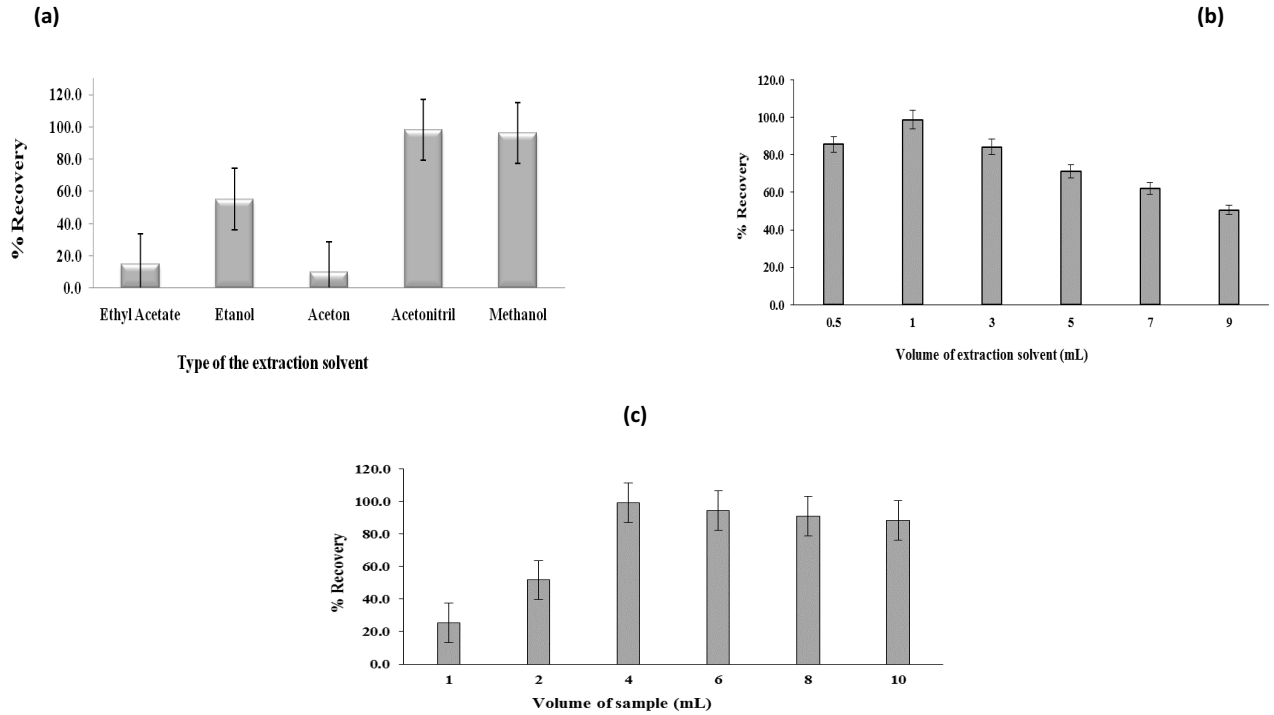

Fig. 1. (a) Effect of extraction solvent on extraction efficiency of 2,4-D (SALLE conditions: volume of extraction solvent, 1 mL; sample volume, 6 mL; pH = 2; volume of salting-out solution, 6 mL NaCl (%5 (w/v))), (b) effect of volume of extraction solvent on extraction efficiency of 2,4-D (SALLE conditions: extraction solvent, ACN; sample volume, 6 mL; pH = 2; volume of salting-out solution, 6 mL NaCl (%5 (w/v))), and (c) effect of sample volume on extraction of 2,4-D (SALLE conditions: extraction solvent, ACN, 1 mL; sample pH = 2; volume of salting-out solution, 6 mL NaCl (%5 (w/v))).

LLE as well as other LPME techniques. Therefore, acetonitrile was applied as an extraction solvent in this study.

3.1.2. Effect of volume of extraction solvent

One of SALLE's principal advantages is the application of the least amount of organic solvents in which the target material should be extracted with the lowest quantity of solvent. The volume of the applied extraction solvent can affect the upper organic phase's volume, the replicability of the results, and the extraction efficiency. Therefore, 0.5–9 mL of acetonitrile was used in this study (Fig. 1b). The results of the effect of different volumes of extraction solvent on the extraction efficiency are reported in Fig. 3. As can be seen, by increasing the volume of acetonitrile from 0.5 to 1 mL, the extraction efficiency of 2,4-D increased, later showed a decreasing trend at volumes higher than 1 mL. When the acetonitrile volume was 0.5 mL, the phase separation was not easy, and it was very difficult to take the upper organic phase separately. Similarly, at higher volumes of acetonitrile, above 1 mL, the volumes of the organic phase get increased but decreased the analyte enrichment due to dilution and hence further higher volumes were not examined. The peak areas of the target analytes decreased when the volume of

