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Reversed-phase high-performance liquid chromatography of pyrrolidinedithiocarbamate complexes of mercuric species using amperometric and coulometric detection

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Abstract

A reversed-phase high-performance liquid chromatographic method has been developed for the determination of organic and inorganic mercury species as pyrrolidinedithiocarbamic complexes using electrochemical detection. These complexes were prepared off-line and separated using as mobile phase a mixture of methanol–water (75:25) containing KNO_3 as supporting electrolyte. The organomercury complexes were detected in both amperometric and coulometric mode, at +1.15 V and +0.90 V respectively. The detection limits ranged from 0.16 ng to 2.8 ng.

Keywords: Complexation; Environmental analysis; Water analysis; Pyrrolidinedithiocarbamates; Organomercury compounds; Mercury

1. Introduction

Elemental speciation is the differentiation and determination of the different physico-chemical forms of an element. This is of great importance and interest to environmental analytical chemists because elemental toxicity is inherently dependent on the chemical form. Metals that are innocuous in themselves or as inorganic forms often become toxic when bound to organic groups. Mercury and organomercurials are a good example. The alkyl derivatives are especially toxic and their toxicity depends on their structure symmetry. The alkyl and aryl mercury have high germicide activity while the symmetric organomercurials are inactive, whereas methylmercury (MeHg) compounds are more toxic than their mercury(II) analogues [1,2]. The rate of synthesis of organomercury species in a water sample depends on concentration of available Hg(II), composition of the microbial population, pH, temperature, redox potential and effect of other metabolic or chemical processes.

Because the difference in toxicity between inorganic and organic species of mercury and the effects of mercury on the environment are related not only to its concentration but also to its chemical form, analytical techniques are needed to separate, identify and determine the organomercury compounds. Several workers have described methods for the determination of dissolved mercury in natural waters. These methods are based on wet digestion of the sample followed by reduction of mercury and detection by the cold vapour atomic absorption technique to determine total mercury, and though in

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some cases the reducing agent used reduces only inorganic mercury under typical conditions, these methods do not allow us to distinguish between different mercury species [3–6]. Electroanalytical determination of mercury and organomercury compounds has been described by some workers using different electrodes such as gold [7], gold plated [8] or mercury electrodes [9,10]. In the last while there has been a growing interest in the use of modified electrodes [11].

The most common analytical techniques used for determination of the amount of organomercurials include some chromatographic techniques such as gas chromatography with electron-capture detection that usually involves the conversion of organomercury species following by multiple extractions with organic solvents [1,12,13]. Flow injection analysis including on-line preconcentration and gas chromatography have been also described [14] and a capillary electrophoresis method has recently been published [15]. Typical methods for organomercury speciation and analysis include high-performance liquid chromatography (HPLC) separation with no electrochemical detection methods [16–23] and HPLC methods followed by electrochemical detection using, e.g., neutral 2-mercaptoethanol complexes [24–28].

The purpose of the present work is to determine the optimum conditions for the electrochemical detection using carbon electrodes coupled to a reversed-phase high-performance liquid chromatographic system. The separation occurs through pyrrolidinedithiocarbamate complexes of inorganic mercury and the organomercurials MeHg, ethylmercury (EtHg) and phenylmercury (PhHg). The method was applied to the determination of organomercury compounds in Jarama river water.

2. Experimental

2.1. Reagents

All reagents were of analytical reagent grade obtained from commercial sources. The standard inorganic and organic mercury solutions were obtained from the chloride salts (Chem. Service, USA). They were stored cool at 4°C as 1000 mg/l in ultrapure water solutions of Hg^{2+}, $MeHg^{+}$, $EtHg^{+}$ and $PhHg^{+}$ and prepared weekly. The standard injected solutions were prepared every day in the mobile phase. Sodium pyrrolidinedithiocarbamate (PDTC, Fluka) at 0.01 *M* water solution was prepared once a week and stored cool. The HPLC solvents were of HPLC grade (Carlo Erba, Italy).

