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Development of a switchable solvent liquid phase extraction method for the determination of chlorthiamid, ethyl parathion, penconazole and fludioxonil pesticides in well, tap and lake water samples by gas chromatography mass spectrometry

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ABSTRACT

This study presents a switchable solvent liquid phase extraction (SS-LPE) method for the enrichment of four pesticides, and their subsequent determination by gas chromatography mass spectrometry (GC–MS). Box-Behnken experimental design was utilized to attain optimum conditions of the SS-LPE method by navigating a design model developed according to parameter effects and their interactions. Significant parameters were evaluated using analysis of variance (ANOVA), and the optimum variables were selected from a list of combinations presented by the model predictor. The detection limits calculated for chlorthiamid, ethyl parathion, penconazole and fludioxonil were 0.58, 0.73, 0.33 and 0.28 ng/mL. These detection limits correlated to enhancement factors in the range of 60–150 folds. Repeatability based on intra-day and inter-day analysis produced percent relative standard deviations below 10%, a good indicator of high precision. Well water, tap water and lake water samples were employed as real sample matrices to test the trueness of the method and its applicability. The recovery results calculated for the samples spiked at different concentrations were satisfactory (90–105%), and this validated the method for real sample applications.

1. Introduction

Throughout history, chemicals derived from natural and anthropogenic sources have been used to deter pests that pose danger to humans, plants and animals, and these are referred to as pesticides [1]. Large scale production of synthetic pesticides commenced in the mid-1940s after the successful use of dichlorodiphenyltrichloroethane (DDT) to control pathogen vectors such as mosquitoes [2]. Disease prevention, and high yield/quality of agricultural products are two main advantages of pesticide usage [3], but reports of their adverse effects on hosts (non-target organisms) has called for strict regulations on their safe applications in the environment [4]. Exposure to pesticides in the environment or in the line of work (occupational exposure) could result in chronic and acute health effects such as eczema, diabetes, skin diseases, digestive tract diseases, respiratory tract diseases, neurotoxicity, reproductive disorders and cancers of different organs [5–7]. Monitoring of pesticides in environmental and food samples is therefore very crucial, but this requires very sensitive analytical methods due to the occurrence of pesticides at low concentration levels.

Ethyl parathion is a typical insecticide belonging to the organophosphate chemical class. This class of pesticide acts by inhibiting acetylcholinesterase (AChE), a cholinergic enzyme that is essential for the nervous system of humans and insects [8,9]. Ethyl parathion is no longer approved for use on vegetables and fruits due to its high acute toxicity for both humans and animals [10]. Fungicides are specific chemicals that are used to terminate the internal energy and membrane cells of fungi [11]. Fludioxonil and penconazole are two active fungicides that are widely applied to crops to mitigate fungal growth and improve production yield or preservation [12,13]. Application of fungicides post-harvest to prevent fungal growth could result in residual

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levels that could cause harm to consumers. Chlorthiamid belongs to the benzonitrile group of pesticides and it was used as an effective herbicide against a wide range of grass and broad-leaved weeds. Chlorthiamid is the parent compound of dichlobenil, which undergoes non-biological or biotic degradation into 2,6-dichlorobenzamide, commonly referred to as BAM [14,15]. BAM is relatively stable and resists degradation, and it leaches through the soil beds to contaminate ground water. Thus, chlorthiamid/dichlobenil has been banned from use in order to prevent contamination of groundwater with the degradation product BAM [15].

Gas chromatography when coupled to a mass spectrometry detector (GC–MS) forms a powerful analytical instrument that determines analytes with high precision and accuracy [16]. Similar to other instruments, direct measurement of most samples is not possible by the GC–MS system and this calls for an efficient sample treatment procedure such as extraction. A great deal of effort has been put into developing miniaturized extraction methods (commonly referred to as micro-extraction) that overcome setbacks of the conventional liquid–liquid and solid phase extraction methods including multiple and tedious steps, large sample and waste volumes, large solvent consumption and low extraction output [17]. After mitigating these drawbacks, recent researches have focused on the use of green solvents that comply with the concepts of green chemistry. Some solvents that have been classified and utilized as green solvents in extraction methods include ionic liquids, deep eutectic solvents and supercritical fluids [18].