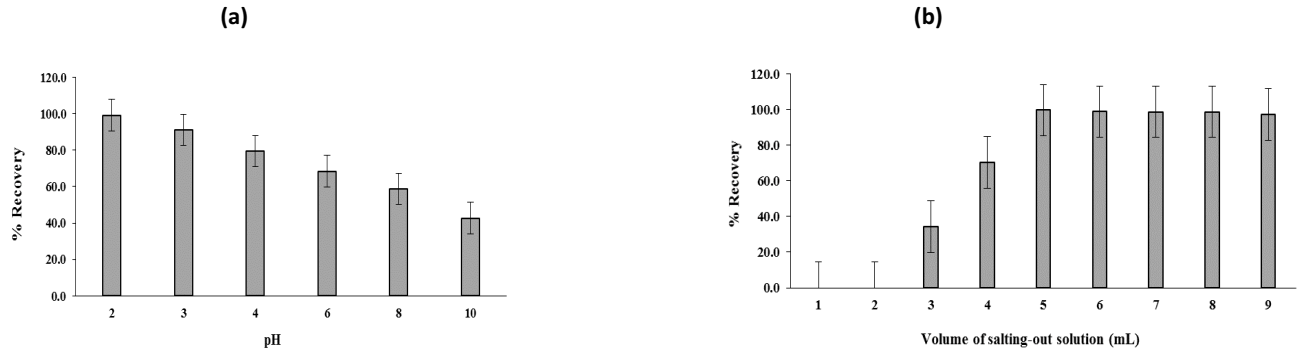

Fig. 2. (a) Effect of solution pH on extraction efficiency of 2,4-D (SALLE conditions: extraction solvent, ACN, 1 mL; sample volume, 4 mL; volume of salting-out solution, 6 mL NaCl (%5 (w/v))) and (b) effect of salting-out solution volume on extraction efficiency of 2,4-D (SALLE conditions: extraction solvent, ACN, 1 mL; sample volume, 4 mL; pH = 2; salting-out solution, NaCl (%5 (w/v))).

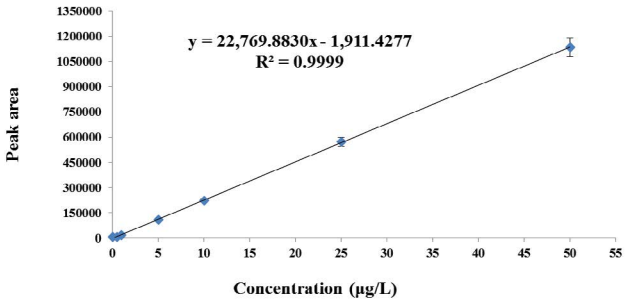

Fig. 3. Calibration curve of 2,4-D at different concentrations (0.01 to 50 µg/L).

acetonitrile is greater than 1 mL which may be attributed to dilution of the organic phase [35]. Hence, 1 mL with 98.69% extraction efficiency was selected as an optimum volume.

3.1.3. Effect of sample volume

Since the applied method's sensitivity has a direct relationship with the volume of the sample, it is expected to observe higher extraction of materials by increasing the sample volume. So, some experiments were carried out to find the effect of different sample volumes (1–10 mL) on the extraction efficiency of 2,4-D. Results revealed that by increasing the sample volume to 4 mL, the sensitivity of measurement was increased, which led to the enhancement of the extraction efficiency. However, in the volumes above 4 mL, no changes in the extraction efficiency were seen (Fig. 1c). At lower sample volume, phase separation was not observed, maybe due to insufficient volume ratio of the sample to that of organic solvent, and it was very difficult to take the upper organic phase separately. On the other hand, with an increase in the sample volume, beyond 2 mL, all the target analytes' peak areas showed increasing tendencies. However, above 4 mL of the sample volume, the instrumental responses showed decreasing extraction tendencies, which may be caused by the dilution effect caused by increased sample volume. On the other hand, in the volumes above 4 mL, the volumes of the organic phase get decreased, and it was very difficult to take the organic phase separately, and hence further higher volumes were not examined. Therefore, 4 mL was selected as an optimum volume with 99.09% extraction efficiency.

3.1.4. Effect of pH solution of 2,4-D

Adjustment of the pH solution of analytes decreases the solubility in water and increases the extraction efficiency. The pH of the analyte solution should be lower than their pKa values to obtain the unionized forms of target analytes that have a higher tendency to distribute into the organic phase. The effect of varying pH values of the sample solution on the extraction efficiency was studied in the range of 2.0–10.0. The results showed that the extraction efficiency of 2,4-D was maximum at pH 2 (Fig. 2a). At higher pH values, the extraction efficiency of 2,4-D decreases due to the hydrolysis of 2,4-D. Therefore, pH 2, with 99.19% extraction efficiency, was chosen as an optimum pH. When pH was low, the neutral species of 2,4-D was so nonpolar that their solubility in water is extremely low, the 2,4-D was easy to be extracted into the organic phase, then the value of recovery increased. At acidic conditions, they are in cationic form, which is important for their retention during the extraction. Similar results have been reported in the literature [25].

