

Micelle-based restricted access ion-pair microextraction of phosphate at trace levels in water samples for separation, preconcentration and determination

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Abstract

A new and simple micelles-rich restricted access supramolecular solvent-based liquid phase microextraction method (RA-SUPRASs-LPME) based on the ion-pair complex formation of phosphate (PO_4^{3-}) ions with ammonium heptamolybdate and malachite green in acidic medium was developed. The phosphate ion concentration after microextraction of the ion-pair complex to the hexagonal aggregates of decanoic acid (DA) was measured with micro-volume UV-Vis spectrophotometer at 625 nm. All analytical parameters which are effective on the method such as acid type and concentration, supramolecular solvent volume, amount of the components forming the complex, sample volume, were optimized. The preconcentration factor (PF), limit of detection (LOD) and limit of quantification (LOQ) for the developed method was found to be 15, 9.6 and 32.1, respectively. The RA-SUPRASs-LPME method was finally applied for the analysis of the phosphate content of different types of water samples.

Keywords: Microextraction, restricted access supramolecular solvent, UV-Vis spectrophotometer, phosphate, ammonium heptamolybdate, malachite green.

Introduction

While the importance of phosphate was low in the previous centuries, phosphate has been of great importance in the last century in response to the rapid increase in world population and the need to the phosphate-based productions (1-3). Around 85% of phosphate rock is used for fertilizer production in the world and the remaining 15% is used in detergent, feed, paper, alloy, matches, food, water treatment, defense industry and chemical industry. While around 15% of phosphate consumption in developed countries is used outside the fertilizer industry, this level is between 0 and 4% in less developed countries (1-3).

Accurate analysis of phosphate in water samples and providing quantitative information of pollution levels is vital. The different methods have been developed for the separation-enrichment and determination of phosphate in water samples (1-5). Pena-Pereira et al. developed a method based on the enrichment of trace levels of phosphate by suspended drop microextraction and determination by micro-volume UV-Vis spectrophotometer. The method based on the ion-pair formation between 12-molybdophosphate and malachite green was extracted with micro-drop methyl isobutyl ketone followed by spectrophotometric analysis. They have been applied this method for the determination of phosphate in different freshwater samples (3).

Zaruba et al. provided a dispersive liquid-liquid microextraction based on solidification of a floating organic drop (DLLME-SFO) for the separation and preconcentration of orthophosphate at trace levels. The method mainly includes three steps; (I) formation of molybdantimonatophosphoric heteropoly blue (HPB) complex of phosphate, (II) mi-

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croextraction of the HPB complex to 55 μL of undecane-1-ol and (III) spectrophotometric analysis of the HPB complex (6).

Ara et al. combined carrier mediated three phase solvent bar liquid phase microextraction (TPSB-LPME) method with high performance liquid chromatography (HPLC) for simultaneous separation, preconcentration and analysis of dexamethasone sodium phosphate (DSP) at trace levels in human urine, plasma and bovine milk samples. In this method, dexamethasone sodium phosphate in acidic aqueous medium was firstly extracted into octane-1-ol phase containing 5 % (w/v) of Aliquat-336 as carrier located in the pores of a hollow fiber. Procedure was completed with the back extraction of the dexamethasone sodium phosphate into an alkali receiving phase (pH=10, 5 μL of 0.65 M NaClO_4) located inside the lumen of the fiber. Analyses were carried out by injection of receiving phase into HPLC (7).

Divrikli et al. developed a solid phase extraction method for the separation and preconcentration of phosphate prior to its UV-Vis spectrophotometric determination. The method based on the formation of phosphomolybdate and its reduction to molybdenum blue. The phosphate complex retained on the Amberlite XAD-4 adsorbent was eluted with 10 mL acetone and analyzed with UV-Vis spectrophotometer at 732 nm (8).

