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DLLME METHOD FOR EXTRACTION OF SOME PRIMARY ALIPHATIC AMINES FROM AQUEOUS SAMPLES FOLLOWED BY GAS CHROMATOGRAPHY-FLAME IONIZATION DETERMINATION

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ABSTRACT

The paper presents a new method based on simultaneous derivatization for the extraction and pre-concentration of some aliphatic amines prior to gas chromatography-flame ionization detection (GC-FID). Primary aliphatic amines are derivatized and extracted simultaneously by a fast reaction with chloroform (derivatization agent/extraction solvent) under mild conditions. The mixture of chloroform and aqueous sample solution is rapidly sucked into a 10-mL glass syringe and then is injected into a test tube with conical bottom and the procedure is repeated three times. After centrifuging the resulted cloudy solution, the derivatized analytes in the sedimented phase are determined by GC-FID. The influence of main factors on the efficiency of derivatization/extraction procedure is studied. Under the optimal conditions, the enrichment factors (EFs) for aliphatic amines are obtained in the range of 397–499 and limits of detection (LODs) are between 1 and 3μg L^{-1}. The obtained extraction recoveries ranged from 50 to 72% and the relative standard deviation (RSD) was less than 4.8% for intra-day (n = 6) precision. The method is successfully applied to determine some aliphatic amines in environmental water samples.

KEYWORDS: aliphatic amines, diffusion liquid-liquid microextraction, DLLME.

INTRODUCTION

Prior to separation and detection by chromatographic, electrophoretic, and mass spectrometric (MS) techniques, a sample preparation step is normally required to transfer the analytes to a suitable medium, isolate them from the major sample matrix, and enrich them to a concentration level detectable by the separation system (Ivanova and Havezov, 1995; Cristina et al., 2009; Burnham et al., 1972). In a majority of cases, sample preparation is still carried out by traditional techniques, such as liquid–liquid extraction (LLE), solid phase extraction (SPE), and purge and trap. Yet the development gap between sample preparation and separation steps still appears to increase in terms of sophistication and performance. Micro extraction techniques represent an important contribution to the improvement of sample preparation performance, which especially addresses the issues of miniaturization, automation, on-site analysis, and time efficiency. Actually, different types of micro extraction techniques were reported in the literature a long time ago (Muray, 1979), but the field gained in significance with the invention of solid-phase micro extraction (SPME) in 1990 (Arthur and Pawliszyn, 1990), which later became commercially available. In 1990, Arthur and Pawliszyn introduced a new method termed solid phase micro extraction (SPME). A polymer-coated fiber, on which the investigated compound adsorbs, is placed in the sample or its headspace (Arthur and Pawliszyn, 1990).

2006 Pedersen and colleagues presented Hollow Fiber Liquid Phase Micro extraction method (Pederson-Bjergaard and Rasmussen, 2008). This is the first method was used to extract the mineral composition, extraction and pre-concentration of analyze in complex samples made possible. This method is based on properties of the matrix applied in the form of three-phase and biphasic (Luciano et al., 2010). Until the 2006 Yaghoub Asadi and colleagues invented a new method called diffused liquid - liquid micro extraction (DLLME) to remove many of the problems associated with previous methods micro-extraction (Rezaee et al., 2006). Aliphatic amines measurement techniques are chromatography and sometimes electrophoresis. Mainly liquid chromatography and in some cases gas chromatography and in a few cases, isolated these materials have been using capillary electrophoresis. In works relating to the measurement with the GC, different extraction methods primarily (SPE) and diffusion solid phase extraction (DSPE) is used for water samples.
In addition, various detectors include Nitrogen- phosphorus detector (NPD), flame ionization detector (FID) and mass spectrometry (MS) was used to detect the output column. To measure the LC after extraction by solid phase and

derivatization of these compounds, UV and MS detection was performed with detectors. Some of these things are mentioned below:
Sanghi et al. Some of aliphatic amines in water samples in the syringe after derivatization by Penta Fluor benzoyl chloride reagent with GC / MS were measured in water samples (Singh *et al.*, 2011).