3.1.5. Effect of volume of salting-out solution

Increasing the volume of salting-out solution in the samples decreased the volume of the organic phase and therefore resulted in higher concentrations of 2,4-D in the organic phase [16] and this parameter is known as ionic strength. Here NaCl is used as a salting-out reagent due to its high ionic strength per unit concentration in the aqueous phase and good extraction efficiency. The effect of the volume of salting-out solution on the extraction efficiency of 2,4-D was studied at different volumes of 5% w/v NaCl solution (1–9 mL). The obtained findings from (Fig. 2b) indicated that the optimum amount of NaCl for the extraction of analysts and the phase separation was considered to be 5 mol. At the lower amount of NaCl, the extraction of analyte was not complete while a much higher amount of NaCl might result to reverse the extraction of analyte due to an increase in polarity of the aqueous phase. The reason for this observation would be the enhanced viscosity of the sample with an increased volume of salt, leading to a decreasing permeability factor of the analyte [16]. The addition of salt is often used to decrease the solubility of hydrophilic compounds in the aqueous phase through a salting-out effect and consequently increase the partition of analytes into the organic phase. Hence, 5 mL of 5% w/v NaCl solution with 99.69% extraction efficiency was selected as an optimum salt solution volume.

3.2. Validation of method

In each applied method, the ensure reliability and accuracy are very important. The results from the evaluation and validation of procedures are as follow:

3.2.1. Calibration curve

Different concentrations of 2,4-D solution (0.01, 0.5, 1, 5, 10, 25 and 50 µg/L) were prepared to depict the calibration curve; each sample was injected 3 times a day. The retention time was obtained at 3.5 min. The calibration curve was depicted based on the obtained data with a coefficient of determination R^2 = 0.9999, and equation y = 22,769.883x–1,911.4277 (Fig. 3). Results indicated a significant linear relationship between the calibration curve area, 2,4-D peaks and the standard solution in which the corresponding amounts are given in Table 1a. *F*-test was used to find the linear relationship between each factor (x) and the corresponding response (y). Since the amount of calculated F (74878.3) was higher than significant F (1.07004 E-09), it can be said that there is a linear relationship between x and y values. Moreover, a *T*-test was applied to validate the slope's suitability and intercept of the calibration curve (Table 1b). There is no systematic error regarding the values of calculated t (–2.066382235) lower than the obtained t value from

Table 1a
Characteristics of the 2,4-D calibration curve

Intercept (*a*)	–1,911.43	Slope (*b*)	22,769.88
Standard deviation of intercept	2,315.74	Standard deviation of slope	107.45
Regression standard deviation	4,870.96	Regression coefficient (R^2)	0.9999
Degree of freedom (df)	5	$F = \frac{SS_{regression}}{SS_{residuals}}$	44,904.99
Sum of squares of residuals ($SS_{residuals}$)	1,186	Sum of squares of regression ($SS_{regression}$)	1.06543 E+12

Table 1b
Evaluation of slope and intercept of a calibration curve based on the analysis of variance test

	t-Stat	*P*-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept (*a*)	–2.066382235	0.107670623	–9,404.971226	1,378.971496	–9,404.971226	1,378.971496
X-Variable 1(*b*)	273.6390435	1.07004E-09	22,597.37374	23,060.63717	22,597.37374	23,060.63717

the table and *P*-value > 5. The results of the slope of the calibration curve (*P*-value < 0.05 and *t* > *t*-critical) showed that the null rejection assumption and there is a significant difference between the slope of the calibration curve and zero. At higher sensitives (the slope of calibration curve), the potential of determination of low concentration is also high. The calibration curve and the corresponding equation were used to determine the concentrations of 2,4-D in the solution. The calibration curve accuracy was confirmed by measuring the standard solutions and corresponding quality control. After ensuring the linear relationship between standard solutions and the calibration curve area, the accuracy of the experiments was tested. The results of the validation test revealed the high accuracy of the experiments.

3.2.2. Determination of limit of detection and limit of quantification

To determine this quantity in the present study, a concentration about 5 times the estimated detection limit was used as the starting point to select the desired concentration for determining the limit of detection (LOD) [37,38]. The specific amount of analyte was dissolved in distilled water to obtain a 0.01 μg/L concentration. 7 sub-samples were obtained for 3 d and analyzed to ensure that LOD represents a routine experimental measurement. Repeated measurements were obtained in the range of 1 to 5 times the estimated LOD, and the recoveries of the specified excess values were in the range of 50% to 150% with an RSD% less than 20% (Fig. 4a). The corresponding limit of quantification (LOQ) and RSD values were 0.01 μg/L and 9.79%, respectively, and LOD and RSD values were 0.004 μg/L and 12.91%, respectively. After the measurement of LOD and LOQ, the accuracy of the method was performed. To confirm of detection limit, control matrix samples with 0.004 μg/L concentration and control matrix samples were tested in the same conditions. Based on the results, the mean of the indicated samples at the detection limit level was greater than the maximum value of control and the LOD was confirmed. To confirm the quantification limit, the matrix samples were labeled with a concentration of about 1 to 2 times the quantification limit. As the recoveries of the results were in the range of 85% to 115%, the LOQ was also confirmed (Fig. 4b).

