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Automated determination of aliphatic primary amines in wastewater by simultaneous derivatization and headspace solid-phase microextraction followed by gas chromatography–tandem mass spectrometry

Anna Llop, Eva Pocurull*, Francesc Borrull

Department of Analytical Chemistry and Organic Chemistry, Rovira i Virgili University, Sescelades Campus, Marcel·lí Domingo s/n, Tarragona 43007, Spain

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ABSTRACT

This paper presents a fully automated method for determining ten primary amines in wastewater at ng/L levels. The method is based on simultaneous derivatization with pentafluorobenzaldehyde (PFBAY) and headspace solid-phase microextraction (HS-SPME) followed by gas chromatography coupled to ion trap tandem mass spectrometry (GC–IT-MS–MS). The influence of main factors on the efficiency of derivatization and of HS-SPME is described in detail and optimized by a central composite design. For all species, the highest enrichment factors were achieved using a 85 μm polyacrylate (PA) fiber exposed in the headspace of stirred water samples (750 rpm) at pH 12, containing 360 g/L of NaCl, at 40 °C for 15 min. Under optimized conditions, the proposed method achieved detection limits ranging from 10 to 100 ng/L (except for cyclohexylamine). The optimized method was then used to determine the presence of primary amines in various types of wastewater samples, such as influent and effluent wastewater from municipal and industrial wastewater treatment plants (WWTPs) and a potable water treatment plant. Although the analysis of these samples revealed the presence of up to 1500 μg/L of certain primary amines in influent industrial wastewater, the concentration of these compounds in the effluent and in municipal and potable water was substantially lower, at low μg/L levels. The new derivatization–HS-SPME–GC–IT-MS–MS method is suitable for the fast, reliable and inexpensive determination of primary amines in wastewater in an automated procedure.

1. Introduction

Aliphatic primary amines such as methylamine, ethylamine, *n*-butylamine and cyclohexylamine are important intermediates in the production of dyestuffs, pharmaceuticals, corrosion inhibitors and polymers [1–6]. Some aliphatic amines are produced in quantities of more than one million tons per year in Western Europe [7]. In addition to their industrial applications, amines may occur as biodegradation products of proteins and aminoacids or other nitrogen-containing compounds [6,8,9]. Most of them are toxic, sensitizers and irritants to the skin, mucous membranes and the respiratory tract [10,11]. Moreover, aliphatic amines can react with nitrite, forming carcinogenic nitrosamines [3,4,12]. Amines may cause environmental contamination and have been detected in biological fluids and environmental samples, usually at trace levels [5]. Their determination is important in the chemical and pharmaceutical industries [13,14] and they have often been found in foods [9,15] and wines [8]. Even though amine content in water is not currently regulated, European legislation establishes content values of 0.5 and 1 mg/L for ammonium and Kjeldahl nitrogen for consumption water, respectively, and between 15 and 85 mg/L for wastewater [3]. Up to now, little information has been available on the occurrence of aliphatic amines in wastewater and surface water. Some researchers have detected aliphatic amines at the ng/L to μg/L levels in river water [6], in lake water [4] and in wastewater samples [1,12,13].

Analysis of aliphatic amines in aqueous samples has traditionally been difficult due to the particular physicochemical properties of aliphatic amines, such as high volatility and polarity, basic character and high solubility in water [1,12]. The most widely used techniques for determining amines in water samples are gas chromatography (GC) and liquid chromatography (LC). GC analysis is often problematic because of the high polarity of amines and their hydrogen-bonding properties, which result in tailing peaks and memory effects [4]. Furthermore, aliphatic amines exhibit poor chromatographic performance and do not have any structural features that could allow their detection without derivatization. They undergo α-cleavage, usually resulting in a base peak at *m/z* 30 ($CH_2{=}NH_2^+$) that provides little scope for confirmation of identity or quantification through selected ion monitoring

* Corresponding author. Tel.: +34 977558492; fax: +34 977558446.
E-mail address: eva.pocurull@urv.cat (E. Pocurull).