Ballesteros et al.Some of aliphatic amines in water samples by solid-phase extraction method by using various absorbents by using GC / MS measurements were made (Sancheza et al., 2009). Farajzadeh and colleagues obtained extraction some aromatic amines by using derivatization of butyl chloroform with liquid - liquid micro- extraction was performed with air support. This method is very simple and extraction and fro concentrated of analytes can be done concurrently (Farajzadeh and Nouri, 2007). Cunha and colleagues some of Biogenic amines in the samples alcohol-free drinks based on liquid-liquid micro extraction with GC / MS measurements were made (Almeida et al., 2012). Ortiz and colleagues have-been measured some of aromatic amines by using Headspace analysis of emissions from polymer materials by using GC / MS (Ortiz *et al.*, 2014).

The purpose of this study is to provide an efficient and relatively fast method for analyzing a number of the type I aliphatic amines in different water samples. Most methods is used for the analysis of the type I aliphatic amines mainly difficult step is the extraction of analytes because these compounds are relatively polar and their extraction efficiency is low.In this study, in order to prepare a sample of diffusion micro-extraction liquid - liquid extraction and preconcentration some kind of aliphatic amines in water samples prior to analysis by GC-FID is used. Who do very simple and is done in a short time and doesn`t need to have sophisticated features and devices. The advantages of this method include high separation power, allowing the analysis of mixtures of analytes with high sensitivity and relative reduction of analysis time point. Taking advantage of this method is expected to achieve lower detection limits and high levels of enrichment factors. In order to confirm the results of measurements of real samples by GC-FID and GC-MS method was used for analysis.

MATERIALS AND METHODS

2- Experimental

2.1. Reagents and solutions

Methyl amine,Ethyl amine,Propyl amine, n-Butyl amine, iso-Butyl amine, chloroform (as derivatization agent/extraction solvent), acetonitril, methanol, sodium chloride, hydrochloric acid, sodium hydroxide and NH_3were purchased from Merck (Darmstadt, Germany). A stock solution of aliphatic amines (each 1000 mg L^{-1}) was prepared in acetonitril and held in a refrigerator at 4 ◦C. Working standard solutions were daily prepared by appropriate dilutions of the stock solution with HPLC-grade water. A solution of analytes (5 mg L^{-1}) in chloroform was used for the calculation of the enrichment factors and extraction recoveries.

2.2. Samples

Tap water was collected from our laboratory just before analysis. Well water was picked up from local area (Tabriz, Iran). Other samples including river water (Tabriz, Iran), municipality wastewater and paint processing unit wastewater (Tabriz, Iran) were also tested. All of the samples were directly subjected to the derivatization/extraction procedure without dilution.

2.3. Instrumentation

A gas chromatograph (GC-2014, Shimadzu, Kyoto, Japan) equipped with a split/splitless injector system, and an FID was used for separation and determination of the selected aliphatic amines. Helium (99.999%, Gulf Cryo, United Arab Emirates) was used as the carrier gas at a constant linear velocity of 30 cm s^{-1} and make up gas (30 mL min^{-1}). The injection port was held at 300 ◦C and used in splitless mode with a purge time of 1 min. Separation was carried out on a DB-5 capillary column (30 m × 0.25 mm i.d., and film thickness 0.25 m) (Supelco, USA). The oven temperature was programmed as follows: initial temperature 70 ◦C (held 2 min) then was raised to 300 ◦C at a rate of 10◦C min^{-1}, and held at 300 ◦C for 1 min. The total time for one GC run was 23 min. The FID temperature was maintained at 300 ◦C. Hydrogen gas was generated with a hydrogen generator (OPGU-1500S Shimadzu, Kyoto, Japan), for FID at a flow rate of 40 mL min^{-1}. The flow rate of air for FID was 300 mL min−1. pH measurements were performed with a Metrohm pH meter model 654 (Herisau, Switzerland). A D-7200 centrifuge from Hettich (Kirchlengern, Germany) was used in DLLME.