Another example is switchable polarity solvents (SPSs), which are produced by converting nonpolar amines into their polar analogues in water by protonation with carbon dioxide [19]. The uniqueness of SPSs lies in their easy change in physical property, from nonpolar to polar and vice versa. This unique property of SPSs can be utilized in other chemical applications such as catalysis reactions, synthesis and purification of compounds [20,21]. The experimental steps involved with the use of SPSs in extraction are simple and few, and the entire procedure carries very low toxicity [21]. In addition, disperser solvents are not required in switchable solvent based extractions due to complete miscibility with water. After an appropriate mixing period, sodium hydroxide solution or an inert gas is added to stimulate switching of the SPS into its original nonpolar state and an ensuing separation from aqueous solution [22]. SPS based extraction methods are simple, economical, high yielding and comply with green analytical chemistry in terms of safe chemical usage and low waste generation [19]. Experimental design (multivariate optimizations) offers the advantage of evaluating the effects of individual parameters of a method and interaction between the parameters. Compared to univariate optimizations, multivariate optimizations require fewer experiments and the developed quadratic models can be used to predict/estimate parameter values that give optimum extraction outputs [23,24].

This study was therefore aimed at utilizing Box-Behnken design to maximize the extraction output of a switchable solvent liquid phase extraction (SS-LPE) method for four pesticides. The analytes are chlorthiamid and ethyl parathion as two obsolete pesticides, and penconazole and fludioxonil as two active pesticides. The SS-LPE method was coupled with GC–MS to significantly boost the signals of the analytes to enable their determination at trace levels.

2. Materials and methods

2.1. Instrumentation

A 6890 model gas chromatograph system equipped with a 5973 model mass spectrometry detector (both purchased from Agilent Technologies, CA – USA) was used to detect the analytes in this study. The analytes were eluted through a capillary column (HP-5MS) having a length, inner diameter and film thickness of 30 m, 250 μm and 0.25 μm, respectively. Starting with an initial oven temperature of 70 °C, the temperature was ramped to 180 °C at 60 °C/min and then to 300 °C at 40 °C/min, where it was held for 120 s. Ultrapure helium (99.999%) was used as carrier gas at 1.0 mL/min constant flow rate, and 1.0 μL of all samples/standards were injected into the inlet port (250 °C) in the splitless mode. The fragment ions (m/z) used to qualify/quantify fludioxonil, penconazole, ethyl parathion and chlorthiamid were 127/248, 248/159, 109/291 and 172/170, respectively. Electron impact ion source with the standard ion energy of 70 eV was used to produce fragmented ions of the analytes. The MS quadrupole temperature was 230 °C, the MS source temperature was 150 °C and the transfer line temperature was 280 °C. Other apparatus used in the study were EBA 20 model centrifuge (Andreas Hettich GmbH, Tuttlingen – Germany) and M1010002 model vortex mixer (ISOLAB, İstanbul – Turkey).

2.2. Chemicals and reagents

Ethyl parathion, fludioxonil, penconazole, and chlorthiamid were purchased from Dr. Ehrenstorfer with percent purities greater than 98%. All four analytes were dissolved in acetonitrile (gravimetrically) to prepare stock solutions, from which specific volumes were taken into a volumetric flask to prepare a 0.10 g/L mixed standard stock solution. Working standard solutions, intermediate standard solutions and calibration standard solutions were subsequently prepared by diluting the mixed standard stock solution in proper ratios. Ultrapure deionized water (resistivity 18.2 MΩ.cm) was used to prepare aqueous standard solutions, and for rinsing all laboratory glassware. The tertiary amine (N,N-dimethylbenzylamine) used in synthesizing the switchable solvent and sodium hydroxide used as trigger agent for the deprotonation process were purchased from Merck (Darmstadt, Germany). Solid CO_2 was purchased from an ice cream shop in İstanbul, Turkey. The procedure reported by Erarpat et al. for the preparation of switchable solvent was employed in this study [22].