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(SIM) [2]. LC analysis is also difficult due to the low absorptivity of aliphatic amines in UV–vis and also because they do not have fluorescent properties. In order to improve the properties of amines, several GC and HPLC methods have been developed that involve a derivatization step prior to chromatographic analysis [1,3]. Derivatization reactions of amines have been reviewed by Kataoka [16], and derivatization with pentafluorobenzoyl chloride or pentafluorobenzaldehyde (PFBAY) followed by GC is often preferred over LC in environmental samples due to its superior selectivity and sensitivity [5,13,16–18]. In conventional methods, liquid-liquid extraction (LLE) [1,6,19] and solid-phase extraction (SPE) [2,3,15,20] are often used to isolate and preconcentrate aliphatic amines from aqueous phase into organic phase prior to or following derivatization. Solid-phase microextraction (SPME), developed in the early 1990s by Pawliszyn et al. [21] is uniquely capable of incorporating extraction and concentration in a singe step. The technique offers many advantages: it is a solvent free and efficient technique, it has a high concentrating efficiency, it is simple to perform and it is easy to automate [4,7–9,11–13,21,22].

The main aim of this study was to develop a rapid, automated and sensitive method that could be applied to potable, municipal and industrial wastewater samples from various wastewater treatment plants (WWTPs) in order to determine ten primary amines. This paper presents an analytical procedure that enables the precise determination of amines using simultaneous derivatization with PFBAY and HS-SPME followed by separation and detection by gas chromatography coupled with an ion trap tandem-in-time mass spectrometry detection system (GC–IT-MS–MS). IT-MS–MS was selected as the detector because of its ability to perform simultaneous quantitative analysis and characterization of trace level compounds, and because the use of MS–MS detection, rather than single MS, was expected to increase the selectivity of the determinations for complex matrices such as wastewater samples. This study showed, for the first time, the determination of primary amines at ng/L levels in various types of wastewater by means of fully automated derivatization–HS-SPME–GC–IT-MS–MS. The influence of various parameters on the efficiency of the derivatization and of SPME is described in detail and optimized by a central composite design, and the method's performance is compared to that of previously reported methods.

2. Experimental

2.1. Reagents and solutions

Methylamine (MA) (40 wt.% in H_2O), ethylamine (EA) (70 wt.% in H_2O), isopropylamine (IPA), isobutylamine (IBA), *n*-butylamine (BA), isoamylamine (IAA), amylamine (AA), *n*-heptylamine (HA), 2-phenylethylamine (PEA), cyclohexylamine (CA), methyl-d_3-amine hydrochloride (dMA) that was chosen as a surrogate standard, 2,3,4,5,6-pentafluorobenzaldehyde (PFBAY) that was the derivatization reagent and sodium chloride were supplied by Aldrich (Steinheim, Germany). The purity of all standards was greater than 98%. Sodium hydroxide and hydrochloric acid were obtained from Scharlau Chemie (Barcelona, Spain). Acetonitrile and ethyl acetate were purchased from SDS (Peypin, France) and were HPLC grade. Ultrapure water was obtained using a Milli-Q purification system (18.2 MΩ cm) (Millipore, Bedford, MA, USA).

Individual stock standard solutions of each aliphatic amine and PFBAY solution were prepared in acetonitrile at a concentration of 2000 mg/L. A stock standard solution of dMA at 2000 mg/L was prepared in pure water and acetonitrile (1:1). The working mixed solution of 1 mg/L was prepared weekly by diluting different amounts of each stock standard solution with acetonitrile. All solutions were stored in darkness at 4 °C.

