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Determination of aliphatic amines in water by gas chromatography using headspace solvent microextraction

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Abstract

The possibility of applying headspace microextraction into a single drop for the determination of amines in aqueous solutions is demonstrated. A 1 μl drop of benzyl alcohol containing 2-butanone as an internal standard was suspended from the tip of a micro syringe needle over the headspace of stirred sample solutions for extraction. The drop was then injected directly into a GC. The total chromatographic determination was less than 10 min. Optimization of experimental conditions (sampling time, sampling temperature, stirring rate, ionic strength of the solution, concentration of reagents, time of extraction and organic drop volume) with respect to the extraction efficiency were investigated and the linear range and the precision were also examined. Calibration curves yielded good linearity and concentrations down to 2.5 ng ml^{-1} were detectable with R.S.D. values ranging from 6.0 to 12.0%. Finally, the method was successfully applied to the extraction and determination of amines in tap and river water samples. This system represents an inexpensive, fast, simple and precise sample cleanup and preconcentration method for the determination of volatile organic compounds at trace levels.

Keywords: Gas chromatography; Aliphatic amines; Headspace solvent microextraction

1. Introduction

Short-chain aliphatic amines are presented widely in the aquatic environment due to their wide spread use in several industrial, chemical and manufacturing applications. These compounds are generated in the production process of plastics, dyes, drugs, anti oxidants and explosives [1] and by the petrochemical industry [2]. Aliphatic amines are also common components of biological systems as degradation products of organic materials such as amino acids and proteins. In addition to hygienic problems due to stinging smell, these compounds may be hazardous to human health as they are sensitizers and irritants to skin, eyes, mucus membranes and respiratory tract. In addition, they can react with certain nitrogen-containing compounds to form nitrosamines, which are potentially carcinogenic substances. Consequently, there is increasing interest in the detection of aliphatic amines in various matrices [3].

Chromatographic techniques are generally used for the determination of aliphatic amines in aqueous media and a number of methods, using either gas chromatography (GC) or Liquid chromatography (LC) are available. A common feature of such methods is the requirement for analyte preconcentration, as these compounds are usually present at trace levels. Analyte enrichment is usually accomplished by using extraction techniques. However, unlike many other compounds of environmental relevance, preconcentration of aliphatic amines by extraction methods is a difficult task, owing to their high-polarity and water solubility [4-7]. In addition in LC methods, chemical derivitization should be used to enhancing the sensitivity, because aliphatic amines have no UV or fluorescent properties. However, in most of these methods, major drawbacks are the substantial sample handling involved which

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introduces a possible source of errors and the long analysis times. In most of these procedures, the reaction times are typically 20–60 min, but reaction times up to 180 min have also been reported [8]. Moreover, multiple liquid–liquid extraction of the derivitized analytes is required to achieve acceptable recoveries and then large volume of the organic solvents must be evaporated before the chromatographic steps. In addition, in some instances, the excess of reagent must be re-extracted or eliminated [4,7]. As a result, the analysis is very tedious and time-consuming. Because of the above mentioned disadvantages, solvent-free sample preparation methods, or those employing less organic solvents are becoming more and more important.

In the last few years, efforts have been directed toward miniaturizing the liquid–liquid extraction procedure by greatly reducing the solvent to aqueous phase ratio, leading to the development of solvent microextraction methodologies. This technique is not exhaustive and only a small fraction of the analytes is extracted/preconcentrated for analysis [9]. Solvent microextraction can be performed with the simplest devices, i.e. a conventional micro syringe [10]. Moreover, because of a wide choice of polar extraction solvents, headspace liquid phase microextraction seems to be even a more attractive technique. The solvents need not even be water immiscible as in direct liquid phase microextraction from aqueous solutions.

The use of a single liquid drop has been reported only very recently for headspace analysis into a single drop for determination of organic compounds [11,12].

The aim of this work was to develop a simple and sensitive method using a very small volume of solvent to achieve preconcentration in headspace analysis of aliphatic amines in aqueous matrices.