2.3. SS-LPE procedure

The switchable solvent was vortexed for 10 s before each experiment to facilitate homogeneity for repeatable results. Exactly 8.0 mL of the sample solution was pipetted into a plastic centrifuge tube (15 mL) followed by the addition of 1.0 mL switchable solvent and vortex mixing for 30 s. Sodium hydroxide (1.2 mL of 0.50 M) was then added for deprotonation and the cloudy solution obtained was vortexed for 15 s to facilitate homogenous mixing and rapid deprotonation. The extraction tube was then placed in a centrifuge device operating at 6000 rpm for 120 s to allow the analyte-rich amine phase to settle as the upper layer in the tube. Approximately 60 μL of the final extract was pipetted into GC micro-vials for automatic injections.

2.4. Samples

Three different aqueous samples were used to ascertain the method's applicability and quantification accuracy in real samples. The samples were tap water, well water and lake water. The polypropylene plastic bottles used to collect the samples were rinsed thoroughly with the sample before filling them full without air spaces. The samples were stored in cool and dry wooden cabinets in the laboratory, and filtered through 0.45 μm hydrophilic polytetrafluoroethylene (PTFE) syringe filters before using in recovery experiments.

3. Results and discussions

The GC–MS system performance was evaluated prior to the method development using calibration plots developed from standard solutions (acetonitrile-based) prepared in the range of 0.10 mg/L–100 mg/L. The univariate and multivariate optimization experiments were performed with 200 ng/mL and 50 ng/mL aqueous standard solutions, respectively.

3.1. Univariate optimization of sodium hydroxide concentration

With the aim of limiting the parameters of the experimental design to three, the concentration of sodium hydroxide was predetermined by the univariate (step-by-step) optimization approach. In addition to setting the design parameters at three, the effect of sodium hydroxide concentration and volume could be redundant in the design experiment. That is, the effect observed for the volume of a specific concentration (e.g. 2.0 mL of 1.0 M solution) would be equal to the effect observed for half the volume and double the concentration (1.0 mL of 2.0 M) for the same final volume of sample. Thus, concentrations of sodium hydroxide including 1.0, 2.0, 3.0 and 5.0 M were tested to evaluate their effects on deprotonation. On an average for all four analytes, the results obtained for 1.0 M sodium hydroxide was about 2.0, 4.0, 5.0 folds higher than the results obtained for 2.0, 3.0 and 5.0 M , respectively. This clearly indicated that concentrations lower than 1.0 M would yield higher peak area values. To test this theory, 0.25 and 0.50 M solutions were tested with the other concentrations but there was no observable amine phase for the former concentration. The results obtained are depicted in Fig. 1 and the linear decrease in peak area values can be clearly seen, where the peak area values of the 0.50 M sodium hydroxide solution was about 4.0 times higher than 1.0 M.

3.2. Box-Behnken experimental design

Parameters fitted into the Box-Behnken experimental design were volume of switchable solvent, volume of 0.50 M sodium hydroxide solution and vortex mixing period, denoted by the respective alphabets A, B and C. The design experiment was constructed around three levels of each parameter including a low level (denoted as −1), a mean level (denoted as 0) and a high level (denoted as + 1). Different combinations for these parameter levels resulted in twelve (12) experimental runs in addition to six (6) replicate runs for the center points of the parameters (A0, B0 and C0), making a total of 18 experimental runs and 54 experimental data (triplicates for each run). The center points were distributed randomly through the twelve original combinations to assess variations that occur at different periods of the experimental run. The effect of the three independent variables (X_1, X_2 and X_3) on response (Y) was determined with the quadratic polynomial expression given in equation 1 (constant: β_0, linear coefficients: β_1, β_2, and β_3, coefficients of interaction: β_{12}, β_{13}, and β_{23}, and quadratic coefficients: β_{11}, β_{22}, and β_{33}) [25].