2.2. Instrumentation

GC–MS analysis were performed using a Varian 3800 gas chromatograph (Varian, Walnut Creek, CA, USA) connected to a Varian 4000 ion trap mass detector. The GC was equipped with a 1079 programmable vaporizing temperature (PTV) injector, a Merlin high-pressure microseal and a 0.8 mm i.d. insert liner (Varian). A fused silica capillary column (3 m × 0.25 mm i.d.) from Supelco (Bellefonte, PA, USA) was used as a guard column connected to a ZB-5 analytical column (30 m × 0.25 mm i.d.; 0.25 μm film thickness) from Torrance (CA, USA). Helium (99.9999%) from Carburos Metálicos (Tarragona, Spain) was used as a carrier and collision gas at a flow rate of 1 mL/min. Varian Workstation software was used for instrument control and data processing.

The 85 μm polyacrylate (PA) and 60 μm polyethyleneglycol (PEG) fibers used in this study were purchased from Supelco. The fibers were conditioned prior to use according to the supplier's instructions by inserting them into the GC injector.

In the derivatization and HS-SPME optimization, the experimental design matrix and data analysis were performed using the Statgraphics statistical computer package "Statgraphics Plus 5.1" (Manugistics, Inc., Rockville, MD, USA). A CombiPAL autosampler (CTC Analytics, Zwingen, Switzerland) was used for the derivatization and extraction.

2.3. Analytical methods

2.3.1. Optimization of chromatographic separation

We optimized the chromatographic separation of the compound derivates by following previous studies [8,13]. Thus, each compound, including the surrogate, was individually derivatized by adding 50 μL of amine (2000 mg/L) and 75 μL of PFBAY (2000 mg/L) in a 20 mL vial that had previously been filled with 10 mL of Milli-Q water at pH 12 adjusted with NaOH (1 M). A stir bar was added and the vial was then sealed and placed in a water bath at 80 °C for 30 min. The mixture was then cooled to room temperature and 2 mL of ethyl acetate was added to the vial in order to extract the derivates by LLE. Finally, 1 μL of the ethyl acetate extract was injected into the GC.

The injector temperature was set at 250 °C and the analyses were done in splitless mode. The column oven was programmed as follows: the temperature was initially set at 60 °C, was increased by 20 °C/min to 135 °C, was then increased by 30 °C/min to 290 °C, and was held for 1.08 min. The total run time was just 10 min. The transfer line, manifold and trap temperatures were 280, 60 and 240 °C, respectively. A filament-multiplier delay of 3.40 min was established in order to prevent instrument damage. The analytes were ionized by electron impact (70 eV). The MS–MS process was carried out by collision-induced dissociation (CID) with resonant or non-resonant excitation, depending on the compound, since the response obtained was adequate and more reproducible. Table 1 shows the optimal MS–MS parameters for each compound.

2.3.2. Derivatization and headspace solid-phase microextraction (HS-SPME)

PA and PEG fibers were thermally conditioned in accordance with the manufacturer's recommendations by inserting them into the GC injector port. The used fibers were cleaned by heating them at 250 °C for 10 min prior to extraction, and a blank test was performed to check for possible carry-over. The entirely automated extractions were performed by a commercial autosampler CombiPAL commercial autosampler mounted on the GC–MS system.

Standards and filtered water samples were adjusted to pH 12 by the dropwise addition of NaOH (1 M). Since some precipitate substances appeared, the samples were filtered again through a 0.45 μm nylon membrane filter (Whatman, Maidstone,

Table 1
Retention time and MS–MS parameters for the studied primary amines using the proposed method.

Segment	Compound	Retention time (min)	Parent ion (*m*/*z*)	*m*/*z* range	CID parameters		Product ions[a] (*m*/*z*)
					Storage level (*m*/*z*)	Amplitude (V)	
1	dMA[b]	3.67	211	81–221	80.2	1.2	190, **183**, 161
	MA	3.66	208	81–218	80.2	1.2	188, **181**, 158
2	EA	4.12	208	80–218	80.2	71	**181**, 161, 158
	IPA	4.29	222	86–232	85.6	78	**181**, **145**, 175
3	IBA	5.04	208	80–218	80.2	72	**181**, 161, 158
	BA	5.31	208	80–218	80.2	72	188, **181**, 158
	IAA	5.64	208	80–218	80.2	72	**181**, 158
	AA	5.82	250	96–260	96.4	79	**187**, 181
4	CA	6.52	248	96–258	95.6	88	233, 228, **181**, **151**
5	HA	6.73	250	96–260	96.4	80	207, **187**, **181**
6	PEA	7.44	208	80–218	80.2	70	**181**, 158

[a] Quantification ions (*m*/*z*) are shown in bold type.
[b] Surrogate standard.