2.2. Apparatus

The HPLC system consisted of a Gilson Model 302C pumping system, equipped with a membrane damper, a Rheodyne Model 7125 injector equipped with a 20 μl loop, and a Spectra Physics SP4290 integrator. As detector was used a Metrohm Model 461-VA amperometric detector equipped with a Metrohm Model 656 amperometric flow cell, of less than 1 μl volume, or an ESA Model 5010 coulometric cell of 2 μl volume. A glassy carbon electrode (Metrohm 6.0805.010) or a carbon paste electrode (Metrohm, 6.0807.000), of 7 mm^2 geometrical surface area, were used as working electrode in amperometric detection.

2.3. Chromatographic conditions

The column was a 150×4 mm pre-packed reversed-phase column containing 5 μm Spherisorb C_{18} particles (Tracer, Spain). The mobile phase was methanol–water (75:25, v/v) containing 0.05 *M* KNO_3, as supporting electrolyte, in amperometric detection and 0.02 *M* in coulometric detection. The detector used was operated at +1.15 V versus an Ag–AgCl reference electrode in amperometric mode and at +0.90 V versus a Pd solid-state reference electrode in the coulometric one. Both column and capillary tubes were of polyether ether ketone to prevent interfering reactions of equipment parts with the complexation reagent.

2.4. Electrode pre-treatment procedure

Before each set of measurements (ca. twenty measurements throughout the day) the glassy carbon surface electrode was polished with filter paper and rinsed with ultrapure water before installation in the electrochemical detector. However, the surface of the coulometric glassy carbon electrode was cleaned

daily by flushing the cell with 5 ml of 6 *M* nitric acid and then with water. After treatment the amperometric electrode or coulometric cell were attached to the system. Then the detector system was connected and the working potential was applied up to obtain a low and stable baseline after injection of samples.

2.5. Procedure for sample extraction

A 100 ml water sample containing 1 m*M* PDTC and 0.01 *M* KNO_3 were left in darkness for 30 min to complete the complexation reaction between PDTC and the mercuric compounds. Then the sam-