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} {X_1}^2 + \beta_{22} {X_2}^2 + \beta_{33} {X_3}^2 \quad (1)$$

In order to obtain adequate signals for all experimental runs, aqueous mixed standard solutions were used for the multivariate optimization experiment. Analysis of variance (ANOVA) in the *Design-Expert 7.0.0* software was used in the evaluation of the results. Significance of the parameters was determined according to *P*-values being equal to or less than the value of 0.05, and *F*-values bigger than *F*-critical values at 95% confidence level. The model developed using the response values of the four analytes was deemed significant with regards to obtained *P* and *F*-values of < 0.0001 and 83.49, respectively. Agreement between values predicted by the model and experimented values can be seen in the probability plot of Fig. 2 with a coefficient of determination (R^2) value of 99%. Adjustment of the R^2 value based on the number of model parameters resulted in a 98% adjusted R^2 value, which varied by about 1.0%. These are strong indicators of the models reliability and accuracy.

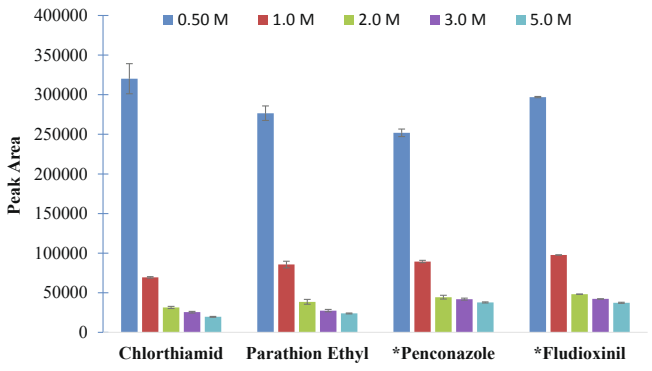


Fig. 1. A plot of sodium hydroxide concentrations against integrated peak area values, n = 3 error bars (*depicts peak area values scaled down three folds).

Table 1 summarizes parameters and interaction between parameters that were significant to the model according to *P*-values ≤ 0.05. In addition to the significance of parameters, their main effects were either determined as positive or negative, where positive main effect refers to the high level (+1) of the parameter variable influencing the yield of extraction and negative main effect refers to the low level (−1) of the parameter variable influencing extraction yield. Switchable solvent volume and sodium hydroxide volume were both significant to the model and recorded negative main effects on extraction yield. This observation can be justified by the principle of analyte enrichment, where the low volumes resulted in equally low volume analyte-rich amine phase after separation. Vortex mixing was not significant to the model (*P*-value = 0.9941) but it recorded a positive main effect. Switchable solvent volume and sodium hydroxide volume interaction (Fig. 3) was significant to the model as well as the quadratic effect of switchable solvent volume (A^2) and vortex period (C^2). The predictor tool of the experimental design was used to derive optimum variables for the three parameters as 1.0 mL (switchable solvent volume), 1.2 mL (sodium hydroxide volume) and 30 s (vortex mixing period).

3.3. Analytical figures of merit

Using the peak area values obtained in the final optimization step as a guide, aqueous standard solutions were prepared in the range of 1.0 ng/mL–1.0 μg/mL, and analyzed under optimum conditions of the SS-LPE-GC–MS method. For all four analytes, the linear dynamic range (LDR) was selected as the plot of linearity with coefficient of determination (R^2) values greater than 0.9997. The lowest calibration standard of each analyte was measured seven times to calculate standard deviation (SD), which was in turn used to evaluate the method's precision (% RSD) and to determine the limits of detection and quantification (LOD and LOQ). The expressions 3xSD/slope and 10xSD/slope were respectively used to calculate the LOD and LOQ values. As given in Table 2, the LOD values of the analytes fell below 1.0 ng/mL, and enhancement in detection power (EDP) values in the range of 60–150 folds were determined by comparing with the LOD values obtained by direct GC–MS analysis ($EDP = LOD_{GC-MS}/LOD_{SS\text{-}LPMS\text{-}GC-MS}$). The LOD values recorded for the analytes in this study are comparable to limits (4.0–6.0 μg/kg) reported for the analytes in the literature (Table 3) using highly sensitive instruments such as gas chromatography/tandem triple quadrupole mass spectrometry (GC-TQMS) and ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS) [26–28].

