Application of ionic liquid-based microwave-assisted extraction of malachite green and crystal violet from water samples

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Abstract

A simple, environmentally friendly and efficient technique, ionic liquid-based microwave-assisted extraction was first used to determine malachite green and crystal violet from water samples coupled to high-performance liquid chromatography. The key parameters influencing extraction efficiency were investigated, such as the type of ionic liquids, the volume of ionic liquid, extraction time, and so on. Under the optimum conditions, good reproducibility of the extraction performance was obtained (relative standard deviation, 1.0% for malachite green and 5.9% for crystal violet, n=5). Good linearity (0.10-25 µg L^{-1}) was observed with correlation coefficients between 0.9991 and 0.9964. The detection limits of malachite green and crystal violet were 0.080 and 0.030 µg L^{-1}, respectively. The proposed method had been successfully applied to determine malachite green and crystal violet in real water samples with recoveries ranging from 95.4 to 102.8%. Compared with the previous technologies, the proposed method required less extraction time (2 min), and provided lower detection limits and

higher enrichment factors. Moreover, there was no volatile and hazardous organic solvents released. Based on these simple, environmentally friendly, rapid and highly efficient results, the proposed approach provides a new and promising alternative for simultaneously extracting trace amounts of MG and CV from water samples.

1 Introduction

The triphenylmethane dyes, malachite green (MG) and crystal violet (CV) have been widely used by aquaculture farmers around the world in fungicides, parasiticides and antiseptics to treat and prevent fungal diseases and parasitic infections in fish since the 1930s [1, 2]. However, the use of MG and CV in aquaculture has recently raised serious concerns because it has been reported that MG and CV may cause human carcinogenesis, mutagenesis and other toxic effects [3]. Consequently, both MG and CV are on the Food and Drug Administration's (FDA's) priority list for fish drugs [4]. And the use of these dyes is also controlled by guidelines laid down in each of the individual member state within the European Union. Though the use of MG and GV has been banned in several countries, the hazardous compounds are still being used in many parts of the world due to their low cost and high effectiveness [5]. Therefore, in response to concerns regarding the health risks associated with the use of MG and CV, it is very important to develop sensitive methods for their detection in water samples.

Up to now, various methods were proposed to detect MG and CV in water samples, such as solid-phase extraction (SPE) with LC/MS/MS measurement [6], magnetic solid phase extraction (MSPE) by spectrophotometric analysis [3], maghemite nanoparticles preconcentration followed by spectrophotometric titration, capillary electrophoresis-Raman spectroscopy (CE-RS) [7], and ELISA [8]. However, most of these methods cannot be used widely for determining both chemicals. Drawbacks include the fact that they are time-consuming, labor-intensive, require large volumes of water samples to pre-concentrate and complicated pretreatment procedure. In contrast, dispersive liquid-liquid microextraction (DLLME) is a popular method to extract the triphenylmethane dyes [9-11] because of its easy handling, miniaturized sample preparation and cost-effectiveness. Nevertheless, this technology still needs volatile and hazardous organic compounds as solvents or co-solvents, which are harmful to human health and the environment. In addition, DLLME is time-consuming [9]. Therefore, it is very essential to develop simple, fast, environmentally friendly and sensitive methods to simultaneously determine MG and CV in water samples.

Recently, microwave-assisted extraction (MAE) has been accepted as a potential and powerful alternative to conventional extraction techniques. The advantages of MAE include higher extraction efficiency, faster extraction processes, lower solvent and power consumption. MAE has also become a routine extraction technique for clinical, food and environmental product analysis [12-15], especially in the area of organic analysis [16]. However, MAE's only deficiency is its use of volatile organic

solvents which are detrimental to the environment and human health [17]. Sample extraction technologies are under pressure to eliminate or minimize the use of toxic organic solvents, which means MAE must find an efficient and environmentally friendly solvent.