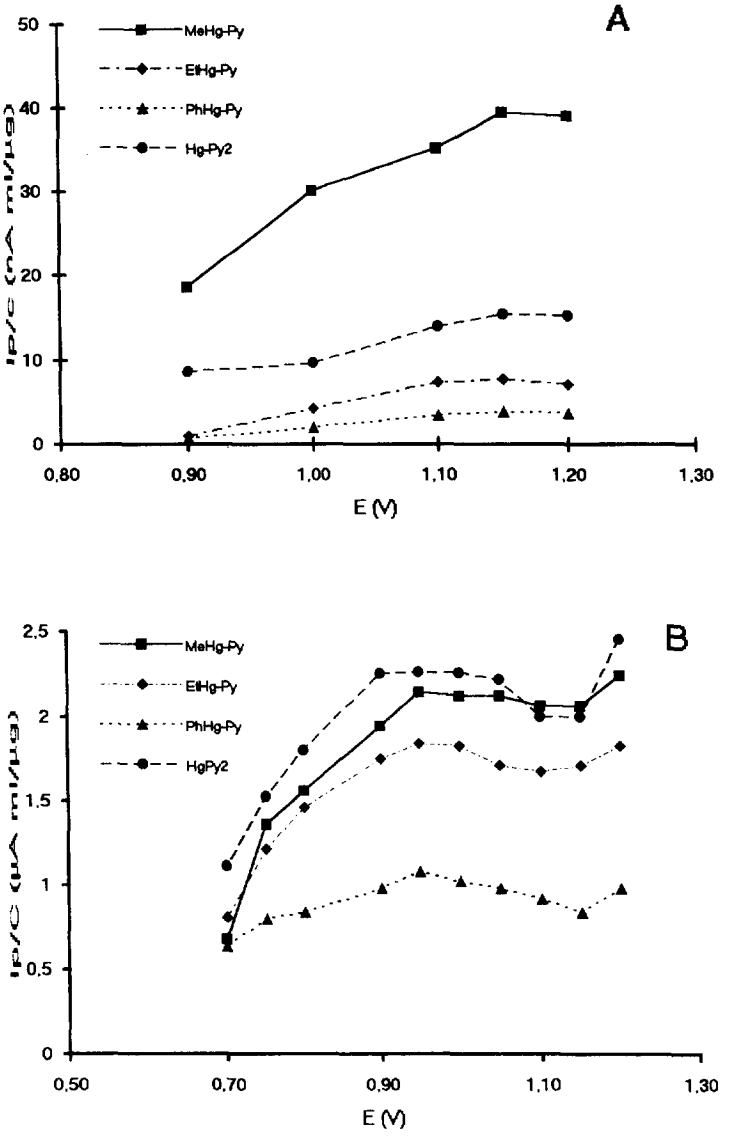

Fig. 1. Hydrodynamic voltammograms of complexes studied. (A) Amperometric detection on carbon paste electrode. Concentrations: $MeHg^+$, 0.95 µg/ml; $EtHg^+$, 5.0 µg/ml; $PhHg^+$, 5.0 µg/ml; Hg^{2+}, 2.05 µg/ml. Eluent: 75% methanol, 0.05 *M* KNO_3 as supporting electrolyte. (B) Coulometric detection. Concentrations: $MeHg^+$, 0.50 µg/ml; $EtHg^+$, 0.52 µg/ml; $PhHg^+$, 0.50 µg/ml; Hg^{2+}, 0.55 µg/ml. Eluent: methanol–water (75:25), 0.02 *M* KNO_3 as supporting electrolyte.

ple was passed with a syringe through a Sep-Pak C_{18} cartridge (Waters), which was prewashed with methanol and water. The Sep-Pak was washed with 5 ml of water and the extract was eluted with 2 ml of methanol. The eluted sample was evaporated to dryness and the residue was reconstituted in 100 μl of mobile phase and a 20 μl aliquot was injected into the chromatograph.

3. Results and discussion

3.1. Electrochemical studies

Preliminary electrochemical studies shown that an excess of PDTC in samples containing MeHgCl, EtHgCl, PhHgCl and $HgCl_2$ leads to formation of MeHgPDTC, EtHgPDTC, PhHgPDTC and $Hg(PDTC)_2$ complexes, when the pH of sample was higher than 4.0. Also it was observed that the maximum current intensity occured at pH values higher than 6.0.

Although some workers [29–31] suggest the addition of EDTA to the mobile phase to prevent interfering reactions between metal parts of the equipment and metals in the samples, in this study EDTA not was added to both mobile phase and samples to avoid high background and signals by oxidation on carbon electrodes at working potentials.

Table 1
Statistical treatment of calibration graphs and limits of detection.

Compound	Electrode	Sensitivity, (μA ml/μg)	r	L.D.[a] (μg/ml)
$MeHg^+$	acp	0.0585	0.9993	0.03
	agc	0.0389	0.997	0.05
	cgc	2.10	0.9996	0.008
$EtHg^+$	acp	0.0417	0.993	0.05
	agc	0.0280	0.999	0.07
	cgc	1.69	0.9997	0.009
$PhHg^+$	acp	0.0192	0.993	0.1
	agc	0.0139	0.995	0.14
	cgc	0.89	0.999	0.02
Hg^{2+}	acp	0.0251	0.994	0.08
	agc	0.0233	0.995	0.08
	cgc	1.39	0.9991	0.01

[a] L.D., signal-to-noise 3:1.
Injection volume 20 μl. Electrodes: agc=amperometric glassy carbon electrode; acp=amperometric carbon paste electrode; cgc=coulometric glassy carbon electrode.

The excess of ligand added was adequate to avoid these metallic interferences in the sample.

Fig. 1 shows the hydrodynamic curves obtained for the four complexes studied in amperometric and coulometric mode, using as chromatographic conditions methanol–water (75:25), containing 0.01 *M* KNO_3 as supporting electrolyte and 1 m*M* or 0.1 m*M* PDTC in the injected samples, respectively. In amperometric mode it was observed that the four compounds reached maximum signals at potentials higher than +1.10 V. The best signal to noise ratio was obtained at a potential of +1.15 V. However the higher signals in coulometric mode were observed at potentials ranging from +0.90 to +1.00 V. A best signal to noise ratio was obtained at +0.90 V in this detection mode.