3.4. Spike recovery experiments

Environmental samples comprise of diverse organic and inorganic components that have the propensity to alter the extraction and determination of an analyte, resulting in a false positive or false negative result. Generally, matrix interference hinders the determination of analytes, leading to results that are relatively lower than the true value. For this reason, three environmental samples (well water, tap water and lake water) were examined under optimum conditions of the SS-LPE method to ascertain the effect of their respective matrices on the quantification of the four analytes. The qualifier and quantifier ions of

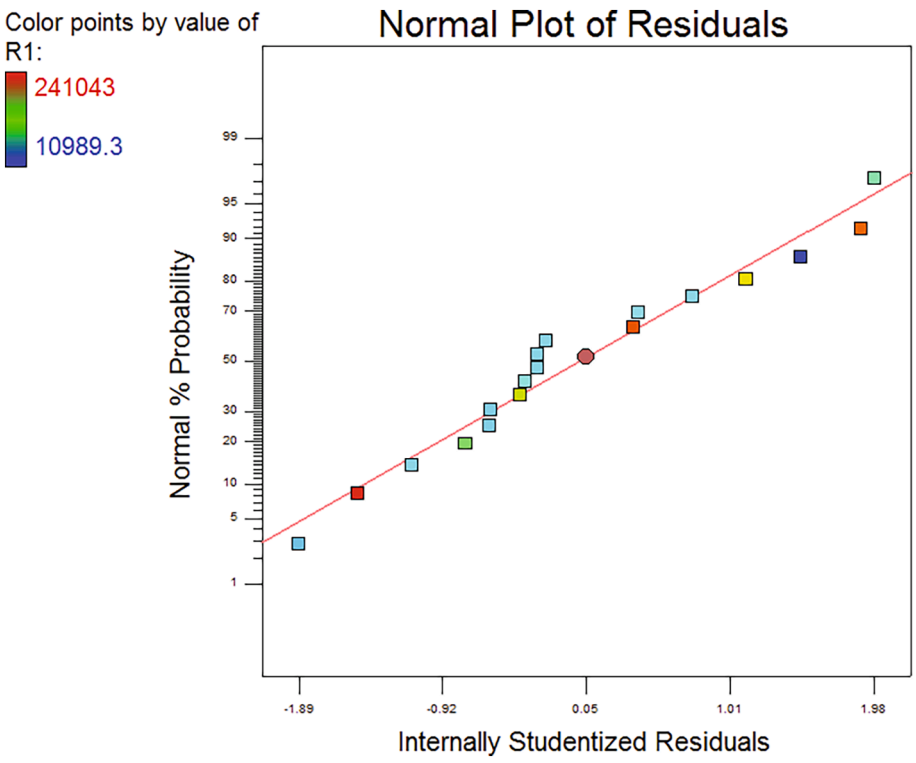


Fig. 2. A normal probability plot depicting internally studentized residuals for the analytes.

Table 1
ANOVA results for average response values of the analytes.

Source	Sum of Squares	Degree of freedom	Mean Square	*F*-Value	*P*-value
Model	7.881×10^{10}	9	8.756×10^{9}	83.49	< 0.0001
A	3.992×10^{10}	1	3.992×10^{10}	380.61	0.0330
B	2.165×10^{10}	1	2.165×10^{10}	206.40	<0.0001
AB	2.130×10^{9}	1	2.130×10^{9}	20.31	0.0015
A^2	3.522×10^{9}	1	3.522×10^{9}	33.58	0.0016
C^2	9.295×10^{9}	1	9.295×10^{9}	88.63	0.0016
Residual	8.390×10^{8}	8	1.049×10^{8}		
Lack of Fit	4.157×10^{8}	3	1.386×10^{8}	1.64	0.2936 (not significant)
Pure Error	4.233×10^{8}	5	8.467×10^{7}		
Cor Total	7.965×10^{10}	17			
R^2	0.9895				
R^2-adjusted	0.9776				

*A: switchable solvent volume, B: sodium hydroxide volume and C: vortex mixing period.

the four analytes were checked at their determined retention times in the chromatograms obtained for blank analysis but no signals were observed. It was deduced that the analytes were absent from the samples, or probably are limits far lower than the detection limit of the SS-LPE-GC–MS method.