In recent years, ionic liquids (ILs) have served as promising alternatives to the traditional organic solvents employed in sample preparation, owing to their excellent properties: negligible vapor pressure, good thermal stability, wide liquid range, tunable viscosity, and miscibility with water and organic solvents as well as good solubility and extractability for various organic compounds. Over the past few years, ionic liquids have been used in various applications, such as solid-liquid extraction [18], solid-phase microextraction [19, 20], liquid-liquid extraction [21], liquid-phase microextraction [22, 23], and aqueous two-phase systems extraction [24, 25]. Moreover, because ILs are a combination of organic cations and various anions ionic liquids can efficiently absorb and transfer microwave energy [26], and thus are used as solvents and co-solvents for MAE. Ionic liquid-based microwave-assisted extraction (IL-MAE) has been reported to successfully extract various environmental pollutants from matrix [27-30]. However, to the best of our knowledge, there are no reports on ionic liquid-based microwave-assisted extraction of MG and CV.

In the present study, a rapid, effective, environmentally friendly and feasible IL-MAE followed by high performance liquid chromatography (HPLC) is proposed to detect MG and CV in water samples. Various influential parameters of microwave-assisted extraction were optimized systematically. The optimized method

was compared with the conventional extraction approaches. The proposed method was then successfully applied to determine MG and CV in water samples.

2 Experimental

2.1 Reagents and materials

Individual standards of malachite green and crystal violet were purchased from Shanghai Dyestuff Chemical Sales Co., Ltd. (Shanghai, China). Standard stock solutions (100 g L^{-1}) were prepared in HPLC-grade acetonitrile and stored at 4 °C. Working solutions (100 ng L^{-1}) were obtained by step-by step daily dilution of the stock solutions with acetonitrile. 1-butyl-3-methylimidazolium hexafluorophosphate ([C$_4$MIM][PF$_6$]), 1-hexyl-3-methylimidazolium hexafluorophosphate ([C$_6$MIM][PF$_6$]) and 1-octyl-3-methylimidazolium hexafluorophosphate ([C$_8$MIM][PF$_6$]) with purity of 99% were purchased from Lanzhou Greenchem ILS, LICP, CAS (Lanzhou, China), Shanghai. HPLC-grade methanol and acetonitrile were purchased from J. T. Baker (Phillipsburg, NJ, USA). All other chemicals were purchased from Beijing Chemicals Co. Ltd (Beijing, China) with analytical grade or better. Ultrapure water was prepared by a Milli-Q SP reagent water system (Millipore, Milford, MA, USA) throughout the experiments.

2.2 Apparatus

A domestic microwave-assisted extraction system (Midea, Shunde, China) with a 2450 MHz magnetron was used. It was modified in our laboratory with the addition of a

water condenser. The whole system was run at atmospheric pressure and could be

employed at the maximum power of 700 W. The pH-meter (Mettler Toledo,

Switzerland) with combined glass electrode and a universal pH indicator (pH 0-14) was

used for the pH measurement. A centrifuge model 3k15 (Sigma, Germany) was used

for the phase separation.

2.3 IL-MAE procedure

Briefly, 20 mL of work solution containing 100 ng L^{-1} of target compounds and

5.0% (w/v) sodium chloride were extracted by IL (500 µL)-MAE. The key parameters

were systematically optimized, including the type of ILs, the volume of IL, extraction

time, microwave power, solution pH and ionic strength. After IL-MAE, the extracts

cooled with ice water to room temperature. Through centrifugation at 3000 rpm, the

upper aqueous phase was removed with a syringe. 150 µL of the residue was diluted to

1 mL by the buffer to decrease viscosity and 20 µL was injected into the HPLC system

for analysis.

2.4 HPLC analysis

An Agilent 1200 LC system consisting of a quaternary pump and a UV/vis

detector set at 618 and 588 nm was used for analysis of MG and CV, respectively. The

analytes were separated on an Agilent Zorbax Eclipse plus C18 column (250 mm×4.6

mm I.D, 5 mm particles) with a mixture of ammonium acetate buffer (0.01 mol L^{-1}, pH

4.5) using an acetonitrile/water mobile phase (20/80, v/v) at a flow rate of 1.0 mL min^{-1}

, and 20 µL injection volume. The chromatographic peaks of the analytes were confirmed by comparing their retention time with the reference standard.

2.5 Determination of enhancement factors

The enhancement factor (EF) was evaluated as follows [9]:

$$EF = \frac{C_{after}}{C_{before}}$$

where C_{before} was calculated from the calibration curve by directly injecting standard solutions into the HPLC system, and C_{after} was determined by the proposed procedure under the optimal conditions and calculated from the standard curve.