The technique is very inexpensive and good precision and sensitivity is obtained with short analysis time.

2. Experimental

2.1. Instrumentation

Separation, identification and quantification were carried out on a Chrompack CP-9002 (Chrompack, Middleburg, The Netherlands) gas chromatograph system equipped with a flame ionization detector. A CP-Cil 5^{TM} capillary column (cat. no. 7700) of 10 m × 0.25 mm i.d. × 0.12 μm film thickness from Chrompack was employed.

The inlet was operated in split mode with a split ratio of 10:1 and an on-column flow rate of carrier gas (Nitrogen) 1.2 ml min^{-1}. The temperature of the injector and of the detector was 190 and 160 °C, respectively. The column temperature programme was initial temperature 35 °C then increased to 50 °C at 3 °C min^{-1} to 125 °C at 50 °C min^{-1}. A 10 μl Hamilton 7105 syringe (Hamilton, Reno, NY, USA) was used to suspend the drop of benzyl alcohol and inject it into the GC. Samples were stirred in 5.0 ml flat-bottom vials containing Teflon-lined septa using an electronic magnetic stirrer (VWR Scientific, West Chester, PA, USA). In order to eliminate air, micro syringe was washed many times with the solvent solution used for microextraction [13]. The syringe was clamped in a fixed position relative to the vial to consistently place the needle tip in the headspace of the vial. The vial was thermoastated at the desired temperature with a water bath.

2.2. Chemicals

All the target analytes, benzyl alcohol and 2-butanone were of analytical-reagent grade, obtained from Merck KGa (Darmstadt, FRG) and were used without further purification. A stock aqueous standard solution (1.0 g l^{-1}) of ethylamine, 2-propanamine, 1-propanamine, *N*-ethyl-ethanamine and 1-butanamine was prepared. The stock standard solution was refrigerated at 4 °C. Working standard solutions were prepared daily at various concentrations ranging from 2 ppb to 1.5 ppm by diluting the stock standard solution with doubly distilled water. The organic extractant was benzyl alcohol containing a fixed amount of 2-butanone as internal standard (0.100 %, v/v).

2.3. Extraction procedure

The experimental set-up is illustrated in Fig. 1. A 2 ml aliquot of the standard mixture was placed in the 5 ml vial with a 6 mm stir bar (VWR Scientific). The Hamilton 7105 syringe was rinsed and primed at least 20 times with the solvent/internal standard. After the uptake of the 1 μl (unless

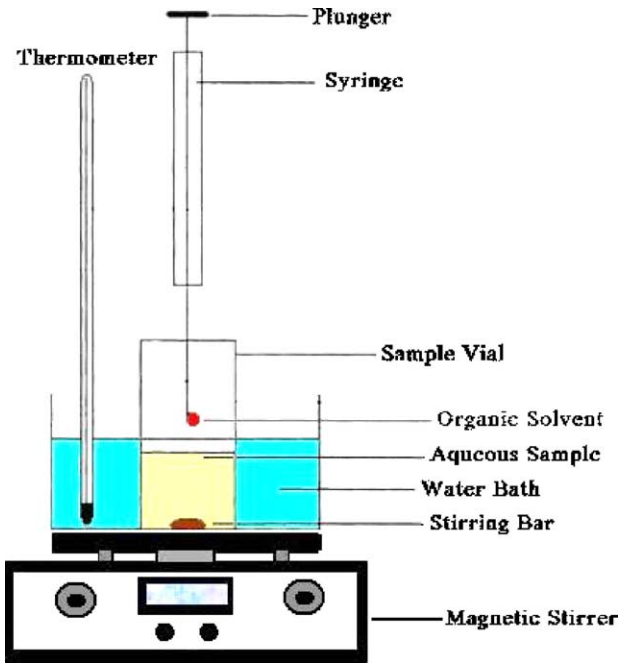

Fig. 1. Schematic representation of the headspace solvent microextraction system for preconcentration into a single microdrop (not to scale).

otherwise stated) of solvent, the needle was used to pierce the vial septum, and the syringe was clamped into place so that the tip of the needle was located in a consistent position in the headspace. The syringe plunger was depressed, exposing the drop, and stirring commenced for various times at various stirring rates during extraction. After stirring, the drop was retracted and injected into the GC for analysis. The analytical signal was the peak area ratio of the analyte to the internal standard.