UK). Of these filtered water samples, 10 mL was poured into 20 mL headspace vials containing 3.6 g of sodium chloride and a magnetic stirring bar. dMA (100 μL of 1 mg/L) was added to the sample as a surrogate and PFBAY was then added to the vial as a derivatization reagent (200 μL of 2000 mg/L). The vial was immediately sealed tightly using a screw cap with a PTFE silicone-faced septum and placed in the tray for SPME. When the temperature of the heat/stir accessory reached 40 °C, the vial was automatically transported there and was stabilized for 1 min. The fiber was then introduced through the septum and kept in the headspace of the vial for 15 min at 40 °C. During the extraction, the sample was magnetically stirred at 750 rpm. Subsequently, the fiber was withdrawn into the SPME syringe needle, which was then pulled out of the sample vial and immediately inserted into the GC injection port for desorption. The desorption was conducted at 250 °C for 8 min. Finally, the compound derivates were analysed by GC–MS at the same conditions described in Section 2.3.1.

2.4. Sampling

Several types of wastewater samples were collected from three different industrial wastewater plants (A, B and C), from a municipal wastewater plant (D), and from a potable water plant (E), all located on the outskirts of Tarragona (Spain). These waters had different origins and matrix complexities and had also undergone different treatment processes, such as conventional activated sludge (CAS) treatment or membrane bioreactor (MBR) treatment. For each sample, 500 mL was put in a glass bottle, acidified with hydrochloric acid (pH 3), filtered through a 0.45 μm nylon filter (Whatman, Maidstone, UK) and stored at 4 °C until analysis.

The industrial WWTP A is a CAS treatment plant that treats a mixture of wastewater from three different chemical plants that make products of various types, such as surfactants, vinyl acetate and plastics (isocyanides, polyurethanes and ABS). The industrial WWTP B is an MBR treatment plant that uses ultrafiltration membranes to treat wastewater from industrial plants of all sorts. The industrial WWTP C is an MBR treatment plant that uses ultrafiltration membranes to treat wastewater from the distillation of used oil. The municipal WWTP D is a CAS treatment plant that uses reverse osmosis after secondary treatment. It treats water from a population of approximately 35,000 inhabitants. The potable water plant E is a CAS treatment plant that uses carbon filters in the last process to obtain a high-quality effluent. Samples were taken from the influent of the biological reactor (of the CAS and MBR plants) and from the effluent (treated water from the secondary treatment) of each WWTP and from the potable water plant. In WWTP D, samples were also taken from the permeate of the reverse osmosis membranes.

3. Results and discussion

3.1. GC–MS–MS optimization

Derivatization with PFBAY and LLE with ethyl acetate was performed for each compound as explained in Section 2.3.1 in order to identify the derivates and then optimize their separation. The derivates were identified by MS operating in full-scan mode in the range of 50–500 *m*/*z*. The mass spectra of the PFBAY-imine-derivates showed typical fragment ions at *m*/*z* 208 and 181. The *m*/*z* 181 ion corresponds to the fragment $[CH–C_6F_5]^+$. When the aliphatic amine was unsubstituted in the α-position, we observed an *m*/*z* 208 product fragmentation ion, which in most cases was the base peak ion. This ion was one of the typical α-cleavage product ions, and it corresponded to $[CH_2–N{=}CH–C_6F_5]^+$ [8,13]. This ion was observed in all cases except for dMA, IPA and CA. The base peak of dMA was *m*/*z* 211. This agreed with the molecular structure of the compound, which has three deuterated protons. The other amines substituted in the α-position, IPA and CA, did not have the *m*/*z* 208 ion, but in the case of IPA we observed the *m*/*z* 222 base peak ion corresponding to the loss of a methyl group, $[M\text{-}CH_3]^+$, and in the case of CA, we observed the *m*/*z* 248 base peak ion, corresponding to the loss of an ethyl group, $[M\text{-}C_2H_5]^+$. AA and HA had *m*/*z* 250 as a base peak the ion, corresponding to the loss of a propyl group, $[M\text{-}C_3H_7]^+$.