2.6 Collection of the real samples

Four water samples were selected for validating the proposed method including pond water, lake water, river water and sewage treatment plant (STP) effluent. The pond water was collected from fish farm water. The lake water was collected from Taihu Lake. The river water was collected from Changjiang River. The STP effluent was collected from Xianlin sewage treatment plant ((Nanjing, China). The collected water samples were filtered though 0.45 µm micro-pore membranes and then stored at 4 ∘C in the dark before analysis.

3 Results and Discussion

3.1 Optimization of IL-MAE conditions

In order to obtain better extraction efficiency, the key parameters that could affect the extraction performance were optimized, including the type of ILs, IL volume, extraction time, microwave power, pH, ionic strength and centrifuging time. Each measurement was replicated three times.

3.1.1 Selection of ILs

The extraction efficiencies of the ILs were investigated and the results are shown in Fig. 1. It can be seen that the extraction capacity of [C_8MIM][PF_6] to MG and CV was best among the ILs studied, which may be due to its relatively lower solubility in water (0.2 g/100 mL) than [C_6MIM][PF_6] (0.75 g/100 mL) and [C_4MIM][PF_6] (1.88 g/100 mL). Consequently, [C_8MIM][PF_6] was used as solvent to extract the analyses.

Figure 1

3.1.2 Effect of IL volume

The volume of extraction solvent is a crucial factor. Large solvent volumes can lead to unnecessary waste and decrease the extraction concentration of the target compounds in solvent, while small volumes may lead to incomplete extraction. In investigating the influence of IL volumes, a series of IL volumes ranging from 150 to 1000 µL were studied. As shown in Fig. 2a, the peak areas increased with the increasing of IL volume up to 500 µL, and then decreased. Therefore, an extraction volume of 500 µL was selected in further extraction.

Figure 2

3.1.3 Effect of extraction time

Extraction time is one of the most important factors in most extraction processes because mass transfer of analytes between the two immiscible phases needs to overcome phase barrier energy. This transport process takes time to occur and to reach equilibrium [31]. As shown in Fig. 2b, when the irradiation time increased from 1 to 2 min, the extraction efficiency increased dramatically. But when the extraction time was beyond 2 min, the extraction yields remained almost unchanged. These results may suggest that the extraction of both compounds reached equilibrium in 2 min. Hence, 2 min was selected as the optimal extraction time.

3.1.4 Effect of microwave power

The microwave power had a significant effect on the extraction of compounds. As shown in Fig. 2c, the extraction yield of MG and CV obtained at different powers gradually increased as the microwave irradiation power increased from 120 to 700 W. It may be due to the fact that microwave irradiation energy can enhance the penetration of the solvent into the matrix and deliver materials efficiently through molecular interaction with the electromagnetic field and offer a rapid transfer of energy to the solvent and matrix, which allows the dissolved components to be extracted [32]. The maximum microwave irradiation power tested of 700 W was selected as the optimal power for the IL-MAE method of extraction.

3.1.5 Effect of solution pH

Solution pH is another essential factor to influence extraction efficiency particularly when acidic or basic solutes are extracted by changing the existing state of the analytes. As weak acid dyes (pK_a=6.9 for MG, 0.8 for CV), they would be well extracted at low pH, which could avoid more target compounds being ionized and dissolved in water [9]. As illustrated in Fig. 3a the peak areas increased in the range of pH 1.8–3.0 and decreased with pH values raised from 3.0 to 6.6. So, pH 3.0 was chosen as the optimal pH for this study.

Figure 3

3.1.6 Effect of ionic strength

Generally, NaCl was used to adjust the ionic strength of the solution. For many organic compounds, solubilities of the target analytes in water decrease with the increasing of ionic strength and their partitioning in the extraction phase is enhanced. However, when the salt concentration was too high, the ion exchange between PF_6^- in $BMIMPF_6$ and Cl^- in solution occurred, and the resulting BMIMCl was soluble in water, which could decrease of the amount of IL and cause a poor extraction performance [30]. At the same time, too high of a salt concentration would influence the phase separation [33-35]. As illustrated in Fig. 3b, the extraction efficiency increased with the increasing of NaCl concentration until the salt concentration reached 5.0% (w/v), and then decreased. Therefore, 5.0% (w/v) was selected as the suitable content of NaCl.