The samples were spiked in the same manner as the aqueous standard solutions were prepared. The final spike concentrations for the samples were 20, 50 and 100 ng/mL, and five-point calibration plots developed with aqueous standards (10–200 ng/mL) were used to quantify the analytes in the spike samples. The recovery results (%) calculated for the analytes in each sample are presented in Table 4 and as seen in that table, the results are satisfactory (90–105%). The recovery results recorded for tap water was relatively higher than the well and lake water samples due to its relatively less complex matrix. However, the recovery results suggest that the analytes can be quantified in all three sample matrices with appreciable accuracy. Intra-day and inter-day precision was determined using 50 ng/mL spiked samples as shown in Table 4. Closeness in the results for three replicate measurements for both intra-day and inter-day tests verify the method's high precision based on repeatability.

3.5. Assessment of green profile

The twelve (12) principles of Green Chemistry proposed by Anastas and Warner set up a framework that boosts processes and chemical systems used for materials and products, while impacting less on humans and the environment [29]. In the context of extraction, the framework suggests the use of less harmful reagents and limiting the amount of wastes generated. Green Analytical Chemistry was therefore geared towards assimilating the principles of green chemistry with analytical methods. Galuszka et al. proposed an analytical tool called Eco-Scale, which uses varying parameters and different stages of a process to estimate the greenness of an analytical method [30]. In this system, penalty points are awarded to chemicals, instruments and wastes generated in the analytical process, and the sum total of the points are subtracted from 100 to obtain an Eco-Scale score. For this study, a penalty point of four (4) was awarded to the aqueous mixed standard solution of the analytes, two (2) for the sodium hydroxide

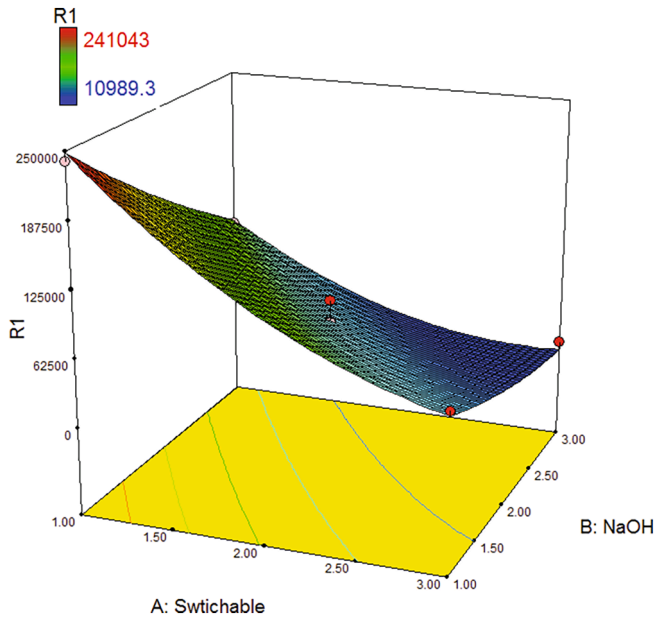


Fig. 3. Response surfaces showing the interaction between switchable solvent volume (A) and NaOH volume (B).

Table 2
Analytical figures of merit obtained for the developed SS-LPE-GC–MS method.

Analyte	LOD, ng/mL	LOQ, ng/mL	R^2	% RSD	LDR, ng/mL	EDP
Chlorthiamid	0.58	1.9	0.9999	7.6	2.0–500	68
Fludioxinil	0.28	0.93	0.9999	9.1	1.0–100	143
Ethyl parathion	0.73	2.4	1.000	8.1	2.0–500	96
Penconazole	0.33	1.1	0.9998	7.2	1.0–200	122

Table 3
Analytical figures of merit reported for the analytes in the literature.