Once all the derivates were identified, a standard mixed solution including each amine and the dMA was derivatized and extracted with ethyl acetate to optimize the chromatographic separation. All derivates were separated in just 10 min using the chromatographic conditions described in Section 2.3.1.

In order to maximize the sensitivity of each compound, MS–MS optimization was performed, taking as a precursor ion the most abundant one that was selective enough. Table 1 shows that the parent ion selected in most of the cases were *m*/*z* 208. The MS–MS was optimized for each compound in order to select an amplitude excitation voltage able to give the maximum abundance of one of the products ions (100%) and a relative abundance of the parent ion between 10 and 20%. The isolation window of the parent ions of dMA and MA was 2 *m*/*z* units; for the parent ions of the rest of the compounds, we used an isolation window of 3 *m*/*z* units. The parent ions were submitted to CID in resonant mode for dMA and MA and in non-resonant mode for the rest of the derivates. The most abundant product ion found in most of the derivates was *m*/*z* 181, which points to the fragment $[CH–C_6F_5]^+$.

Section 2.3.1 describes the optimal chromatographic conditions, and Table 1 summarizes the retention time and the MS–MS parameters used for each compound (segment, parent ion, CID parameters, m/z range and product ions).

3.2. Derivatization and HS-SPME optimization

One objective of this study was to select the best conditions for the simultaneous derivatization and HS-SPME of primary amines from aqueous samples. The performance of microextraction methods, particularly when they also involve a derivatization stage, is potentially affected by many factors. In order to optimize the method, we considered some of the optimal values reported by researchers in previous studies as initial derivatization and headspace microextraction conditions [8,13].

Since primary aliphatic amines and their imine-derivates are polar and hydrophilic compounds, according to the rule 'like dissolves like', polar fibers are preferred. Two types of polar fibers were selected to optimize the extraction: 85 μm PA and 60 μm PEG. PA had been tested by other researchers for the extraction of some of the analytes obtaining good results [13], and PEG is a relatively new coating fiber that has been used to extract moderately and highly polar analytes (aldehydes, ketones, aromatic amines, phenols, alcohols and acids) [23] but to our knowledge has never been tested for the extraction of aliphatic amines. Since PA and PEG fibers have different structures and could therefore be affected differently by some variables, we optimized the extraction of each type of fiber individually. For each type of fiber we had some fixed variables and some variables to optimize. We first fixed the experimental variables that are best established in the literature, such as the sample pH, the derivatizing reagent (PFBAY) concentration, the sample agitation, the extraction mode (direct extraction or headspace extraction), the desorption temperature, and the desorption time. Afterwards, using an experimental design, we optimized the variables that are the most relevant: the fiber coating, the derivatization and extraction temperature, the derivatization and extraction time and the salt concentration. We considered the derivatization and extraction temperature and the time of derivatization and extraction as a single global factor to optimize in each case. A central composite design, which is probably the most widely used experimental design for fitting second-order response surfaces, was used to obtain the optimal conditions for each type of fiber.