2.4. Derivatization/extraction procedure

The pH of solution was adjusted at 11 using a 1 mol L^{-1} NaOH solution and then 5 mL of this solution was placed into a 10-mLglass test tube with conical bottom. then 45 µL chloroform(derivatization agent/extraction solvent) was transferred into the tube. The mixture was rapidly sucked into a 10-mL glass syringeand then was injected into the tube. After centrifuging of cloudy solution for 5 min at 4000 rpm, 1µL of the sedimented organic phase was removed using a 1-LGC micro syringe (zero dead volume, Hamilton, Switzerland) and injected into the GC system for analysis. The derivatization reaction between chloroform and primary aliphatic amines was performed according to the following reaction:

2.5. Calculation of enrichment factor and extraction recovery

The enrichment factor (EF) was defined as the ratio between theanalyte concentration in the sedimented phase (C_{sed}) and the initial concentration of analyte (C_0) within the sample.

$$EF = \frac{C_{Sed}}{C_0} \quad (1)$$

C_{sed} was obtained by comparing the obtained peaks areas after performing the proposed method with those obtained by direct injection of standard solutions of the selected amines in chloroform. The extraction recovery (ER) was defined as the percentage ofthe total analyte amount (n_0) which was extracted into the sedimented phase (n_{sed}).

$$ER = \frac{n_{sed}}{n_0} \times 100 = \frac{C_{sed} \times V_{sed}}{C_0 \times V_{aq}} \times 100 \quad (2)$$

whereV_{sed} and V_{aq} are the volumes of sedimented phase and aqueous solution, respectively.

3. Results and discussion

3.1. Optimization of derivatization agent/extraction solvent volume

The volume of extraction solvent/derivatization agent can effect on the volume of the sedimented phase, and extraction efficiency. Although by increasing extraction solvent volume, the extracted amounts of analytes will increased until all the amount is extracted, but their concentrations in the sedimented phase is diluted. Low volume of extraction solvent enhances enrichment factors, by reducing the volume of sedimented phase. In order to study the effect of extraction solvent volume on the performance of the presented method, different volumes of chloroform (45–85µL at 10-µL intervals) were tested. By increasing the volume of chloroform from 45 to 85 L, the volume of sedimented phase increased and hence the analytical signals decreased rapidly (Fig. 1). Volumes less than 45µL were avoided due to lower volume (3µL) or lack of sedimented phase that make to decrease the repeatability of this method. Relatively high analytical signals along with good repeatability were obtained when 55 µL of chloroform was used. Therefore, in this study, 55µL was selected as the optimal volume of derivatizationagent, which leads to obtain about 10 µL sedimented phase volume.

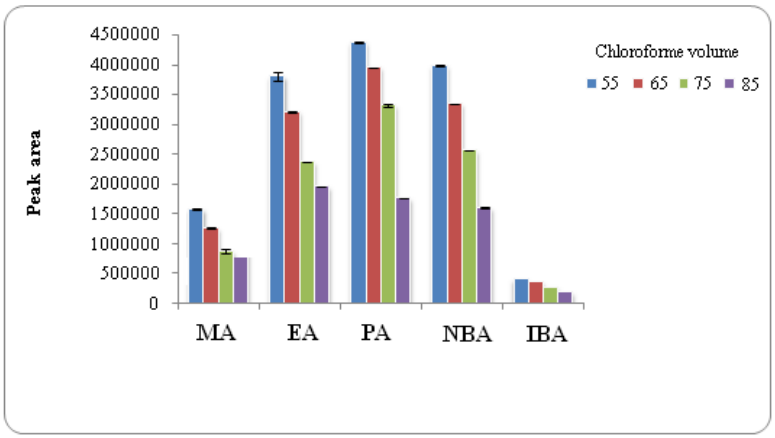

Figure 1: Effect of extraction solvent on extraction efficiency. Error bars represented the maximum and minimum values for the three measurements.

3.2. Optimization of diffuser solvent volume

For study the influence of diffuser solvent volume in this method, investigated different volume of aceto nitrile.In a study of 55 microliter volumes of chloroform as solvent diffuser extraction solvent with various volumes of solvent diffuser (acetonitrile) into an aqueous solution containing 5 mg/L of each of the amines was injected. Therefore volumes of 0.5, 0.75, 1, and 1.5 ml of acetonitrile were studied. While other experimental conditions kept constant (Fig 2). Tests indicated in small volumes of acetonitrile because with large droplets of solvent extraction process DLLME not done well and extracting of analytes done with little efficiency. While values greater than 1 mL of diffusion extraction solvent to take place effectively and extract analytes with high performance occurs. The optimal volume of 1 mL of acetonitrile as a solvent selected player. It should be noted that the values of 1 and 1.5 mL of acetonitrile extraction efficiency is almost identical.