3.2. Chromatographic conditions

It is necessary to have an excess of ligand in the sample solutions to obtain a quantitative formation of complexes. However, as the excess of ligand is oxidized on the electrode, and the solvent front becomes very high and wide, it can be difficult to detect the signal of compounds with low retention times such as the MeHgPDTC complex. A study of the effect of ligand excess on the chromatographic separation and detection indicated that constant and maxima signals are obtained for ligand–metal ratios higher than 4:1, but no signal of the MeHg ion can be observed for ratios higher than 20:1. Accordingly in this study a concentration of 1.0 m*M* PDTC was chosen when the concentration of organomercury compounds was higher than 1 μg/ml, but a 0.1 m*M* PDTC concentration was more appropriate when the concentration levels of mercuric compounds were lower. An increase of the reaction time between PDTC and mercurials up to 15 min was achieved for quantitative formation of complexes.

A study of the effect of supporting electrolyte concentration in the eluent was carried out both in amperometric and coulometric modes of detection. The results indicate an increase of analytical signal and noise when supporting electrolyte concentration increased. The best signal to noise ratios were obtained at 0.05 and 0.02 *M* concentrations for amperometric and coulometric detection, respectively. The pH value of the solutions was tested for pH

higher than 6.0 to obtain the higher signals. No buffer (such as phosphate buffer) was added to the eluent and samples in order to obtain a lower background signal.

3.3. Calibration graphs, sensitivity and precision

The linearities of calibration graphs, for concentrations ranging between 0.10 and 10.0 μg/ml in amperometric mode and 0.02 to 1.0 μg/ml in coulometric mode, were evaluated at the working potentials chosen. Table 1 shows sensitivity, detection limits and linearities in amperometric mode using both glassy carbon and carbon paste electrodes together with coulometric detection. Because of the higher surface area of the carbon paste electrode, detection limits obtained with this electrode were lower than when the glassy carbon electrode was used. Besides, for the carbon paste electrode no pre-treatment of the surface was necessary before each set of measurements, and so following studies were made with this electrode. The detection limits obtained were better than those given in the bibliography using UV detection [18].

When the detector was operated in the coulometric mode, better detection limits (one order of mag-

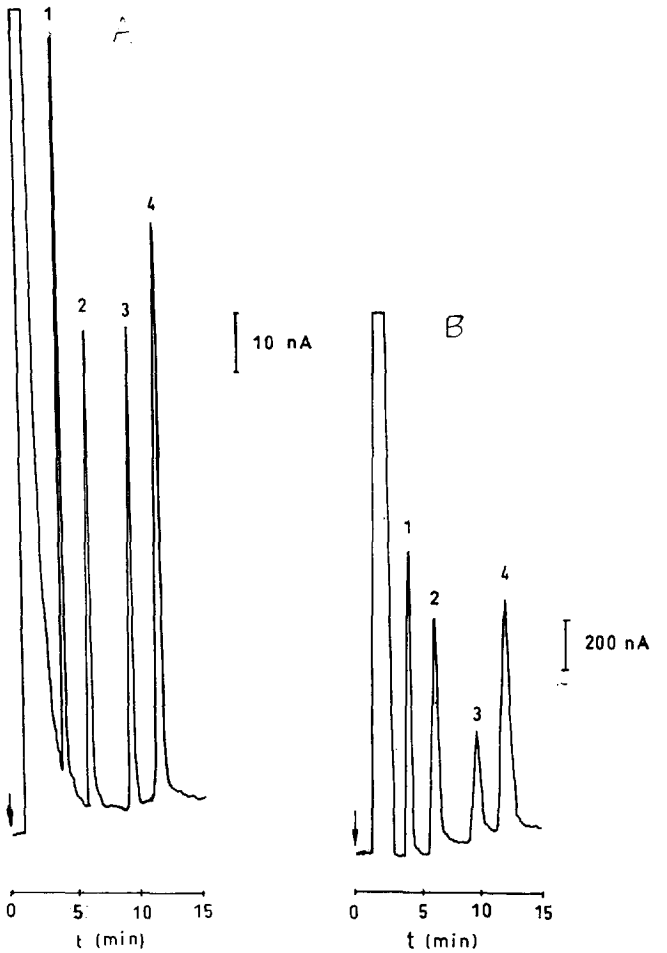

Fig. 2. Typical chromatograms obtained in (A) amperometric detection on a carbon paste electrode at +1.15 V. Concentrations: (1) $MeHg^+$, 0.95 μg/ml; (2) $EtHg^+$, 1.74 μg/ml; (3) $PhHg^+$, 2.73 μg/ml; (4) Hg^{2+}, 2.05 μg/ml; PDTC, 1.0 m*M*. (B) Coulometric detection at +0.90 V. Concentrations: (1) $MeHg^+$, 0.51 μg/ml; (2) $EtHg^+$, 0.51 μg/ml; (3) $PhHg^+$, 0.52 μg/ml; (4) Hg^{2+}, 0.50 μg/ml; PDTC, 0.1 m*M*.