Amines are bases that are easily protonated by water. To perform derivatization with PFBAY, in the first step of the reaction in water to produce the imine-derivates, the analytes must be in their non-ionic form in order to prevent their protonation. The required pH conditions depended on the pk_a values of the protonated conjugated acidic form of the amines. In the studied amines, this value was around 10.6 [13]. We therefore fixed the pH of the samples at 12 by adding NaOH (1 M). Some researchers have tested pH conditions for the derivatization step and observed at excessively high pH (13.5) PFBAY degraded to a geminal diol, which prevents its reaction with amines [8]. The concentration of the derivatization reagent (PFBAY) must be taken into account in the derivatization reaction. PFBAY must be present in greater quantities than the amines in order to have high reaction efficiencies. Since 10 μg/L of amines in 10 mL water was used for optimization, 40 mg/L of PFBAY was used. Agitation has a strong effect on SPME kinetics and speeds up the equilibrium process, and for a given extraction time, responses are higher with agitation than without it. In our studies, we stirred the samples at the CombiPAL autosampler accessory's maximum available speed, which was 750 rpm. It was possible to use direct or headspace sampling for the analysis of the imine-derivates; headspace extraction was selected because derivatization produces highly volatile imines and, furthermore, headspace mode is preferred with high-complexity samples such as wastewater, specifically industrial wastewater. The desorption temperature in the GC injector must be high enough to desorb all of the imine-derivates, but the stability and lifetime of the fiber must also be considered. We took into account the temperatures recommended by the supplier of the fibers and selected 250 °C. The desorption time was set at 8 min in each case in order to avoid possible injector contamination and carry-over effects.

Table 2
Factor levels of the experimental design.

Variable	Low (−1)	High (+1)	Centre (0)
Extraction temperature (°C)	40	80	60
Extraction time (min)	15	60	37.5
Salt concentration (g/L NaCl)	0	360	180

3.2.1. Optimization by a central composite design

The factors selected for each type of fiber (PA and PEG) as potentially affecting the derivatization and extraction were derivatization and extraction temperature, derivatization and extraction time, and salt concentration. In order to study the effect of these three factors, a central composite design (with $\alpha = 1.67$) was created in three orthogonal blocks using surface response. The Statgraphics statistical package was used to generate the experimental matrix and calculate the standardized main effects of the factors considered. The design involved 17 experiments, which were performed in random order in order to protect against the effects of lurking variables. Table 2 shows the values corresponding to the low (−1), high (+1) and centre (0) values for each factor. The individual chromatographic peak area of each derivatized compound was regarded as an experimental response for optimizing. Pareto charts were used to identify the most influential factors. The data obtained in each central composite design were evaluated by ANOVA at the 5% significance level. These results are shown in bar chart format, with the effects sorted in rank order. For instance, Fig. 1 shows the Pareto chart for the area of butylamine derivatized and extracted using PA fiber. The information shown is similar to that in the Pareto chart for other compounds, and also using PEG fiber. In most cases, temperature was the most important parameter in the derivatization and extraction of the compounds. The chromatographic areas were largest when the temperature was at the lowest level (40 °C). The NaCl concentration was the second most important parameter in seven of ten compounds, and the areas were largest when the NaCl concentration was at its highest level (360 g/L NaCl). Time was the least influential factor, and the areas were largest when time was at its lowest level (15 min). These results agree with the fact that the derivatization reaction can proceed rapidly in aqueous solution at room temperature and provide good yields [5,16]. Furthermore, high temperatures could result from a lesser affinity between the fiber and the analytes in the headspace. The results obtained for NaCl concentration were also expected, since salt addition increases extraction efficiency, especially for polar and volatile compounds, such as aliphatic amines. For instance, Fig. 2 shows the response surface graph obtained by plotting derivatization and extraction temperature versus NaCl concentration for a derivati-

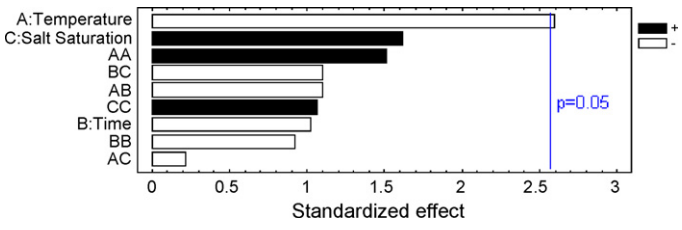

Fig. 1. Standardized Pareto chart of the main effects in the central composite design for BA using PA fiber. The line represents the significant limit.