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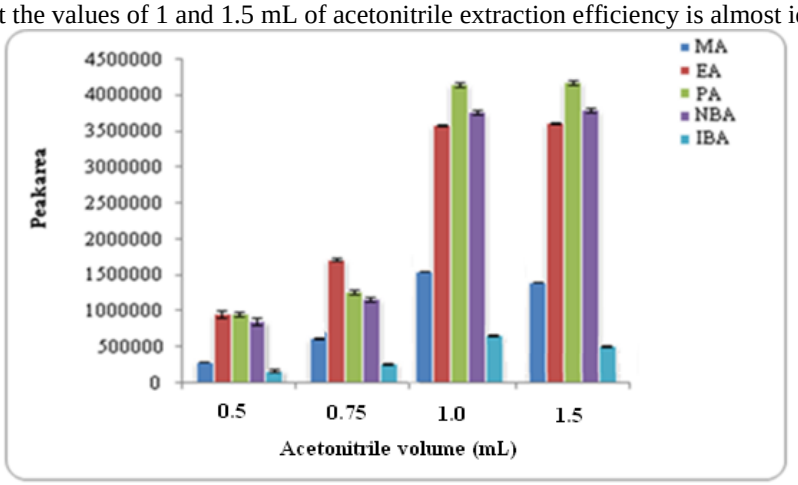

Figure 2: Effect of diffusion solvent on extraction performance. Error bars represented the maximum and minimum values for the three measurements.

3.3. Salt addition effect

Generally, salt addition can improve extraction yield, as a result of "salting out" effect. Therefore, the effect of salt addition on yield of the extraction procedure was investigated at five different concentrations of NaCl from 0 to 20% (w/v). By increasing the concentration of NaCl, the analytical signals were slightly decreased in1% (w/v) NaCl and then start to increase. In the presence of NaCl,different phenomena were occurred. Addition of a salt increased

viscosity of solution, which led to reduce extraction efficiencies. On the other hand, salting out effect increased the extraction efficiency by decreasing solubility of analytes in aqueous phase. Hence it can be concluded that the presence of salt despite the increasing volume of sedimentary phase, resulting in increased efficiency.It should be noted that the percentage of more than 20% due to the large increase in the polarity of the solvent and aqueous phase into the aqueous phase the player has not spread and there is no possibility of DLLME.Hence, all experiments were performed by 10% addition of the salt (Fig 3).

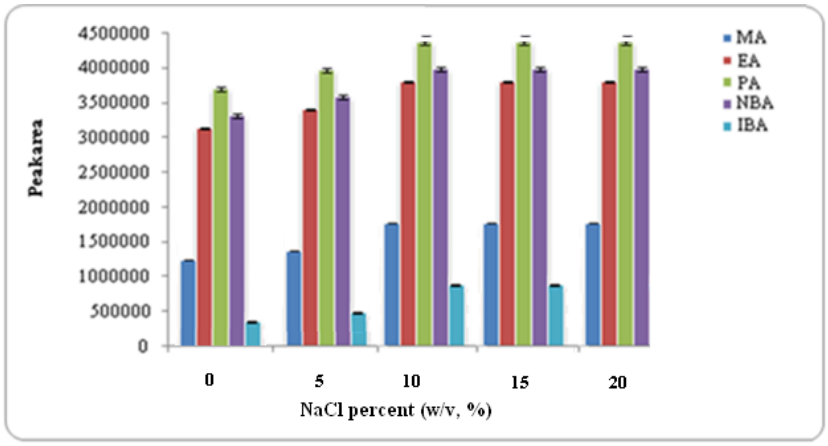

Figure 3: Effect of salt on extraction efficiency. Error bars represented the maximum and minimum values for the three measurements.