Restricted access materials (RAMs) have found a wide range of applications for 20 years. They have mainly used as sorbents for the separation and preconcentration of organic, inorganic and bio-active species without interference from matrix components that are excluded by chemical, physical or physicochemical means (9, 10). The use of RAMs before detection systems eliminate sample pretreatment process have multiple steps (e.g. centrifugation, precipitation, residue dissolution, solvent evaporation, etc.) and tendency to increased sample output (11-13). The latest improvements in this area are related to design of more selective material and the development of simpler synthetic procedures of RAMs. Latest, SUPRASs have been shown to act as restricted access liquids. SUPRASs are classified as nanostructured liquids obtained from colloidal amphiphiles via spontaneous self-assembly and coacervation processes (14, 15). High density of binding sites they present to analytes and the different polar-regions within the nanostructures derived from the amphiphilic character of SUPRASs molecules that make them excellent extractors for analytes in a wide range of polarities (16-18).

In the presented work, the simple and cheap application capability of the phosphomolybdate method for the UV-Vis spectrophotometric phosphate was combined with restricted access features of micelles-rich alkyl carboxylic acid-based SUPRASs for the separation, preconcentration and analysis of phosphate traces in water samples.

Material and Method

Experimental instrumentation

Measurements were made with UV-Vis spectrophotometer (Hitachi, USA) with micro quartz cuvettes. Sartorius PT-10

pH meter (Germany) with glass electrode was used for pH adjustment of the solutions. To provide the supramolecular solvent, Sonorex brand (Germany) DT-255 model ultrasonic water bath was utilized. Centrifuge (Hettich Rotofix 32 A, Buckinghamshire, England) was used to separate the cloudy solution formed at the microextraction stages. Deionised water had 18.2 $\text{M}\Omega\text{ cm}$ resistivity was used during the experimental studies. Milipore MiliQ-Direct 18 model pure water device was used in obtaining deionised water.

Reagents and solutions

Sodium dihydrogen phosphate (NaH_2PO_4) salt obtained from Merck (Darmstadt, Germany) was used in the preparation of the stock solution containing 10^{-3} M phosphate anion. This solution was prepared by dissolving the appropriate amount of NaH_2PO_4 salt in purified water. Decanoic acid was obtained from Sigma-Aldrich. Ion pair formation between ammonium heptamolybdate tetrahydrate and malachite green was carried out by using same method given in literature (19) as follows; 8.6 g of ammonium heptamolybdate tetrahydrate (Merck, Darmstadt, Germany) were taken up in a beaker and dissolved in deionised water (solution-I). Then, 8.6 mL of 98 % H_2SO_4 solution (Merck, Darmstadt, Germany) with a density of $1.83\text{ g}\cdot\text{mL}^{-1}$, 23 mg malachite green and 2.0 g of tartaric acid were added in the solution-I and last volume was completed to 100 mL with deionised water. The resulting mixture was dissolved in an ultrasonic bath and filtered through blue band filter paper. This solution named as auxiliary reagent was used in experimental studies.

Preparation of the micelles-rich restricted access supramolecular solvent

Preparation of the restricted access supramolecular solvent was carried out as follows: 2.5 g of decanoic acid, 0.5 mL of THF and 10 mL of deionised water were placed in a 50 mL centrifuge tube, the resulting mixture was left in an ultrasonic bath for 5 min. In the meantime, nano and micro size cloudy micelles with restricted access properties were obtained. After centrifugation, the micelles-rich restricted access supramolecular solvent phase on the upper side and the water phase on the lower side were obtained. The micelles-rich restricted access supramolecular solvent phase was separated from the aqueous phase by a pipette and taken into another tube.