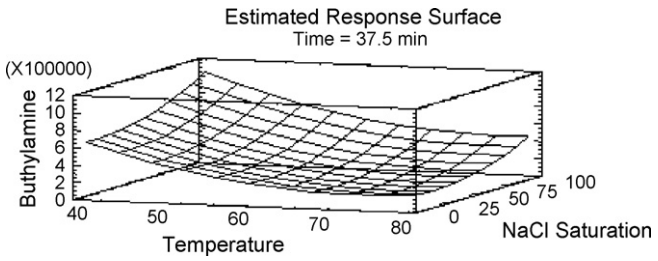

Fig. 2. Estimated response surface for BA obtained using the central composite design by plotting derivatization and extraction temperature versus the NaCl concentration in PA fiber.

zation and extraction time of 37.5 min for butylamine derivatized using PA fiber. The largest areas are found for 40 °C and 360 g/L NaCl. Comparing the best conditions obtained for PA and PEG fibers in each compound (40 °C, 15 min and 360 g/L NaCl), larger response areas were obtained using the PA fiber in all cases (see Fig. 3). PA fiber was therefore used in further experiments.

4. Method validation

The analytical validation of the simultaneous derivatization and HS-SPME–GC–MS–MS method for the analysis of water samples was performed by establishing linear range, repeatability, reproducibility between days, and detection and quantification limits using secondary effluent from municipal WWTP samples as blanks.

In order to improve the reproducibility of the method, the linear range was obtained by analysing spiked concentrations ranging from 0.025 to 20 μg/L of amines and using 10 μg/L of dMA as an internal standard. Five blanks were analysed and the averaged peak area of each compound was subtracted from the peak area of each spiked analysis. The calibration curves by internal standard were linear, with correlation coefficients (R^2) higher than 0.992 for all target compounds (Table 3). The intra-day (repeatability) and inter-day (reproducibility) precision of the method were determined by means of five determinations of the secondary effluent WWTP wastewater samples spiked at the same concentration (1 μg/L). Table 3 shows that the relative standard deviations (RSDs) for intra-day precision ranged from 2 to 7% whereas the RSD values for inter-day precision ranged from 5 to 12%. The limits of detection (LODs) of the method were defined for a signal-to-noise ratio of 3 for all compounds and ranged from 10 to 100 ng/L. The LOD of cyclohexylamine was one order of magnitude higher than that of the other compounds (2500 ng/L). The limits of quantification (LOQs), calculated as the concentration of the lowest point of the calibration curve, ranged from 0.025 to 7.5 μg/L. The LODs and LOQs obtained in our study were comparable to and slightly better than those obtained in other studies determining only some of the studied amines and using different detection systems or different extraction techniques, such as gas chromatography-flame ionization detection (GC-FID) and liquid-phase microextraction (LPME) [5,13]. It should be noted that we included a larger number of compounds and used MS–MS detection, which allowed a more accurate identification of compounds.