3.1.7 Effect of centrifuging time

Centrifugation controls the phase separation and is a crucial step in the proposed method. Although the turbidity phenomenon was easy to generate when cooling with ice water, it was difficult to separate $[C_8MIM][PF_6]$ from the aqueous solution without centrifugation. The effect of centrifugation time on the extraction efficiencies is investigated and illustrated in Fig. 3c. The extraction efficiencies of the compounds increased with centrifugation time from 1 to 5 min, and then decreased with any further increase of centrifugation time. This may be due to the fact that $[C_8MIM][PF_6]$ was not fully separated from water when the centrifuging time was too short, and longer centrifuging time resulted in heat generation leading to the dissolving of parts of the $[C_8MIM][PF_6]$ and the loss of sensitivity. Therefore, 5 min was chosen for the optimal centrifuging time.

3.2 Method evaluation

Important parameters such as linear range, precisions and detection limits were determined under the optimal conditions to evaluate the method performance. As illustrated in Table 1, for the studied analytes, good linearity with correlation coefficients (R^2, 0.9991 for MG and 0.9964 for CV) were achieved with satisfactory precisions (RSD, 1.0% for MG and 5.9% for CV, n=5). The detection limits (LODs) were determined using a signal noise ratio of 3 (S/N = 3) and were found to be 0.080 µg L^{-1} for MG and 0.030 µg L^{-1} for CV, which display the potential of the method for the

determination of low concentrations of MG and CV. The results show that the proposed

approach could be used as an effective pretreatment technique to enrich and separate

the target analytes from water.

Table 1

3.3 Comparison between IL-MAE and other extraction methods

Extraction and determination of MG and CV in water samples by the proposed

method were compared with those of other previous methods (Table 2). The proposed

method shows performed better than the other methods with reference to shorter

extraction time, lower RSDs and detection limits, and better enrichment factors. The

excellent extraction performance of IL-MAE to MG and CV may owe to the following

reasons:

(1)   Heating by microwaves can decrease the viscosity of ILs drastically and

increase the diffusion rate. Thus, the extraction time is shorter.

(2)   Solvents have higher capacity to extract analytes at higher temperature.

Thus, the heating by microwaves can also improve extraction efficiencies.

(3)   Microwave irritation cause ILs to completely disperse in the aqueous

phase, thereby increasing the chance for mass transfer of the target

compounds into the IL phase. This could be one reason for higher

extraction efficiencies.

(4) $[C_8MIM][PF_6]$ has excellent extraction capability for organic compounds.

This is another reason for higher extraction efficiencies.

In particular, there are almost no volatile and hazardous solvents released in this

approach, which shows that the proposed method is not only more environmentally

friendly than previous methods but also meets sample extraction technology

requirements for the elimination of toxic organic solvents.

3.4 Applications

In order to test the reliability of the proposed approach, this method was applied to

the simultaneous determination of MG and CV in different field samples. As presented

in Table 3, MG was detected in pond water and STP effluent, and CV was detected in

STP effluent. Good recoveries ranged from 95.4 to 102.8% which confirmed that the

IL-MAE method is able to determine the trace concentrations of MG and CV in real

samples coupled with HPLC.

Table 3

4 Concluding remarks

The presented method explored for the first time ionic liquid-based

microwave-assisted extraction as a high-performance and powerful preconcentration

method for the simultaneous extraction of trace amounts of MG and CV from water.

The proposed method required less extraction time (2 min) and provided lower

detection limits (0.080 µg L^{-1} for MG and 0.030 µg L^{-1} for CV, S/N = 3) and a higher

enrichment factor (291 for MG and 330 for CV) compared with previous extraction

technologies. In addition, the imidazolium-based IL, which is non-volatile, innocuous

[36] and potentially recyclable [37], was employed as an extraction solvent wherein almost no volatile and hazardous organic solvents were released meets the requirements for green and environmentally benign extraction and does not generate secondary pollution. Furthermore, it does not require special approaches making it very simple and easy to use.

Due to its good repeatability and precision (RSD, 1.0% for MG and 5.9% for CV, n=5), the green and effective IL-MAE method showed excellent results in the simultaneously analysis of trace amounts of MG and CV in real environmental water. In addition, it seems possible to extend this method to the extraction of MG and CV in other similar samples by varying the extraction conditions.

The authors have declared no conflict of interest.