3.2.3. Precision of results

The precision method was carried out using the intra-day (1 d) and inter-day (3 consecutive) precisions approach. The results' repeatability is illustrated by coefficient variations (CV%), which equals with RSD, obtained by the following equation: $CV\% = RSD\% = (SD/\bar{X}) \times 100$.
where CV is coefficient variation, SD is the standard deviation of results, and $\bar{X}$ is the average of replication results.

To investigate the intra-day precision, the spiked solution with 0.5, 5, and 25 μg/L was prepared, and the extraction was performed, and samples were injected into the HPLC. Furthermore, to study the inter-day precision, artificially spiked solutions with three concentrations (0.5, 5 and 25 μg/L) were also injected into the HPLC instrument three times for each. RSD and recovery percentages of 2,4-D at three different concentrations with 7 replications for intra-day and inter-day are reported in Tables 2 and 3. The percentages of recovery and RSD for extraction of 2,4-D obtained were 91.71%–99.84% and 0.22%–6.74%, respectively. Regarding the obtained results, it is obvious that the applied method has high precision and the replicability recoveries on different days in the acceptable range.

3.2.4. Accuracy of results

The accuracy of results can be obtained by the determination of the recovery percentages of analytes [36]. 20 μL of spiked solutions in three concentration levels (0.1, 2, and 30 μg/L) were injected into the instrument using a syringe. The obtained recovery percentages and RSD were 98.2%–104% and 0.19%–6.72%, respectively (Table 4). The results showed the suitability of the applied method for measurement of 2,4-D.

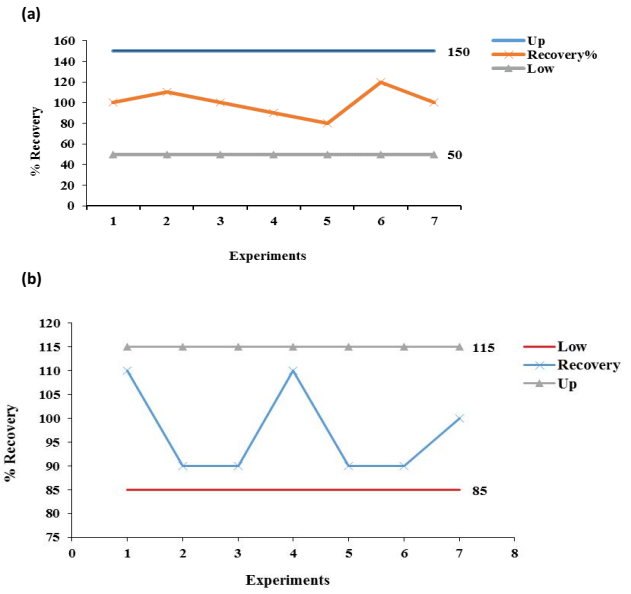

Fig. 4. (a) Determination of recovery percentage limit of added 2,4-D for measurement of LOD and LOQ (initial 2,4-D concentration: 0.01 μg/L) and (b) determination of recovery percentage limit of added 2,4-D for accuracy of LOQ (initial 2,4-D concentration: 0.01 μg/L).

3.3. Determination of concentration of 2,4-D in Ahwaz wastewater

The detection of the 2,4-D present in real samples was performed by plotting sample sub-peak surface calibration curve to the standard sub-peak level as the analytical response for the concentration calculation. The SALLE was applied for the determination of 2,4-D in influent and effluent of Ahwaz water treatment plant No 2. Fig. 5 represents the chromatogram of 2,4-D in a drinking water of real sample with unspike and real sample with spiked 2,4-D.

Table 4
Results of the accuracy of recovery of 2,4-D

Spiked concentration (ppb)	Average recovery percentage	RSD%
0.1	104	6.72
2	98.2	2.34
30	99.91	0.19

According to the chromatogram (Fig. 5), it is obvious that the applied method for the extraction of pesticides was appropriate. According to the results of water samples at the inlet and outlet of Ahwaz water treatment plant No. 2, in none of the studied samples, the concentration of 2,4-D was not higher than the national standard of 1053 (30 ppb) and the World Health Organization (70 μg/L) which does not pose a threat to public health. Furthermore, the accuracy of the method was performed by spiking analytes into the samples using Laboratory Fortified Matrix and Laboratory Fortified Matrix Duplicate methods. The related results are shown in Fig. 6. In these samples, the recovery percentages of 2,4-D were 95.98% and 115% using Laboratory Fortified Matrix and Laboratory Fortified Matrix Duplicate methods, respectively. Moreover, according to the graph and obtained results, the analyte was categorized in the range of 93.99% to 117.35% mineralization and the difference between Laboratory Fortified Matrix and Laboratory Fortified Matrix Duplicate was less than 20%. Therefore, the obtained results are under control, the method was accurate, and there is no need to revise the matrix effects. The obtained results of this research are in line with the results of Chamkasem and Morris [38] and Wen et al. [25].