RA-SUPRASs-LPME method

The developed RA-SUPRASs-LPME method is shown schematically on Figure 1. In this method, 600 μL of 4.5 M H_2SO_4 and 1.0 mL of the auxiliary reagent forming the previously prepared were added to 15 mL of model solution medium (standard solution) containing known concentration of phosphate ions. 5 minutes were allowed in order to allow completing the complex formation. Then, 200 μL of the micelles-rich restricted access supramolecular solvent was added into this solution and the resulting mixture was sonicated 5 min. Formation of a cloudy solution was observed which indicated formation of the nano and micro-sized extraction droplets (17). This solution was centrifuged

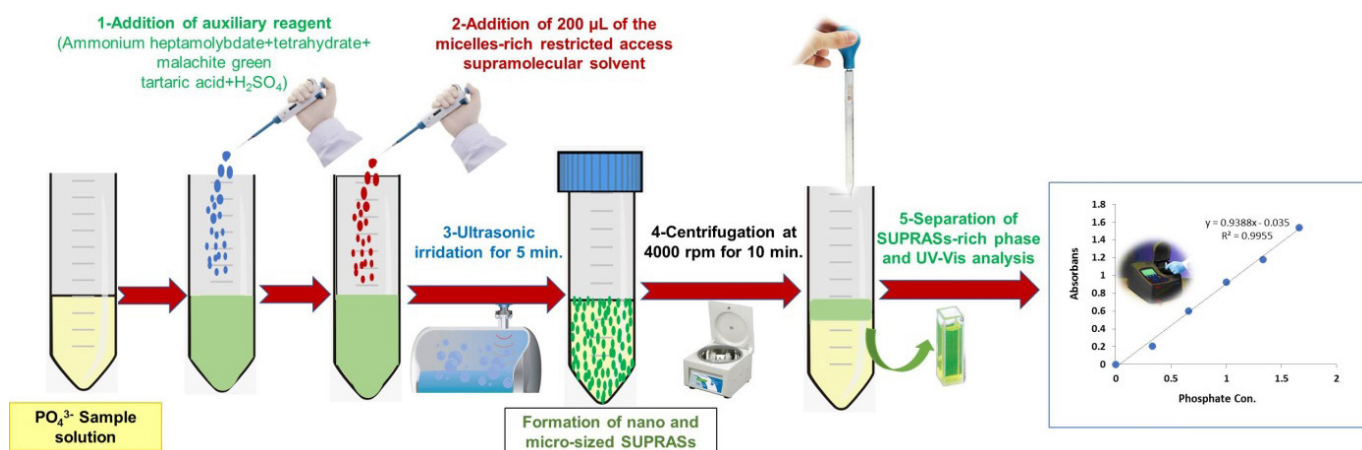


Figure 1. Graphical presentation of the presented RA-SUPRASs-LPME method.

at 4000 rpm for 10 min. The upper extraction solvent phase was transferred to another centrifuge tube by using a micropipette and the volume was made up to 1.0 mL with ethanol. The final phosphate concentration was measured by UV-Vis spectrophotometer at 625 nm. The same procedures were carried out in blank and standard solutions to obtain calibration curve.

Application to environmental water samples

In order to evaluate the applicability of the developed RA-SUPRASs-LPME method to environmental water samples, lake water in biological wastewater treatment plant exit area (Kayseri city, Turkey), natural spring water from village (Kayseri city, Turkey) and mountain-wrapped lake water sample (Kayseri city, Turkey) were collected by glass bottles and stored at 4 °C. Water samples collected were filtered through a PTFE syringe filter (0.45 µm, Osmonics) and subjected to the RA-SUPRASs-LPME method.

Results

Effect of the concentration of H₂SO₄

Ion-pair complex formation of phosphate ions with ammonium heptamolybdate and malachite green occurs in acidic medium

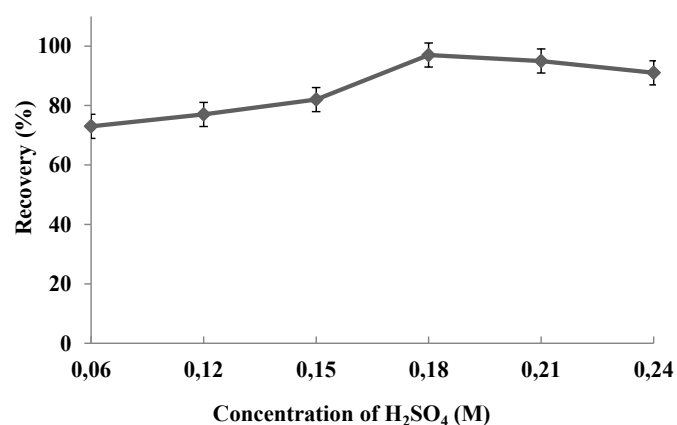


Figure 2. The effect of the concentration of H₂SO₄ on the recovery of phosphate ions in the presented study (N=3).