Analyte	LOD	LOQ	%RSD	Ref.
Chlorthiamid	6.0 μg/kg	–	13.9	[27]
Fludioxinil	4.0 μg/kg	–	6.9	[27]
Ethyl parathion	5.0 μg/kg	10.0 μg/kg	–	[28]
Penconazole	5.0 μg/kg	10.0 μg/kg	–	[28]

trigger agent, three (3) for the waste generated and three (3) for the GC–MS instrument used. Thus, the Eco-Scale score calculated for this study was 88 (100–12) and this confirms the method as an excellent green analytical approach.

4. Conclusion

In this study, Box-Behnken experimental design was used to optimize a switchable solvent liquid phase extraction method for preconcentration of four pesticides from aqueous solution. The experimental design provided a detailed evaluation of individual experimental parameters and interaction of their variables, from which the most favorable conditions were selected to validate the method. The four analytes recorded enhancement factors in the range of 60–150 folds, synonymous with detection limits in the range of 0.20–0.80 ng/mL. None of the analytes were detected in blank analysis of lake water, tap water and well water samples, and the samples were therefore spiked at different concentrations to evaluate the amount that could be recovered. All four analytes recorded percent recovery results in the range of 90–105%. Intra-day and inter-day repeatability tests also validated the method's precision. The method presented in this study can be readily replicated in other laboratories with basic materials and can be incorporated into routine laboratory analysis.

Table 4
Percent recovery results of the analytes spiked in aqueous samples.

Analyte	Spike level	Lake water (%)	Tap water (%)	Well water (%)
Chlorthiamid	25 ng/mL	90.8 ± 5.4	96.6 ± 8.1	96.4 ± 8.6
	*50 ng/mL	91.0 ± 6.5	98.8 ± 8.3	90.7 ± 3.8
	**50 ng/mL	92.5 ± 5.3	98.5 ± 4.3	92.6 ± 3.9
	100 ng/mL	93.3 ± 5.1	97.9 ± 6.5	95.9 ± 7.2
Fludioxinil	25 ng/mL	96.5 ± 5.2	102.4 ± 7.4	96.2 ± 8.4
	*50 ng/mL	93.0 ± 4.7	100.2 ± 1.5	96.6 ± 8.9
	**50 ng/mL	92.5 ± 7.5	99.7 ± 5.5	97.2 ± 5.3
	100 ng/mL	95.3 ± 6.4	104.1 ± 6.7	97.3 ± 4.7
Ethyl parathion	25 ng/mL	93.8 ± 6.6	98.9 ± 9.4	101.2 ± 9.3
	*50 ng/mL	92.6 ± 1.8	96.3 ± 4.5	95.2 ± 3.6
	**50 ng/mL	91.8 ± 6.4	97.7 ± 4.2	98.4 ± 4.9
	100 ng/mL	91.5 ± 6.5	97.1 ± 5.9	100.6 ± 4.1
Penconazole	25 ng/mL	94.0 ± 7.0	101.4 ± 9.2	96.6 ± 6.9
	*50 ng/mL	93.1 ± 7.5	99.3 ± 6.3	94.9 ± 2.6
	**50 ng/mL	92.4 ± 5.8	98.6 ± 4.7	96.1 ± 7.2
	100 ng/mL	93.6 ± 8.9	102.9 ± 2.9	103.5 ± 6.8

*: intra-day precision test, **: inter-day precision test performed with 50 ng/mL standard solutions, n = 3 standard deviations.

CRediT authorship contribution statement

Ayşe Evrim Bulgurcuoğlu: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing - original draft. **Büşra Yılmaz Durak:** Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization. **Dotse Selali Chormey:** Data curation, Methodology, Validation, Writing - original draft, Writing - review & editing. **Sezgin Bakırdere:** Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing - review & editing.

Declaration of Competing Interest

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

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