3.4. Effect of pH

The selected aliphatic amines forms ammonium salts in an acidic medium, hence their derivatization is known to bepH-sensitive. The influence of pH on the efficiencies of the derivatization reaction and extraction was investigated within a pH range of 9–12 (Fig. 4). It was found that high derivatization /extraction efficiencies were obtainable at pH as above of 11. At pH values 9 and 10, the analytical signals for the tested amines were decreased. It can be attributed to protonation of amines (pKa = 10.6–10.8) at neutral and acidic pHs. It is noted that the target amines did not derivatized at pH value 9 or less. As shown in Fig. 4, the better derivatization and extraction efficiency wouldbe obtained at pH 11 in the cases of most analytes used.

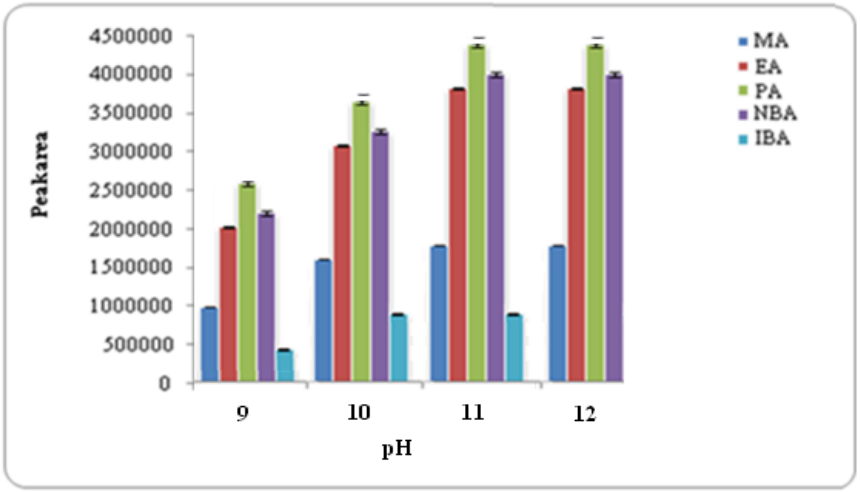

Figure 4: Effect of pH on the extraction efficiency. Error bars represented the maximum and minimum values for the three measurements.

3.5. Optimization of centrifuging time and speed

Centrifugation is a mandatory process to achieve the collection of extract ant droplets. The effect of time and speed of centrifuging were examined in the ranges of 3-10 min and 2000–5000 rpm, respectively. The obtained results showed that these parameters exerted a minor effect in the performance of the extraction and so 4000 rpm and 5 min were selected as centrifuge rate and time, respectively (Fig 5,6).

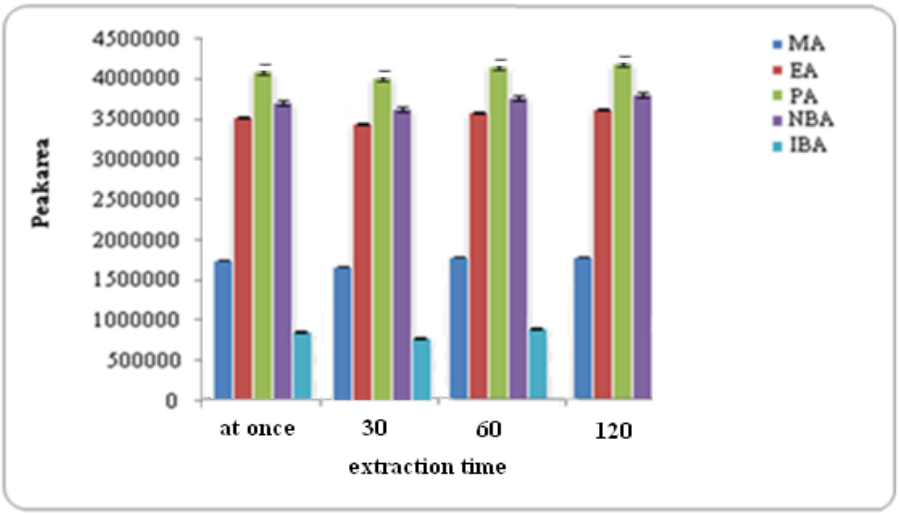

Figure 5: Effect of extraction time on extraction efficiency. Error bars represented the maximum and minimum values for the three measurements.