3. Results and discussion

3.1. Basic principle

The extracting solvent had to conform to two requirements—to extract analytes properly and to be separated from analytes peaks in the chromatogram [11]. In addition, solvent should have a low vapor pressure so as to preclude the evaporation of the drop during the extraction.

Few solvents differing in polarity, boiling point and water solubility were tested. Preliminary trials showed that benzyl alcohol gave the best extraction efficiency, so it was chosen as the extraction solvent for further works. Since aliphatic amines are volatile and also some fluctuations in volumes injection may occur, 2-butanone was added as an internal standard to correct such difficulties and the analytical signal was taken as the peak area ratio of the analytes to it. A typical chromatogram of the standard solution of aliphatic amines after headspace extraction with a drop of benzyl alcohol containing 2-butanone as an internal standard is presented in Fig. 2.

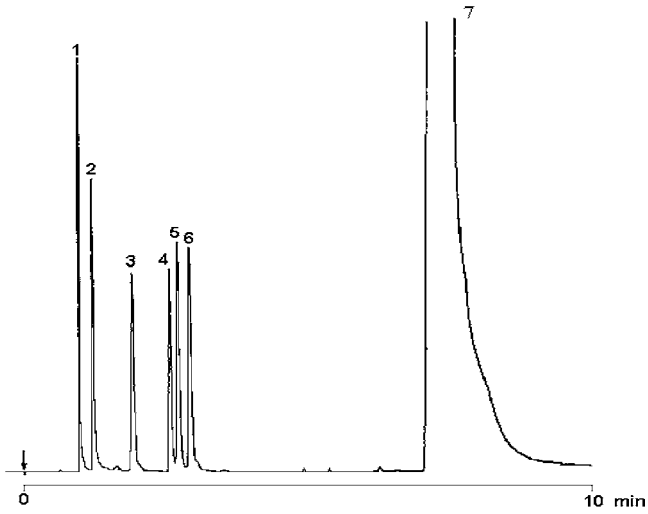

Fig. 2. Chromatogram of a standard solution of aliphatic amines. 1: ethylamine, 2: 2-propanamine, 3: 1-propanamine, 4: *N*-ethyl- ethanamine, 5: 2-butanone (internal standard), 6: 1-butanamine, 7: benzyl alcohol (solvent).

3.2. Optimization of solvent microextraction

The initial object was to develop and optimize solvent microextraction sampling conditions for the extraction of aliphatic amines from water samples. For solvent microextraction, there are several parameters to control the optimum performance of the method, such as extraction solvent, rate of sample agitation, organic drop volume, ionic strength of the solution, sampling time, temperature and concentration of reagents. The effect of each parameter on extraction was examined. The results are as follows.

3.2.1. Extraction solvent

Three different solvents were tested. Solvent selectivity was evaluated for the extraction of a 5 ml sample containing 100 $\mu g\,l^{-1}$ of each analyte in deionized water. The stirred solution (400 rpm) was sampled for 10 min using 1 μl of the appropriate organic solvent. The solvents examined here, varied in terms of water solubility. Longer sampling times and faster stirring rates were avoided. The results are given in Fig. 3.