solution. In our method, H₂SO₄ was used to form acidic sample medium. Hence, the influences of the concentration of H₂SO₄ in the range of 0.06-0.24 M on the extraction efficiencies of the presented RA-SUPRASs-LPME system were investigated. The results are depicted on Figure 2. The quantitative recoveries for phosphate ions were obtained in the concentration of H₂SO₄ in the range of 0.18-0.21 M. Therefore, 0.18 M H₂SO₄ was selected as optimal value for the further step of the presented study.

Effect of the volume of ion pair forming auxiliary solution

Molybdophosphate complex formation related with the phosphate ion concentration and ion pair forming auxiliary solution concentration. If the concentration of ion pair forming auxiliary solution is lower than the minimum concentration that can form complex with phosphate ion, free phosphate ions will be present in the medium and quantitative extraction efficiency will not be achieved. To develop an accurate analytical method for the analysis of phosphate ions, it is necessary to find the optimum concentration of ion pair forming auxiliary solution. The effects of the volume of ion pair forming auxiliary solution including ammonium heptamolybdate and malachite green which prepared according to literature (19)

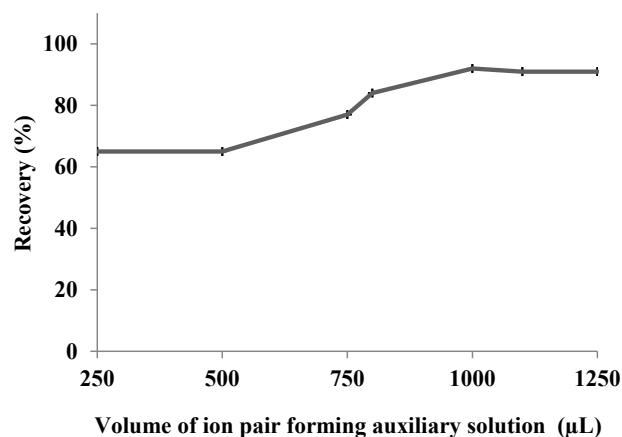


Figure 3. The effect of the volume of ion pair forming auxiliary solution on the recovery of phosphate ions (N=3).

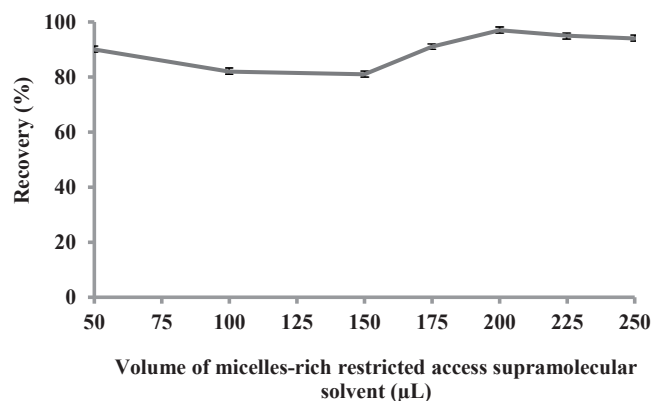


Figure 4. The effect of amount of decanoic acid on the recoveries of phosphate ions in the presented microextraction study (N=3).

were investigated in the volume range of 250-1250 µL. The results are given on **Figure 3**. The quantitative recoveries of phosphate ions were obtained by addition of ion pair forming auxiliary solution in the volume range of 1000-1250 µL. Hence, 1250 µL was selected as an optimal value and was used for further studies.