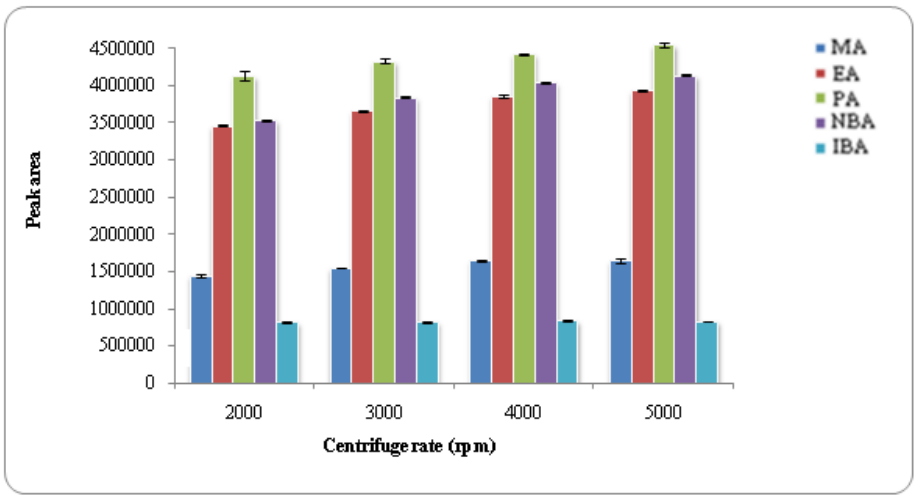

Figure 6: Effect of speed centrifuge extraction efficiency. Error bars represented the maximum and minimum values for the three measurements.

3.6. Quantitative features of the method

The analytical performance of the presented method was validated by obtaining linear range (LR), square of correlation coefficient, LOD, LOQ, EF, ER, repeatability, and robustness. Table 1summarizes most of analytical characteristics of the optimized method. The linearity was studied in the range of 10–2000 µg L^{-1}(8concentrations). The results show that wide linear ranges with goodline arities (R^2> 0.999) were achievable for the selected amines by the proposed method. High enrichment factors ranging from 397 to499 were obtained. Limits of detection and quantification were in the range 1–3 and 0.4–11µg L^{-1}, respectively. The extraction recoveries for the selected amines were in the range of 78–

99%which are acceptable for a micro extraction technique. Precision of the method was determined by analyzing spiked samples at three concentration levels (50 and 100 µg L^{-1}) at the same dayand at four different days. In the case of 50 and 100 µg L^{-1},RSDs were in the range of 3.2–5.2% for intra-day precision (n = 6). The RSD% values when50 and 100 µg L^{-1} of each analytes was added to the solution are given inTable 1.

Table 1: Analytical features of simultaneous derivatization/DLLME followed by GC-FID determination of the selected amines.

Analyte	Calibration equation	LR[a] (µg L^{-1})	LOD[c] (µg L^{-1})	LOQ[d] (µg L^{-1})	R^{2}[b]	PEE[f]	EF±SD[e] (n=6)	RSD[g] (n=6)	
								50 µg/L	100 µg/L
MA	$y = 7.5426\times10^{+4}x - 8649$	10 - 1000	1.5	5.0	0.999	81	406	3.9	3.9
EA	$y=2\times10^{+6}x + 4705$	10 - 1000	1.0	2.5	0.999	99	499	3.2	5.2
PA	$y=1\times10^{+6}x- 635$	10 - 1000	1.3	4.0	0.997	90	452	3.4	3.9
NBP	$y = 9.4285\times10^{+4}x+ 4174$	50 - 1000	2.2	8.0	0.999	96	484	5.2	3.8
IBP	$y = 2.3142\times10^{+4}x- 4037$	50 - 1000	3.0	11.0	0.999	78	397	5.0	4.6

a Linear range.
b Square of correlation coefficient.
c Limit of detection, S/N = 3.
d Limit of quantification, S/N = 10.
e Mean enrichment factor ± standard deviation, EF = C_{Sed}/C_0, (n = 6).
f percent of extraction efficiency
g Relative standard deviation (n = 6, C = 50 and C=100 ng mL^{-1})