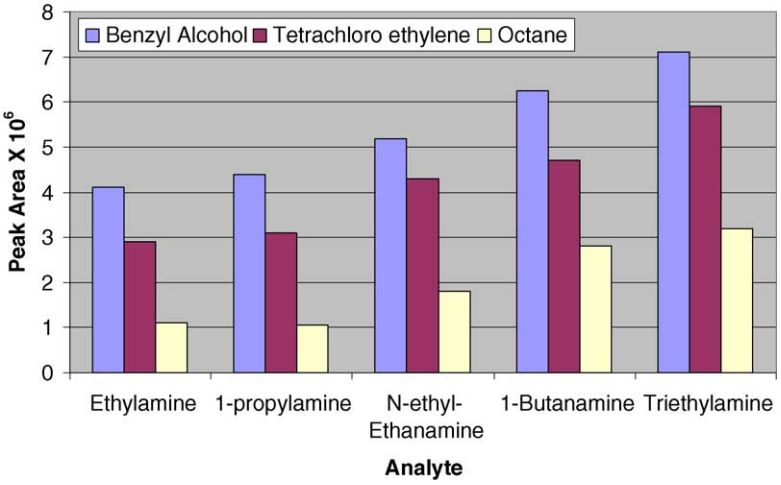

Fig. 3. Relative extraction efficiencies of target analytes using different types of extracting solvent (for experimental conditions, see text).

The extraction efficiency was based on the average peak area counts of each analyte for three replicate analyses. However, the results show that the semi-polar tetrachloroethylene micro drop extracted relatively well, because of its high-vapor pressure, the use of the other polar solvent, i.e. benzyl alcohol, is preferred. Moreover, benzyl alcohol was found to provide better extraction efficiency.

3.2.2. Stirring rate

Sampling agitation enhances extraction and reduces extraction time because the equilibrium between the aqueous and vapor phases can be achieved more rapidly [9]. It is essential to obtain a linear relationship between the concentrations of analytes in the water and in the vapor phase. Mass transfer in the headspace is assumed to be a fast process, because diffusion coefficients in the gas phase are typically 10^4 times greater than corresponding diffusion coefficients in condensed phase [14]. Furthermore, convection is induced in the headspace by the stirring of the aqueous phase. For the purpose of the present study, three replicate analyses were taken at five different stirring rates—0 (static case), 200, 400, 600 and 800 rpm. Faster stirring rates were avoided as they resulted in dislodgement of the organic drop from the needle. The results show clearly that stirring produces a dramatic increase in the analytical signal when compared to the stagnant case (Fig. 4).

This is consistent with the expected behavior of the solvent microextraction based on the film theory of convective-diffusion mass transfer [13]. As can be seen in Fig. 4, the peak areas of all analytes increase with increase in the stirring rates up to 600 rpm. At 600 rpm the peak areas of some analytes remain constant and those of the rest of the analytes continue to increase, hence a stirring rate of 600 rpm was chosen for further works.

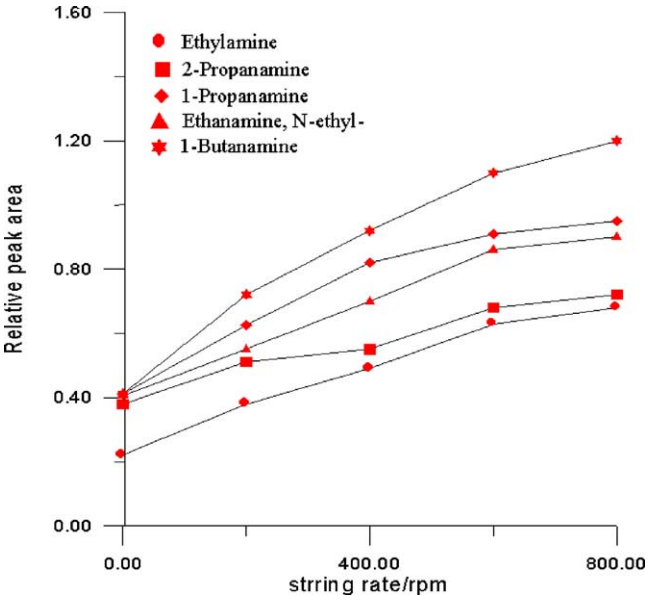

Fig. 4. Relative extraction efficiencies of target analytes with different stirring rates: concentration 100 μg l^{-1}; sampling time 10 min, and; 1 μl organic drop.