Effect of the amount of decanoic acid

Since the main component of the micelles-rich restricted access supramolecular solvent phase is decanoic acid, the increase in the amount of decanoic acid leads to an increase in the nano and/or micro-sized micelles and hence the extraction efficiency. The influences of amounts of decanoic acid on the formation of the micelles-rich restricted access supramolecular solvent phase and the extraction efficiencies of phosphate ions in the presented microextraction system were investigated in the range of 50-250 mg. The results are depicted on **Figure 4**. The quantitative recovery values for phosphate ions were obtained with the addition of decanoic acid in the range of 175-225 mg. For the further studies, 200 mg of decanoic acid was selected as optimal value.

Effect of the volume of tetrahydrofuran

Tetrahydrofuran is one of the most commonly used solvents in the formation of supramolecular solvent systems, since it facilitates the formation of micelles and nanoscale micelles and consequently increases extraction yields (17). Hence we used THF to obtain micelles-rich restricted access supramolecular solvent phase. The effect of THF volume on recovery efficiencies was also investigated. The developed method was applied to the model solutions prepared for this purpose. The results are given on **Figure 5**. The quantitative recovery efficiency was obtained when 250 µL of THF was used. 250 µL of THF was determined as the optimum working value and the experiments were performed on this value in the following studies.

Effect of the sample volume

The application of the method to the highest possible sample volume is an important and critical factor in the separation

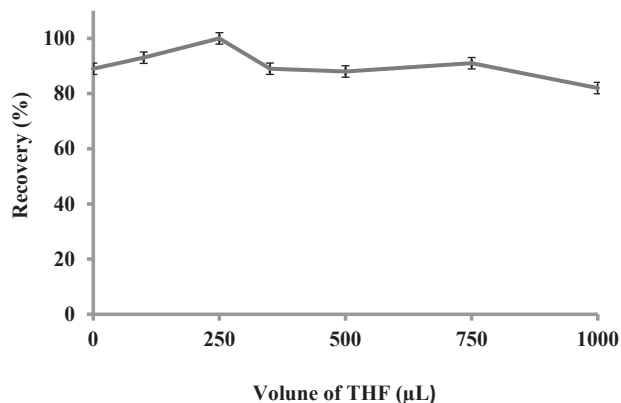


Figure 5. The effect of tetrahydrofuran volume on the recoveries of phosphate ions in the presented microextraction study (N=3).

and preconcentration studies, in enabling the determination of the lowest possible analyte concentration and obtaining a high enrichment factor (20-24). Therefore, the developed RA-SUPRASs-LPME method was applied to the model solutions ranging from 7.5-20 mL. Quantitative recovery efficiencies were obtained up to 15 mL sample volume for phosphate ions. Hence 15 mL of the samples studied was subjected to the developed RA-SUPRASs-LPME method.

Effect of extraction time

In order to form nano or micro-sized extraction droplets, RA-SUPRASs-LPME experiments were carried out in an ultrasonic bath. Model solutions prepared were subjected to ultrasonic irradiation between 1 and 10 min. Quantitative extraction efficiency was obtained with effective cloudy phases occurring at periods of 5 min and above exposure to ultrasonic vibration. Therefore, for next experimental stages, samples were subjected to ultrasonic irradiation for 5 min.

Effect of the foreign ions

The effects of matrix components of the real samples are an important problem in the instrumental detection of analytes (25-32). The effect of some alkaline earth, alkali metals and some anions which could have a matrix effect on the RA-SUPRASs-LPME method was also investigated under optimum conditions. These ions were studied because they are the most common ions found in environmental water samples. The results are given in **Table 1**. It is determined that the matrix ions do not have a disruptive effect on the recovery values of phosphate at the studied levels in **Table 1**. At the matrix ion concentrations above these levels, the recovery values of phosphate are not quantitative. Hence, real samples for the applicability of the RA-SUPRASs-LPME method were selected by considering these results.