3.7. Real samples analysis

The optimum experimental conditions were used to assess applicability of the proposed method for quantitative determination of target analytes by GC-FID and GC-MS. For this purpose, different real samples including tap, river, and well waters and municipality and amico company wastewaters were tested. None of the analytes were detected in tap, and well waters and municipality wastewater. We used of GC-MS for amico company wastewater. n-Butyl amine was found in the chromatograms obtained for amico company wastewater. The concentrations of n-butyl amine and were calculated as 198µg L^{-1} (n = 4). Typical GC-FID chromatograms of these samples are shown in Fig. 7. The results were confirmed by GC–MS for amico company wastewater samples are shown in Fig. 8. The mass data confirmed the presence of n-butylaminein amico company wastewater. According to the obtained MS spectrum of derivatized butyl amine (Fig. 8), this is a derivative fragment ion and the suggested structure for this fragment is $[Butyl–NH2–COOH]^{+}$. This fragmentprobably is produced due to de-butylation of derivatized butyl amine. Also the fragment at m/z = 57 that is observed in it. In spite of GC-FID chromatogram, no peak was observed in GC–MS chromatogram of wastewater sample for n-propylamine which its presence in wastewater sample was not confirmed. In order to evaluate the matrix effect, the samples and de-ionized water were spiked with analytes at three levels (50, 100, and 250 µg mL^{-1} of each amine)and the proposed method was applied to them (three times for each concentration). In all samples good relative recoveries compared to deionized water were obtained which indicate that there were not matrix effects in the analyzed samples(Table 3).

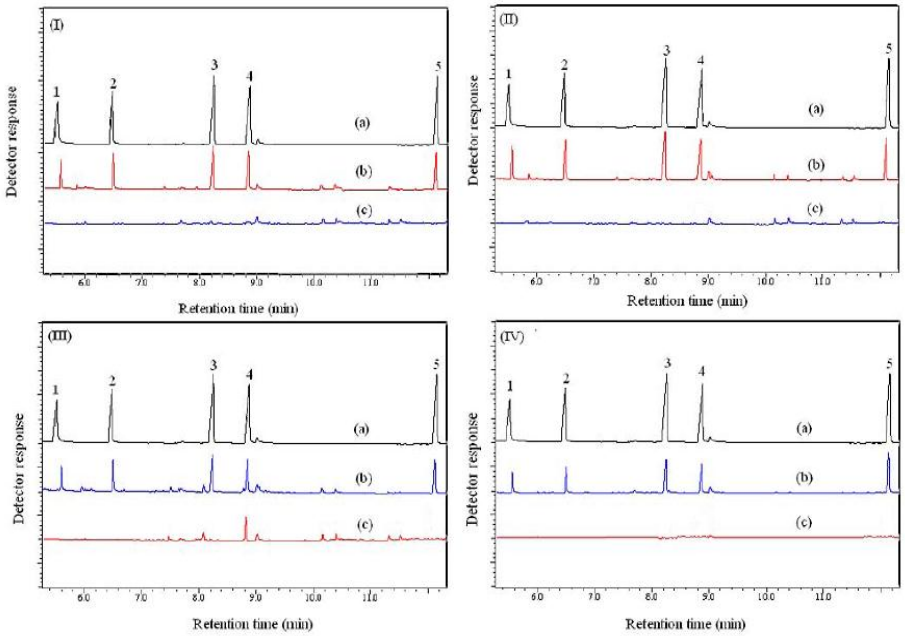

Figure 7: GC-FID chromatogram of the sample (I) Surface water collected from Tabriz West (II) drinking water Tabriz (III) Waste of Paint Company Amico and (IV) Wastewater in. The above forms chromatograms (a) of the standard solution in chloroform to a concentration of 250 milligrams per liter of dissolved analytes, (b) relating to real samples by Spike 250 µg / L of each analyte, and (c) actual sample Spike not. Identification of peaks.1: Methyl amine؛2: Ethyl amine; 3:Propyl amine; 4:*n*-Butyl amine; 5:*iso*-Butyl amine.