3.4. Comparison with other published methods

The proposed method of SALLE for the determination of 2,4-D in water was compared with others as

Table 2
Results of precision of method in for 7 replications for 1 d (intra-day)

	Spiked level					
2,4-D	0.5 μg/L		5 μg/L		25 μg/L	
	Average recovery percentage	(%RSD)	Average recovery percentage	(%RSD)	Average recovery percentage	(%RSD)
	91.71	6.71	99.29	1.37	99.84	0.22

Table 3
Results of precision of method at three different concentrations for 7 replications for 3 different days (inter-day)

	Spiked level					
2,4-D	0.5 μg/L		5 μg/L		25 μg/L	
	Average recovery percentage	(%RSD)	Average recovery percentage	(%RSD)	Average recovery percentage	(%RSD)
	93.81	6.74	97.45	4.45	99.57	0.39

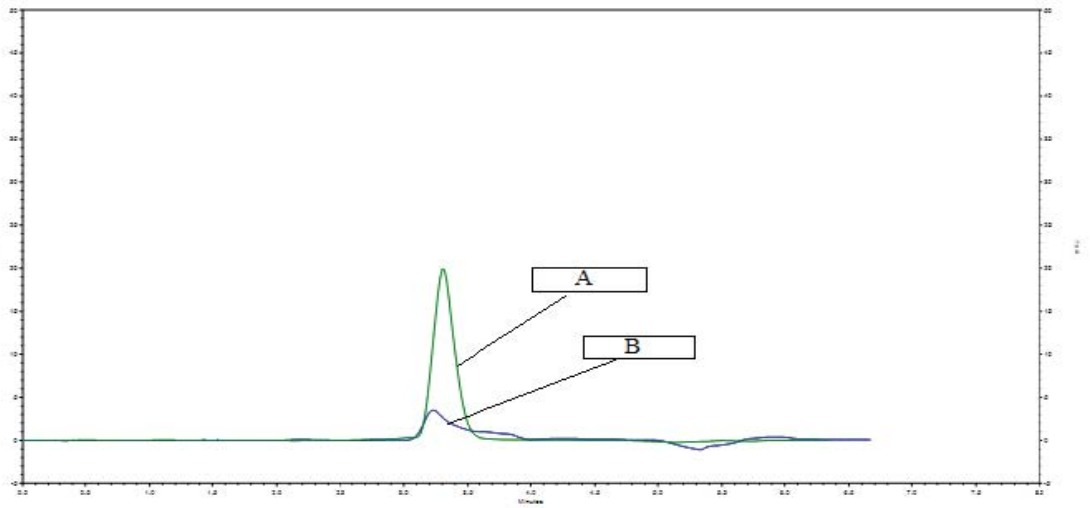

Fig. 5. Typical representative chromatogram of (A) real sample with unspike 2,4-D and (B) real sample with spiked 2,4-D (HPLC operating conditions: mobile phase: methanol–water-acetic acid (80:19.5:0.5, v/v); flow rate 1.5 mL/min; analytical column C18; UV detection at wavelength = 280 nm; injection volume = 20 μL).

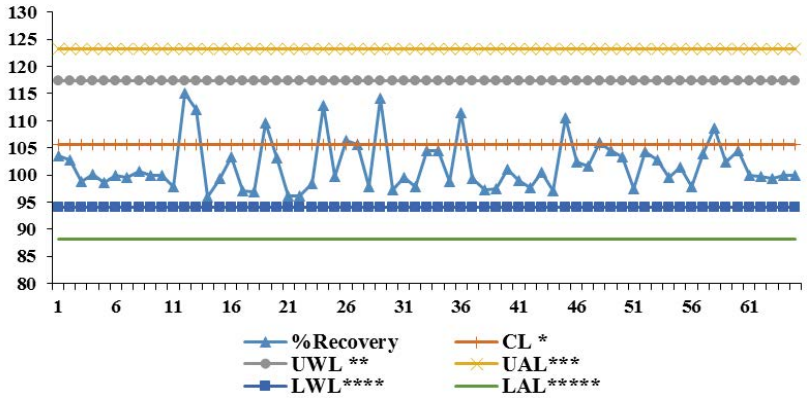

Fig. 6. Recycling evaluation of 2,4-D in real samples for all sampling cases in the range of studied time via performance parameters of enriched binary laboratory matrix (sample volume: 49.5 mL; the standard added volume of 2,4-D: 0.5 ppb; added concentration of 2,4-D: 1,000 ppb). *Control levels; **upper warning limits; ***upper action limits; ****upper warning limits; *****lower action limits.