3.2.3. Sampling temperature

The effect of stirring temperature was studied by exposing an extracting drop for 15 min in the headspace at 10–80 °C. Measurements were performed on aqueous solution containing 100 μg l^{-1} of each analyte. The extraction curve showed that amount of analyte absorbed, increases with increase in temperatures up to 50–60 °C (Fig. 5). This can be explained by the fact that at higher temperatures, the vapor pressure of the analytes and hence their concentrations in headspace increase. Above the temperature mentioned, the amounts of analytes extracted decreases, probably because the partition coefficients to the extraction phase decreases. Therefore, the optimum sampling temperature for a fixed extraction time of 15 min was 50 °C.

3.2.4. Sampling time

A series of spiked-water samples (100 μg l^{-1}) were prepared and the variation of the analytical signal for each analyte was studied as a function of exposure time (Fig. 6).

As can be seen in Fig. 6, relative peak areas increase with increase in exposure time without reaching equilibrium. On the other hand, we noticed that with increase in the exposure time, the drop volume increases significantly and after a 45 min exposure time, it grew from an initial of 1–5.0 μl and consequently, lost touch with the needle. This is because benzyl alcohol is potentially hydrophobic and absorbs water together with the analytes.

It was not also possible to increase the initial drop volume using a 2 μl drop in the tip of the needle, after a 15 min exposure time, the volume of the drop become too large to remain fixed to the needle tip and the drop become detached.

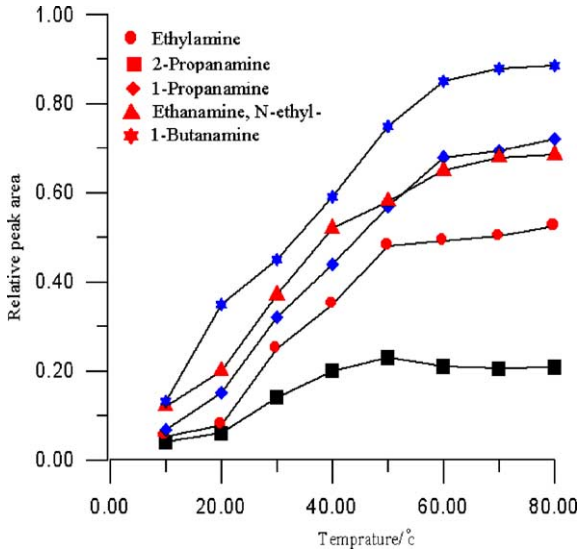

Fig. 5. Effect of sampling temperature on the relative peak areas of each analyte (for more details, see text).

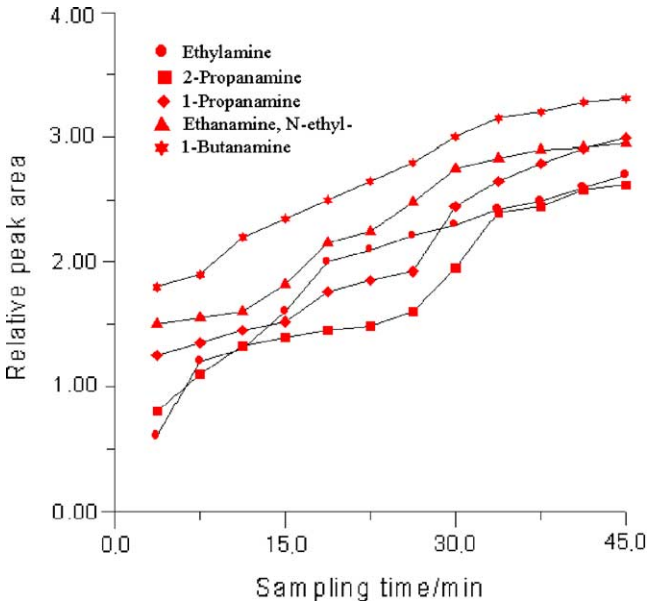

Fig. 6. Extraction time profiles for determination of optimum sampling times: concentration 100 μg l^{-1}; stirring rate 600 rpm, and; 1 μl organic drop.