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Figure captions

Figure 1. Effect of the type of ionic liquid. Experimental conditions: IL volume: 500

µL; extraction time: 2 min; microwave power: 700 W; pH: 3.0; ionic strength: 5.0%
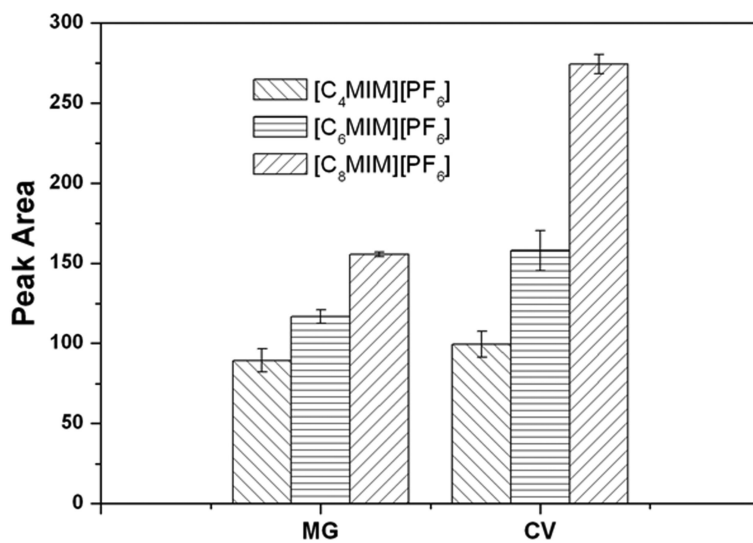
(w/v); centrifugation time: 5 min.

Figure 2. Effect of IL volume (a), extraction time (b) and microwave power (c) on the

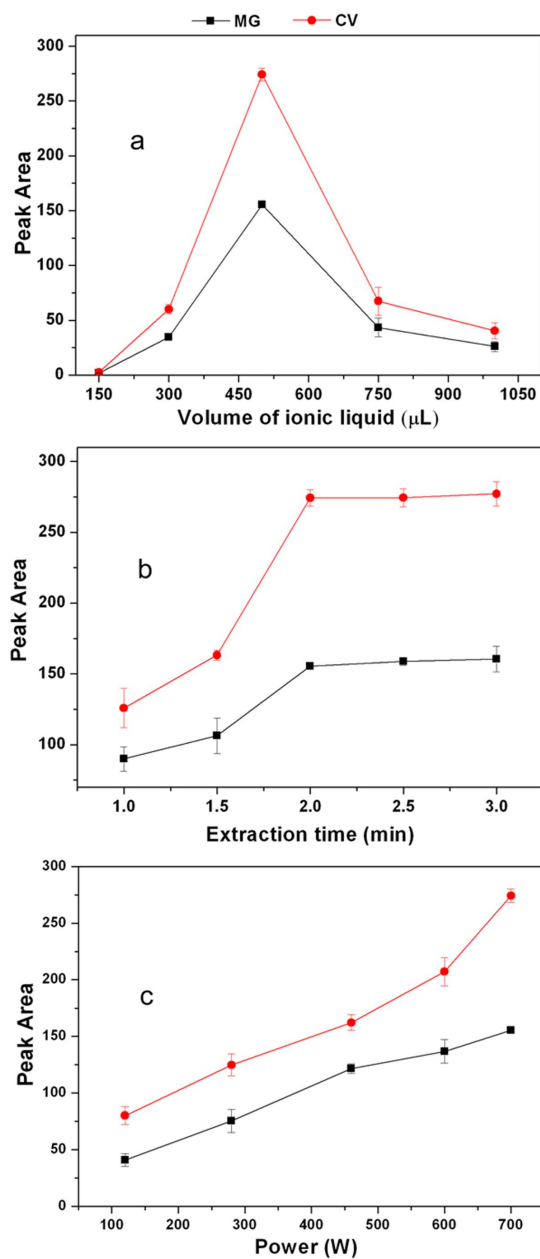extraction efficiency of target analytes.