Table 5
Comparison between efficiency of the proposed method for extraction of 2,4-D with the other methods

Method of extraction	Instrument	Volume of extraction solvent (mL)	Correlation coefficient (R^2)	Limit of detection (μg/L)	Recovery range (%)	References
SALLE	HPLC	1	0.9999	0.004	98.2–104	Proposed method
SALLE	Ultraviolet visible spectrophotometry	2	0.9979	0.75	83–100	[25]
QuEChERS	LC-MS/MS	10	0.998	0.72	86–107	[37]
SPE	LC-MS/MS	4	0.9994	0.003	70–112	[39]
SPE	LC-MS/MS	5	0.99	0.15	95–81	[40]

summarized in Table 5. The method was found to be simple, cost-effective and provides a cleaner chromatogram with good selectivity and reproducibility. This method does not quantify for as great several analytes across several chemical classes but can detect the most number of phenoxyacetic acid herbicides in comparison to other similar methods. This proposed method offered a wider linear range with LOD and LOQ compared to other work. Based on the experimental findings, the proposed technique can be considered one of the preferred alternatives, having a

promising future for selective and quantitative extraction of trace-level pesticide pollutants considered in this study and others having polar chemical natures and/or ionizable pollutants contaminating various environmental water systems.

4. Conclusion

In this research, the SALLE method was successfully used for pre-concentration and extraction of 2,4-D from water samples. The applied time to prepare the sample without the deleterious effect on the method's sensitivity was minimal. Furthermore, the use of excessive amounts of toxic organic solvents such as chlorinated organic solvents are avoided. A good detection limit and linearity were successfully obtained. The proposed method has several advantages, such as simplicity, high accuracy and sensitivity, and cost-effectiveness. This method can be applied for the measurement of other substances in different media. In addition, the analysis of real samples showed that the concentration of 2,4-D was not higher than the national standard of 1053 (30 ppb) and the World Health Organization (70 μg/L) which does not pose a threat to public health. Finally, it can be concluded that this technique can be used as an appropriate, quick, and sensitive method for measurement and monitoring of 2,4-D in water samples.

Acknowledgments

This paper was obtained from the Ph.D. thesis of Mrs. Naghmeh Orooji which was supported financially by the Islamic Azad University of Ahvaz and Ahvaz Water and Wastewater Company. Furthermore, we express our sincere thanks to Dr. Haghighi pour (Ahvaz Abfa Company) for his assistance in this thesis and also the staff of Ahvaz water and wastewater plant No.2 for Compliance with Ethical Standards.

Funding

This research was funded by the Islamic Azad University of Ahvaz and Ahvaz Water and Wastewater Company.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors. Providing helps in laboratory works.

References

[1] S.V. Bhat, S.C. Booth, E.A.N. Vantomme, S. Afroj, C.K. Yost, T.E.S. Dahms, Oxidative stress and metabolic perturbations in *Escherichia coli* exposed to sublethal levels of 2,4-dichlorophenoxyacetic acid, Chemosphere, 135 (2015) 453–461.

[2] A. Romero-Natale, G. Rebollar-Pérez, I. Ortiz, M.G. Tenorio-Arvide, R. Munguía-Pérez, I. Palchetti, E. Torres, A simple spectroscopic method to determine dimethoate in water samples by complex formation, J. Environ. Sci. Health., Part B, 55 (2020) 310–318.

[3] A.H. Smith, E.O. Lingas, M. Rahman, Contamination of drinking-water by arsenic in Bangladesh: a public health emergency, Bull. World Health Organ., 78 (2000) 1093–1103.

[4] M. Golshan, B. Kakavandi, M. Ahmadi, M. Azizi, Photocatalytic activation of peroxymonosulfate by TiO_2 anchored on cupper ferrite (TiO_2@$CuFe_2O_4$) into 2,4-D degradation: process feasibility, mechanism and pathway, J. Hazard. Mater., 359 (2018) 325–337.

[5] N. Orooji, A. Takdastan, R.J. Yengejeh, S. Jorfi, A.H. Davami, Photocatalytic degradation of 2,4-dichlorophenoxyacetic acid using Fe_3O_4@TiO_2/Cu_2O magnetic nanocomposite stabilized on granular activated carbon from aqueous solution, Res. Chem. Intermed., 46 (2020) 2833–2857.

[6] J.J. Liu, M. Jiang, G. Li, L. Xu, M.J. Xie, Miniaturized salting-out liquid–liquid extraction of sulfonamides from different matrices, Anal. Chim. Acta, 679 (2010) 74–80.

[7] D.M. Han, W.P. Jia, H.D. Liang, Selective removal of 2,4-dichlorophenoxyacetic acid from water by molecularly-imprinted amino-functionalized silica gel sorbent, Int. J. Environ. Sci., 22 (2010) 237–241.