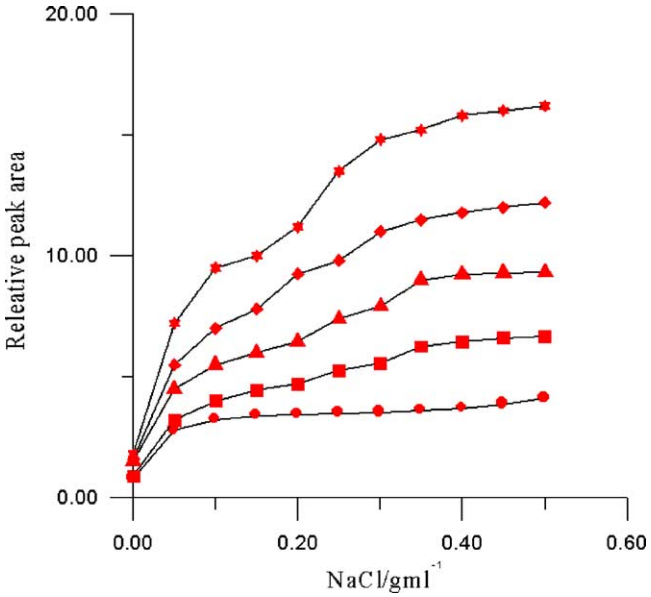

Fig. 7. Effect of salt concentration on the extraction efficiency: concentration 100 μg l^{-1}; stirring rate 600 rpm; sampling time 15 min, and; 1 μl organic drop.

For quantitative analysis, however, it is not necessary for the analytes to reach equilibrium, only to allow sufficient mass transfer into the drop and extract reproducible extraction time [15,16]. Therefore, in further work, a compromise expose time of 15 min allowing incidents of drop detachment to be avoided was chosen, even though the analytes had not reached equilibrium at this time point.

3.2.5. *Ionic strength of solution*

Addition of salt to the solution may have several effects on extraction. More commonly, the presence of salt increases the ionic strength of the solution and affects the solubility of organic analytes [17]. Extraction is usually enhanced with increasing salt concentration and increased polarity of the compound (salting-out effect) [18].

For solvent microextraction, the effect of salt was previously studied [1,19]. These reports concerned dynamic liquid phase microextraction, where a conventional micro syringe was used as a separatory funnel. The results revealed that the presence of salt decreased the extraction efficiency.

The effect of NaCl concentration (ranging from 0 to 0.5 g ml^{-1}) was investigated and the extraction efficiency was monitored. The results, based on triplicate analysis, were presented in Fig. 7 as a plot of relative peak areas versus amount of NaCl.

It is evident that the addition of NaCl promotes transport of the analytes to the headspace and hence to the extracting drop. However, the extraction efficiency did not change any further at NaCl concentrations higher than 0.3 g ml^{-1}. Therefore, in further works, saturated salt conditions with a NaCl concentration of 0.3 g ml^{-1} were chosen.

3.3. *Evaluation of method performance*

Calibration curves were calculated based on examination of 2 ml of aqueous solution of aliphatic amines. NaCl, 0.6 mg, was added to the extraction vials before analysis and sampling with 1 μl of benzyl alcohol at 50 °C and 600 rpm stirring rate for 15 min was carried out. Using five spiked levels, the linear range for all the aliphatic amines investigated were within 1.2 mg l^{-1}. For each spiking level, three replicate analyses were performed. All procedures were carried out in triplicates to evaluate inter-day reproducibility. The correlation coefficient (r^2) ranged from 0.9123 to 0.9418, as shown in Table 1.