Figure 3. Effect of pH (a), ionic strength (b) and centrifugation time (c) on the extraction efficiency of target analytes.
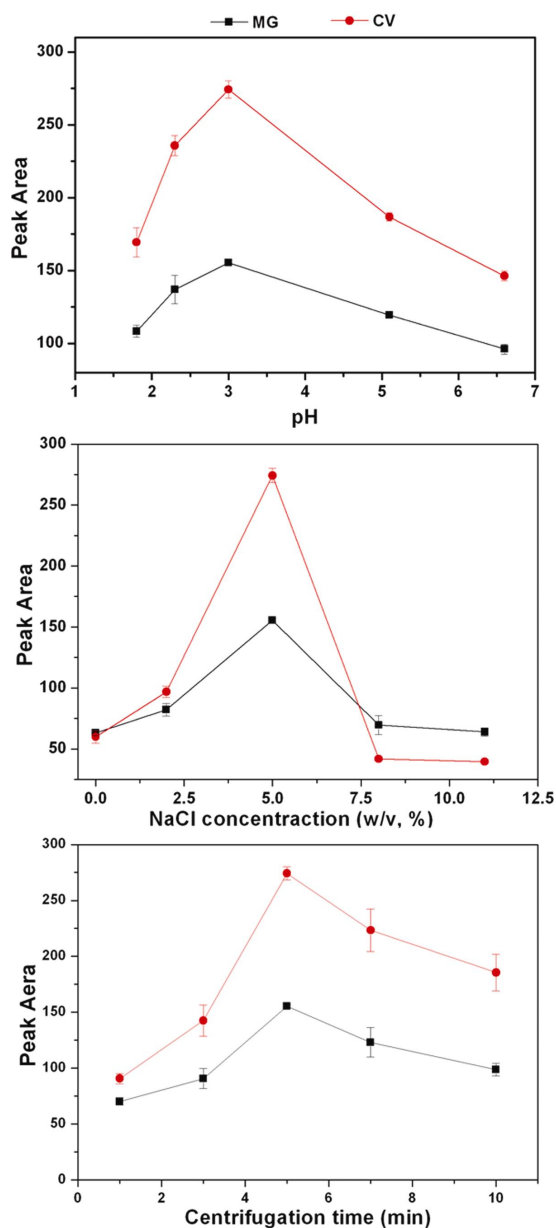
Table 1

Analytical parameters of MG and CV measured with IL-MAE method.

Analytes	Linear range ($\mu g\,L^{-1}$)	R^2	LOD ($\mu g\,L^{-1}$)	EF	RSD (n=5, %)
MG	0.15-25	0.9991	0.080	291	1.4
CV	0.10-22	0.9964	0.025	330	5.9

Table 2

Comparison of the proposed method with the previous studies.

Extraction method	Extraction time	LOD ($\mu g\ L^{-1}$)	Precision (RSDs, %)	EF	Ref
IL-DLLME	50 min	MG: 0.086 CV: 0.030	MG: 9.4 CV: 7.6	MG: 254 CV: 276	[9]
CPE	20 min	MG: 2.9 CV: 4.8	MG: 4.26 CV: 6.02	20	[5]
CPE	20 min	MG: 1.2	MG: 1.1	---	[38]
MSPE	260 min	0.5	4.2	---	[3]
IL-MAE	2 min	MG: 0.080 CV: 0.025	MG: 1.4 CV: 5.9	MG: 291 CV: 330	This study

Abbreviation: CPE, cloud point extraction; MSPE, magnetic solid phase extraction; IL-DLLME, ionic liquid dispersive liquid-liquid microextraction; IL-MAE: ionic liquid-based microwave assisted extraction; RSDs, relative standard deviations.

Table 3

Determination of MG and CV in the real samples by the proposed method.

Samples	Concentrations[a] ($\mu g\ L^{-1}$)		Recoveries[b] (%)		Recoveries[c] (%)	
	MG	CV	MG	CV	MG	CV
Pool water	0.173±0.012	ND	95.8±1.2	97.6±1.6	96.3±2.5	100.5±0.8
Lake water	ND[d]	ND	95.4±2.1	94.2±1.0	89.4±1.7	98.4±2.4
River water	ND	ND	101.2±0.8	98.4±1.3	96.7±0.9	102.8±1.9
STP effluent	0.143±0.006	0.317±0.015	98.2±2.0	100.8±1.8	99.4±3.0	98.5±1.1

a) Average of three determination ± standard deviation.

b) Spiked with 0.2 $\mu g\ L^{-1}$ of MG and CV.

c) Spiked with 0.5 $\mu g\ L^{-1}$ of MG and CV.

d) Not detected.