[8] X.S. Bian, J.Q. Chen, R. Ji, Degradation of 2,4-dichlorophenoxyacetic acid (2,4-D) by novel photocatalytic material of tourmaline-coated TiO_2 nanoparticles: kinetic study and model, Materials (Basel), 6 (2013) 1530–1542.

[9] X.L. Liu, P. Lv, G.X. Yao, C.C. Ma, Y.F. Tang, Y.T. Wu, P.W. Huo, J.M. Pan, W.D. Shi, Y.S. Yan, Selective degradation of ciprofloxacin with modified NaCl/TiO_2 photocatalyst by surface molecular imprinted technology, Colloids Surf., A, 441 (2014) 420–426.

[10] B. Kordestani, R.J. Yengejeh, A. Takdastan, A.B. Neisi, A new study on photocatalytic degradation of meropenem and ceftriaxone antibiotics based on sulfate radicals: Influential factors, biodegradability, mineralization approach, Microchem. J., 146 (2019) 286–292.

[11] Y.Z. Hong, A. Ren, Y.H. Jiang, J.H. He, L.S. Xiao, W.D. Shi, Sol–gel synthesis of visible-light-driven $Ni_{(1-x)}Cu_{(x)}Fe_2O_4$ photocatalysts for degradation of tetracycline, Ceram. Int., 41 (2015) 1477–1486.

[12] X.N. Liu, Y.H. Tang, S.L. Luo, Y. Wang, X.L. Zhang, Y. Chen, C.B. Liu, Reduced graphene oxide and $CuInS_2$ co-decorated TiO_2 nanotube arrays for efficient removal of herbicide 2,4-dichlorophenoxyacetic acid from water, J. Photochem. Photobiol., A, 262 (2013) 22–27.

[13] K. Del Ángel-Sanchez, O. Vázquez-Cuchillo, A. Aguilar-Elguezabal, A. Cruz-López, A. Herrera-Gómez, Photocatalytic degradation of 2,4-dichlorophenoxyacetic acid under visible light: effect of synthesis route, Mater. Chem. Phys., 139 (2013) 423–430.

[14] S. Nasseri, M. Dehghani, S. Amin, K. Naddafi, Z. Zamanian, Fate of atrazine in the agricultural soil of corn fields in Fars province of Iran, J. Environ. Health Sci. Eng., 6 (2009) 223–232.

[15] J. Sherma, Pesticide residue analysis (1999–2000): a review, J. AOAC Int., 84 (2001) 1303–1312.

[16] T. Gezahegn, B. Tegegne, F. Zewge, B.S. Chandravanshi, Salting-out assisted liquid–liquid extraction for the determination of ciprofloxacin residues in water samples by high-performance liquid chromatography-diode array detector, BMC Chem., 13 (2019) 28, https://doi.org/10.1186/s13065-019-0543-5.

[17] M.E.T. Padrón, C. Afonso-Olivares, Z. Sosa-Ferrera, J.J. Santana-Rodríguez, Microextraction techniques coupled to liquid chromatography with mass spectrometry for the determination of organic micropollutants in environmental water samples, Molecules, 19 (2014) 10320–10349.

[18] M.I. Pinto, G. Sontag, R.J. Bernardino, J.P. Noronha, Pesticides in water and the performance of the liquid-phase microextraction based techniques, Microchem J., 96 (2010) 225–237.

[19] A. Asfaram, M. Ghaedi, A. Goudarzi, M. Soylak, Comparison between dispersive liquid–liquid microextraction and ultrasound-assisted nanoparticles-dispersive solid-phase microextraction combined with microvolume spectrophotometry method for the determination of Auramine-O in water samples, RSC Adv., 5 (2015) 39084–39096.

[20] H.Y. Yan, J.J. Du, X.G. Zhang, G.L. Yang, K.H. Row, Y.K. Lv, Ultrasound-assisted dispersive liquid–liquid microextraction coupled with capillary gas chromatography for simultaneous