The ACS Committee report on environmental analysis [20] was used to calculate the limit of detection (LOD) of the method. LOD was determined as the signal of extracted low concentration of the analyte corresponding to three times the standard deviation of 50 successive injections of the blank noise distribution. The results are summarized in Table 1.

Table 1
Figures of merit for solvent microextraction of 2 ml spiked-water samples after exposing 1 μl benzyl alcohol drop for 15 min (n = 3)

Analyte	R.S.D. (%) (n = 3)	LOD (μg l^{-1})	Correlation coefficient (r^2)
Ethylamine	12	25	0.9130
2-Propanamine	11.2	16.3	0.9192
1-Propanamine	6.0	2.5	0.9418
N-ethyl-ethanamine	9.88	7.25	0.9225
1-Butanamine	8.25	5.3	0.9351

Table 2
Accuracy for each aliphatic amine after exposing 1 μl benzyl alcohol drop for 15 min in stirred 2 ml tap water and river water samples spiked with different amounts of each target analytes ($n = 3$)

Analyte	Concentration added (mgl^{-1})	Concentration determined		
		Standard sample	Tap water	River water
Ethylamine	0.06	0.056 ± 0.005	0.059 ± 0.008	0.064 ± 0.008
	0.5	0.49 ± 0.04	0.52 ± 0.04	0.51 ± 0.05
	0.9	0.91 ± 0.07	0.92 ± 0.06	0.92 ± 0.07
2-Propanamine	0.06	0.062 ± 0.004	0.064 ± 0.006	0.06 ± 0.006
	0.5	0.5 ± 0.06	0.5 ± 0.04	0.49 ± 0.05
	0.9	0.92 ± 0.07	0.94 ± 0.04	0.9 ± 0.05
1-Propanamine	0.06	0.061 ± 0.003	0.061 ± 0.004	0.062 ± 0.006
	0.5	0.5 ± 0.04	0.53 ± 0.03	0.54 ± 0.05
	0.9	0.89 ± 0.05	0.87 ± 0.07	0.9 ± 0.05
N-ethyl-Ethanamine	0.06	0.059 ± 0.003	0.058 ± 0.004	0.061 ± 0.003
	0.5	0.59 ± 0.03	0.47 ± 0.05	0.5 ± 0.06
	0.9	0.89 ± 0.04	0.88 ± 0.06	0.89 ± 0.05
1-Butanamine	0.06	0.058 ± 0.002	0.059 ± 0.003	0.062 ± 0.002
	0.5	0.49 ± 0.04	0.5 ± 0.04	0.5 ± 0.05
	0.9	0.89 ± 0.03	0.88 ± 0.05	0.89 ± 0.06

3.4. Application to real water samples

The described method was applied to the analysis of tap and river waters. Samples were spiked to produce three different concentrations 0.06, 0.50 and 0.90 ppm, in order to check the matrix effect on determination. Unspiked samples were also processed.

The chromatograms obtained for the spiked samples are very similar to those obtained for standard solutions and no interferences were observed from other compounds potentially present in the sample (e.g. ammonia). None of the amines investigated were detected in the tested samples. The concentrations for the spiked samples calculated and are summarized in Table 2. As can be observed, the results are comparable to those observed for standard solutions containing the same concentrations of the analytes. This reveals that the performance of the proposed preconcentration procedure was similar for all types of samples tested.

4. Conclusions

The results of this work show that the use of solvent microextraction to enrich and quantifying trace levels of aliphatic amines in water samples is a valid alternative for their determination. The proposed procedure is very simple and rapid. The overall analysis time (sample pre-treatment plus chromatography) is about 25 min. In addition, consumption of toxic organic solvents typically involved in many other methods are not necessary. For solvent microextraction there is no need of dedicated and expensive apparatus. The proposed procedure can be applied with satisfactory accuracy and reproducibility to the determination of aliphatic amines at low- to sub ppm concentrations. No significant differences were observed in the quantification of the analytes between the different types of samples tested.

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