nitude) than in the amperometric mode were observed. Besides, detection limits found in the coulometric mode are in the same order of magnitude as those obtained by cold vapour atomic absorption spectrometry when used as the HPLC detection method [16]. The linearities obtained for coulometric detection and carbon paste electrode amperometric detection were better than those obtained in glassy carbon amperometric mode. Fig. 2 shows typical chromatograms obtained in both amperometric (carbon paste) and coulometric detections, respectively.

Replicate samples of the four compounds were injected at approximately 2.0 μg/ml concentration in amperometric detection, and 0.3 μg/ml in coulometric detection, in order to evaluate the reproducibility of the method. For carbon paste amperometric detection, a relative standard deviation ranging between 1.3 and 2.8% was observed. In coulometric detection, relative standard deviation values ranging from 0.6 to 1.9% were obtained.

3.4. Interference studies

The interference by heavy metals was studied by injecting into the chromatograph several samples containing lead, copper, zinc, iron, cadmium, chromium, nickel and cobalt at the 1.0 μg/ml level of concentration. In the chromatographic conditions, signals corresponding to a copper complex, close to the PhHgPDTC peak, and cobalt and nickel complexes, both close to the EtHg-PDTC peak, were observed. In order to determine the higher level of concentration of these ions that can be present in the solution injected without interference, solutions containing all mercuric compounds studied, in concentrations over 0.50 μg/ml, and several concentrations of the interfering ion, were analysed using amperometric detection. This study showed that copper, cobalt and nickel ions do not interfere in the analysis of EtHg and PhHg when their concentrations are lower than 0.075 μg/ml. For higher concentrations, these interferences can be overcome by using a mobile phase containing 73% of MeOH instead of the 75% used in the method. At this methanol percentage, resolution of all compounds investigated was obtained without a significant loss of sensitivity, but with a subsequent increase of analysis time.

All investigated metals can interfere in the analysis of mercurial compounds reacting with PDTC and reducing the excess of ligand in the sample solution. An increase of the PDTC concentration in the sample avoids this interference.

With heavy metals, several models of inorganic and organic complexants present in natural water, such as chloride, sulphate, cysteine, glycine, nitrilotriacetic acid (NTA) and humic acids (HA), were investigated. No interference was observed in the presence of cysteine, glycine and NTA, in concentrations up to 1 m*M*, where 1 m*M* PDTC was added to the samples. However, a decrease in mercury complex signals was observed in solutions containing 10 mg/l HA and the mercuric ions in concentrations of 0.5 μg/ml, at reaction times lower than 15 min. When this solution was permited to react with 1.0 m*M* PDTC for 30 min, a quantitative formation of complexes was observed. This results indicate a necessary increase in the reaction time between PDTC and mercurials ions in real samples. No interference was observed from chloride and sulphate ions, for concentrations of interferent lower than 1 m*M*.

Table 2
Physico-chemicals parameters of both studied water samples.

Parameter	Sample A	Sample B
pH	7.6	7.5
Disolved oxygen (ppm)	9.3	1.1
Conductivity (μS)	94	92
Alkalinity (mg/l)	281	348
Chloride (mg/l)	81.4	89.2
Ammonia (mg/l)	3.3	4.8
Nitrate (mg/l)	-	17.0
Sulfate (mg/l)	182	169
Phosphate (mg/l)	-	10.3
Calcium (mg/l)	171	118
Magnesium (mg/l)	2.7	4.7
Sodium (mg/l)	17.5	111
Potassium (mg/l)	7.0	24.7
Iron (mg/l)	0.05	0.12
Manganese (mg/l)	0.12	0.17
Cadmium (μg/l)	0.36	0.76
Lead (μg/l)	0.94	1.54
Copper (μg/l)	1.54	3.72
Zinc (μg/l)	11.5	7.8
Organic matter (mg/l)	0.29	2.0
$MeHg^+$ (μg/l)	0.80±0.01	0.83±0.05
Hg^{2+} (μg/l)	0.45±0.03	0.43±0.02

Sampling date: May 1993 [32,33].