Discussions

Phosphate is mainly released to the water environment by industry, municipal resources and over-fertilization. Excessive discharge of phosphate into the nature increases the effect of

Table 1. The effect of alkali, alkaline earth metals and some selected anions on the RA-SUPRASs-LPME method (N =3)

Ion	Added Salt	Studied concentration, mg·L ⁻¹	Recovery, %
Na ⁺	NaNO ₃	2500	102±0
K ⁺	KCl	1000	97±1
Mg ²⁺	Mg(NO ₃) ₂ ·6H ₂ O	250	98±3
Ca ²⁺	Ca(NO ₃) ₂ ·4H ₂ O	500	100±2
Cl ⁻	KCl	1000	97±1
SO ₄ ²⁻	Na ₂ SO ₄	1000	102±0

eutrophication in surface waters due to its biochemical processes that increase plant growth leading to increased concerns. In the presented work, we propose a new methodology including separation and preconcentration of phosphate by using our RA-SUPRASs-LPME method for the spectrophotometric determination of phosphate from natural water samples.

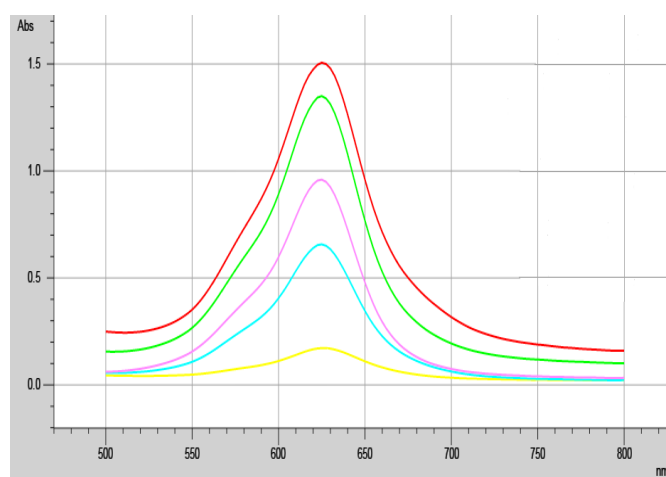
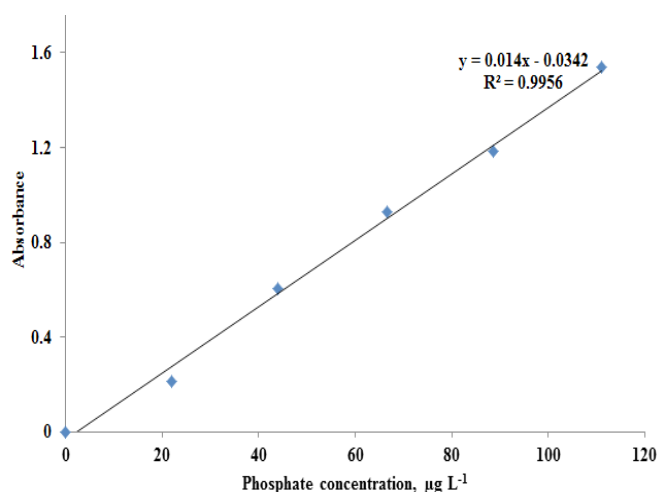
The limit of detection (LOD) was obtained by dividing 3 times the standard deviation (3SD) of the UV-Vis absorbance values obtained from the analysis of seven replicates blank samples to the slope (m) of the calibration curve, while the limit of quantification (LOQ) was obtained by dividing 10 times the standard deviation (10SD) of the UV-Vis absorbance values obtained from the analysis of seven replicates blank samples to the slope (m) of the calibration curve.

The preconcentration factor (PF) was calculated by dividing the highest sample volume by which the method can be applied to the final volume. The reproducibility was investigated on the addition-recovery experiments for the environmental water samples under the optimized conditions and calculated as relative standard deviation (RSD %). LOD, LOQ, and PF values were found as 9.6 µg·L⁻¹, 32.1 µg·L⁻¹, and 15 respectively. The relative standard deviations (RSDs %) were found in range of 0.44 and 3.5 %. The calibration curve with spectrum belong to

increasing phosphate concentration shown on **Figure 6** was obtained under optimized conditions. Coefficient of correlation (R^2) was found as 0.9955.