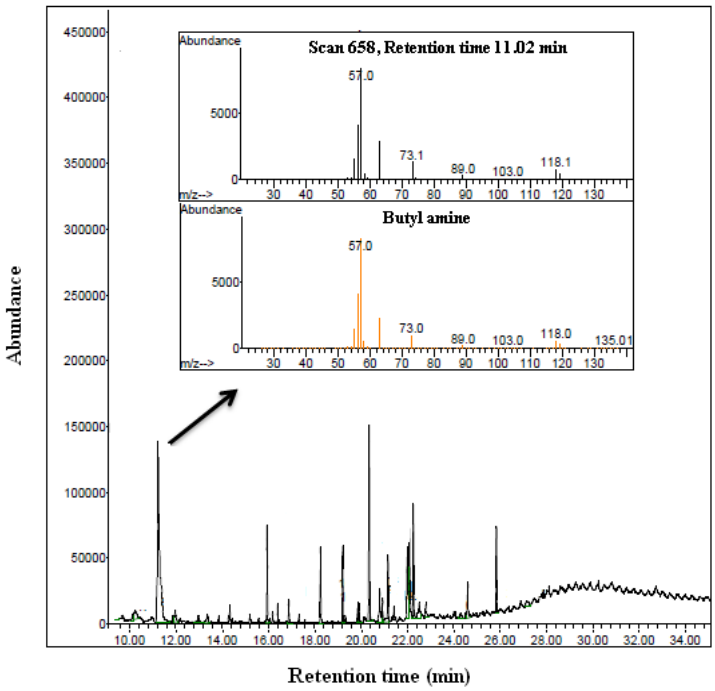

Figure 8: Total ion chromatogram recorded GC-MS and butyl amine mass range. Peak in the 11.02 and mass spectrum of the peak confirmedPresence of butyl amine.

Table 3: Relative recovery values at the concentrations of aliphatic amines.

Analytes	Mean recovery ± standard deviation (n=3)			
	Sample 1[a)]	Sample 2 [b)]	Sample 3 [c)]	Sample 4 [d)]
All samples were spiked with each analyte at a concentration of 50 ng mL^{-1}				
MA	96 ± 3	99 ± 3	96 ± 3	94 ± 4
EA	96 ± 3	99 ± 2	95 ± 4	94 ± 3
PA	98 ± 4	98 ± 2	97 ± 2	95 ± 4
NBA	97 ± 3	97 ± 2	96 ± 3	95 ± 3
IBA	94 ± 4	94 ± 3	97 ± 3	97 ± 3
All samples were spiked with each analyte at a concentration of 100 ng mL^{-1}				
MA	97 ± 2	98 ± 2	95 ± 2	93 ± 3
EA	96 ± 3	97 ± 3	94 ± 3	91 ± 2
PA	97 ± 3	97 ± 2	95 ± 2	91 ± 4
NBA	98 ± 4	98 ± 4	92 ± 2	92 ± 3
IBA	92 ± 3	96 ± 4	93 ± 3	91 ± 2
All samples were spiked with each analyte at a concentration of 250 ng mL^{-1}				
MA	99 ± 1	98 ± 2	96 ± 3	95 ± 2
EA	104 ± 2	99 ± 3	97 ± 2	96 ± 2
PA	102 ± 2	98 ± 3	101 ± 3	97 ± 2
NBA	98 ± 3	97 ± 4	99 ± 3	97 ± 1
IBA	97 ± 2	98 ± 3	98 ± 1	96 ± 3

a) Water b) Wastewater Tabriz
c) Surface water d) Amico painting company`s Wastewater

4. CONCLUSION

In this study, simultaneous derivatization and air-assisted liquid–liquid micro extraction of some aliphatic amines was performed directly in aqueous media. The optimized technique in conjunction with GC-FID and GC-MS was considered as an efficient and economical procedure for analysis of some primary aliphatic amines in different aqueous samples. The developed method has numerous advantages such as rapidness, simplicity, and excellent repeatability. The results revealed that the developed method was suitable for determination of some butyl amine at µg L^{-1} levels in wastewater samples of the Amico painting company.

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