analysis of nine pyrethroids in domestic wastewaters, J. Sep. Sci., 33 (2010) 1829–1835.
[21] Y.L. Liu, Y.H. He, Y.L. Jin, Y.Y. Huang, G.Q. Liu, R. Zhao, Preparation of monodispersed macroporous core–shell molecularly imprinted particles and their application in the determination of 2,4-dichlorophenoxyacetic acid, J. Chromatogr. A, 1323 (2014) 11–17.
[22] X.F. Liu, Q.F. Zhu, H.X. Chen, L.Z. Zhou, X.P. Dang, J.L. Huang, Preparation of 2,4-dichlorophenoxyacetic acid imprinted organic–inorganic hybrid monolithic column and application to selective solid-phase microextraction, J. Chromatogr. B, 951 (2014) 32–37.
[23] J.J. Jiménez, Simultaneous liquid–liquid extraction and dispersive solid-phase extraction as a sample preparation method to determine acidic contaminants in river water by gas chromatography/mass spectrometry, Talanta, 116 (2013) 678–687.
[24] L.F. Huang, M. He, B.B. Chen, B. Hu, Membrane-supported liquid–liquid–liquid microextraction combined with anion-selective exhaustive injection capillary electrophoresis-ultraviolet detection for sensitive analysis of phytohormones, J. Chromatogr. A, 1343 (2014) 10–17.
[25] Y.Y. Wen, C.W. Yu, J. Zhang, C.Q. Zhu, X.S. Li, T.L. Zhang, Z.L. Niu, Determination of 2,4-D in water samples by salting-out assisted liquid–liquid extraction-UV-vis, Int. J. Adv. Res. Chem. Sci., 2 (2015) 8–13.
[26] A. Amraei, A. Niazi, M. Alimoradi, M. Hosseini, Simultaneous spectrophotometric determination of some polycyclic aromatic hydrocarbons using chemometrics methods after their preconcentration by salting-out assisted liquid–liquid extraction, Int. J. Anal. Chem., 6 (2019) 10–18.
[27] J.J. Liu, M. Jiang, G. Li, L. Xu, M.J. Xie, Miniaturized salting-out liquid–liquid extraction of sulfonamides from different matrices, Anal. Chim. Acta., 679 (2010) 74–80.
[28] M. Wang, Z.W. Cai, L. Xu, Coupling of acetonitrile deproteinization and salting-out extraction with acetonitrile stacking in chiral capillary electrophoresis for the determination of warfarin enantiomers, J. Chromatogr. A, 1218 (2011) 4045–4051.
[29] F.-J. Zhao, H. Tang, Q.-H. Zhang, J. Yang, A.K. Davey, J.-P. Wang, Salting-out homogeneous liquid–liquid extraction approach applied in sample pre-processing for the quantitative determination of entecavir in human plasma by LC-MS, J. Chromatogr. B, 881–882 (2012) 119–125.
[30] G.G. Noche, M.E.F. Laespada, J.L.P. Pavón, B.M. Cordero, S.M. Lorenzo, *In-situ* aqueous derivatization and determination of non-steroidal anti-inflammatory drugs by salting-out-assisted liquid–liquid extraction and gas chromatography–mass spectrometry, J. Chromatogr. A, 1218 (2011) 6240–6247.
[31] American Public Health Association (APHA), American Water Works Association (AWWA), Water Environment Federations (WEF), Standard Methods for the Examination of Water and Wastewater, 23rd ed., Washington D.C., 2017.
[32] J.L. Acero, F.J. Benítez, F.J. Real, M. González, Chlorination of organophosphorus pesticides in natural waters, J. Hazard. Mater., 153 (2008) 320–328.
[33] S.C. Wendelken, Method 515.4 Determination of Chlorinated Acids in Drinking Water by Liquid–Liquid Microextraction, Derivatization, and Fast Gas Chromatography with Electron Capture Detection, DIANE Publishing, 1996.
[34] Y.Y. Wen, J.H. Li, F.F. Yang, W.W. Zhang,W.R. Li, C.Y. Liao, L.X. Chen, Salting-out assisted liquid–liquid extraction with the aid of experimental design for determination of benzimidazole fungicides in high salinity samples by high-performance liquid chromatography, Talanta, 106 (2013) 119–126.
[35] Y. Alemayehu, T. Tolcha, N. Megersa, Salting-out assisted liquid–liquid extraction combined with HPLC for quantitative extraction of trace multiclass pesticide residues from environmental waters, Am. J. Anal. Chem., 8 (2017) 433, doi: 10.4236/ajac.2017.87033.
[36] L. Dhooghe, K. Mesia, E. Kohtala, L. Tona, L. Pieters, A.J. Vlietinck, S. Apers, Development and validation of an HPLC-method for the determination of alkaloids in the stem bark extract of *Nauclea pobeguinii*, Talanta, 76 (2008) 462–468.
[37] D. de Beer, E. Joubert, Development of HPLC method for *Cyclopia subternata* phenolic compound analysis and application to other *Cyclopia* spp., J. Food Compos. Anal., 23 (2010) 289–297.
[38] N. Chamkasem, C. Morris, Direct determination of 2,4-dichlorophenoxyacetic acid in soybean and corn by liquid chromatography/tandem mass spectrometry, J. Regul. Sci., 4 (2016) 9–18.
[39] E. Sklivagou, K. Papadopoulou, A. Bakoulis, Determination of acid herbicides in water by LC/MS/MS, Desal. Water Treat., 13 (2010) 320–327.
[40] F.F. Donato, M.L. Martins, J.S. Munaretto, O.D. Prestes, M.B. Adaime, R. Zanella, Development of a multiresidue method for pesticide analysis in drinking water by solid phase extraction and determination by gas and liquid chromatography with triple quadrupole tandem mass spectrometry, J. Braz. Chem. Soc., 26 (2015) 2077–2087.