The applicability of the RA-SUPRASs-LPME method to real samples was tested by determining the phosphate content of various environmental water samples. The developed RA-SUPRASs-LPME method was applied to lake water (lake water-I) in biological wastewater treatment plant exit area, village natural spring water and another mountain-wrapped lake water sample (lake water-II). For this purpose, known concentrations of phosphate ion were added to these environmental water samples, developed method was applied and recovery studies were performed. The results are shown in **Table 2**. When the results are examined, it is seen that recovery results varying between 94% and 102% are obtained. The results show that our method can be applied successfully for selected water samples. In this way, the accuracy of our method has been proved for the studied environmental water samples.

We compared our RA-SUPRASs-LPME method with other methods in the literature in **Table 3**. Although the preconcentration factor and the limit of detection of our method is low, the most important advantages are that high repeatability and the extraction time is generally shorter than other methods so that the extraction equilibrium can be reached in a very short

**Figure 6.** The calibration curve (A) and UV-Vis spectrum belong to increasing phosphate concentration (B).

time due to formation of the very effective nano or micro-sized micelles-rich restricted access supramolecular solvent drops. Also, our method has many advantages in term of low consumption of SUPRASs, high extraction efficiency, short extraction and analysis times, no necessity expensive and complex laboratory equipments in the extraction and analysis stages simplicity, good precision and eco-friendly.

Conclusions

In this paper, a micelles-rich restricted access supramolecular solvent based microextraction method (RA-SUPRASs-LPME) was used for the separation and preconcentration of the phosphate in environmental water samples prior to micro-volume UV-Vis spectrophotometric analysis. Due to some advantages like high extraction efficiency, short extraction and analysis

Table 2. Application of the RA-SUPRASs-LPME method for determination of phosphate in real water samples (N=3).

Sample	Added, $\mu\text{g}\cdot\text{L}^{-1}$	Found, $\mu\text{g}\cdot\text{L}^{-1}$	Recovery, %
Lake water-I	0	264 \pm 2 ^a	-
	66	334 \pm 8	101
	132	402 \pm 14	102
Spring water	0	260 \pm 4	-
	100	358 \pm 2	99
	200	450 \pm 2	98
Lake water-II	0	276 \pm 4	-
	66	326 \pm 2	95
	132	432 \pm 2	94

a: mean \pm SD.

Table 3. Comparison of the RA-SUPRASs-LPME method with other phosphate determination methods in literature studies

Extraction method	Analysis instrument	Samples	LOD, $\mu\text{g L}^{-1}$	RSD, %	PF or EF	Extraction time, min	Ref
DSDME	UV-Vis spectrophotometer	Mineral waters	0.19	2.7	325	7.5	3
-	Online UV-Vis spectrophotometer	Natural waters	1.3	0.8-5.6	-	-	5
DLLME-SFO	UV-Vis spectrophotometer	Tap and river waters	0.7	1.23-6.15	50	10	6
SPE	UV-Vis spectrophotometer	Fruit leaves and natural waters	2.23	2	-	30	8
CPE	UV-Vis spectrophotometer	Tap, well and mineral waters	0.5	1.4	20	20	31
SPE	UV-Vis spectrophotometer	-	0.04	0.38	147.9	60	35
On-line SPE	flow-injection amperometry	Fresh and Marine waters	0.18	5.2-5.9	18	-	36
RA-SUPRASs-LPME	UV-Vis spectrophotometer	Lake and spring water	9.62	0.44-3.5	15	10	This study

DSDME: Directly suspended droplet microextraction, DLLME-SFO: Dispersive liquid-liquid microextraction based on solidification of a floating organic drop, SPE: Solid phase extraction, CPE: Cloud point extraction.

times, green methodology, our method can be used for the routine phosphate analysis in different laboratories.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

Ethical Compliance

This article does not contain any studies involving human participants or animals performed by any of the authors.

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