3.5. Determination of mercury and organomercury compounds in natural water samples

The proposed method was applied to water samples collected from the Jarama river. The samples were filtered through a 0.45 μm filter and, prior to application of the analytical method, the water samples were characterized in order to know possible interferences during the analysis processes. The most significative data are shown in Table 2. Samples studied did not contain a higher level concentration of inorganic salts and metals, however they contain an important amount of organic matter that can interfere in the mercury determination.

Because the mercury concentration in water samples was just lower than detection limits obtained for the method, a Sep-Pak C_{18} cartridge was used in an extraction and pre-concentration step. Methanol was chosen as eluent because it is compatible with the chromatographic mobile phase. The recoveries obtained were higher than 95%, for all studied compounds, when the ionic concentration in the sample was higher than 0.01 *M*. Fig. 3 shows typical chromatograms obtained when 100 ml of water sample were treated by the described procedure. No $PhHg^+$ and copper complexes were observed in the extracts obtained. Time reaction studies confirmed that a reaction time of 30 min was necessary to obtain the quantitative PDTC reaction with the mercuric ions. The concentration of organomercury species obtained by the chromatographic method can be seen in Table 2.

To check the efficiency of the extraction and the accuracy of the method, samples of water were

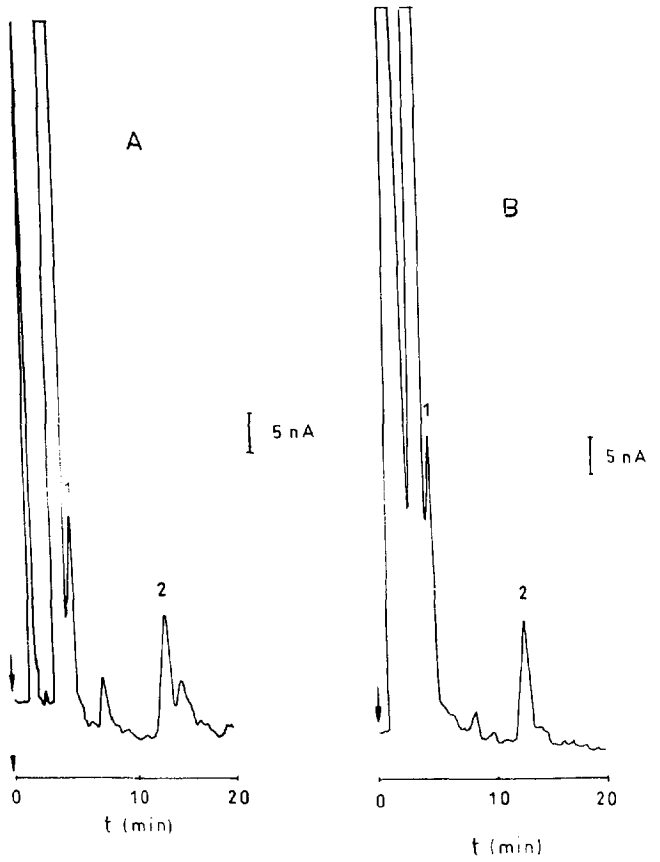

Fig. 3. Chromatograms of two samples of Jarama River water. Amperometric detection on carbon paste electrode. Pre-concentration on Sep-Pak cartridge of 100 ml of water. (1) MeHgPDTC and (2) $Hg(PDTC)_2$.

Table 3
Determination of mercury and organomercuric ions in spiked sample B ($n=4$).

Compound	Concentration (μg/l)			Recovery (%)
	Present	Added	Found	
$MeHg^+$	0.83±0.05	1.00	1.81±0.06	98
$EtHg^{2+}$		1.00	1.02±0.03	102
$PhHg^+$		0.99	1.02±0.03	103
Hg^{2+}	0.43±0.03	1.00	1,39±0.03	96

spiked with known amounts of organomercuric and mercuric standard solutions, extracted by the described procedure and their concentrations measured. An equilibration time of 24 h permitted the mercury species to bind to complexing compounds present in the water sample. The data obtained are shown in Table 3. As can be observed, the recoveries obtained were close to 100%. These results corroborate the ability of the method to achieve these determinations.

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