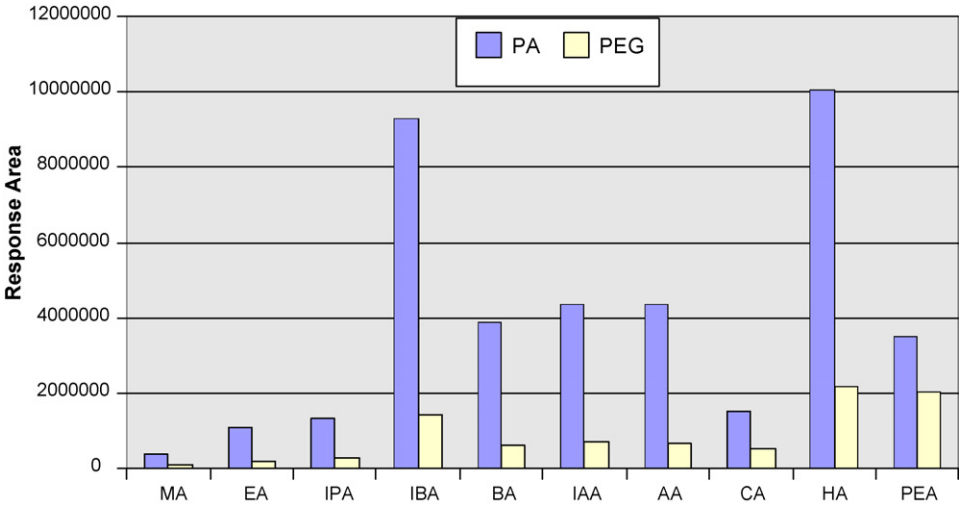

Fig. 3. Comparison of the PA and PEG fibers in the extraction of 10 μg/L of each amine as its PFBAY-imine under the optimal conditions.

Table 3
Method linear ranges, correlation coefficients (R^2), LODs, LOQs, repeatability and reproducibility between days (% RSD). See text for other conditions.

Compound	Linear range (μg/L)	R^2	LOD (ng/L)	LOQ (ng/L)	Repeatability[a] (% RSD)	Reproducibility[a] (% RSD)
Methylamine	0.025–20.0	0.998	10	25	3	7
Ethylamine	0.050–17.5	0.994	10	50	5	11
Isopropylamine	0.100–5.0	0.993	25	100	4	9
Isobutylamine	0.025–10.0	0.997	10	25	3	7
Butylamine	0.025–20.0	0.995	10	25	2	6
Isoamylamine	0.200–20.0	0.998	50	200	3	5
Amylamine	0.750–20.0	0.998	100	750	6	10
Cyclohexylamine	7.500–20.0	0.993	2500	7500	7	11
Heptylamine	0.200–17.5	0.997	100	200	2	5
2-Phenylethylamine	0.025–20.0	0.996	10	25	6	10

[a] $n = 5$; 1 μg/L.

5. Method application

The developed method was used to determine the primary amines in wastewater samples collected from several WWTPs and a potable water plant (see Section 2.4). During the analysis of the industrial wastewater samples, a strong matrix effect was observed. The analysis showed that the most complex samples (industrial wastewater) affected the measured peak area of the internal standard (dMA). In fact, the peak size of the internal standard decreased when the complexity of the samples increased, because the derivatization and extraction processes were influenced by the constituents present in the medium. Thus, the quantification of primary amines by derivatization and HS-SPME turned out to be highly dependent on the composition of the matrix. In order to overcome this difficulty in relatively complex matrices, we diluted the samples and quantified primary amines according to the method of standard addition. For the quantification of each compound, a calibration curve was constructed using least-square linear regression of standard solutions of primary amines to the internal standard. As an example, Fig. 4 shows the derivatization–HS-SPME–GC–MS–MS chromatograms of the primary amines of a non-spiked effluent sample from industrial WWTP A. All compounds appeared in the sample at levels ranging from 0.20 to 25 μg/L.

Table 4 shows the results of the average concentrations of the studied compounds found in each type of sample ($n = 3$). As expected, the levels of most of the compounds were higher for the samples from WWTPs A, B and C (industrial wastewater) than for the samples from WWTP D (municipal wastewater) and potable water plant E. The concentrations of IBA and CA in the influent of WWTP A stand out for their high values, around 1500 μg/L. These values were expected because one of the industries that sends its water to WWTP A uses these compounds to make its products. For the same sample origin, the effluent did not correspond exactly to the treated influent because of differences in hydraulic retention time. Therefore, we were unable to perform a strict comparison between influent and effluent concentrations, but instead only make a comparison in general terms. In most cases, the amine

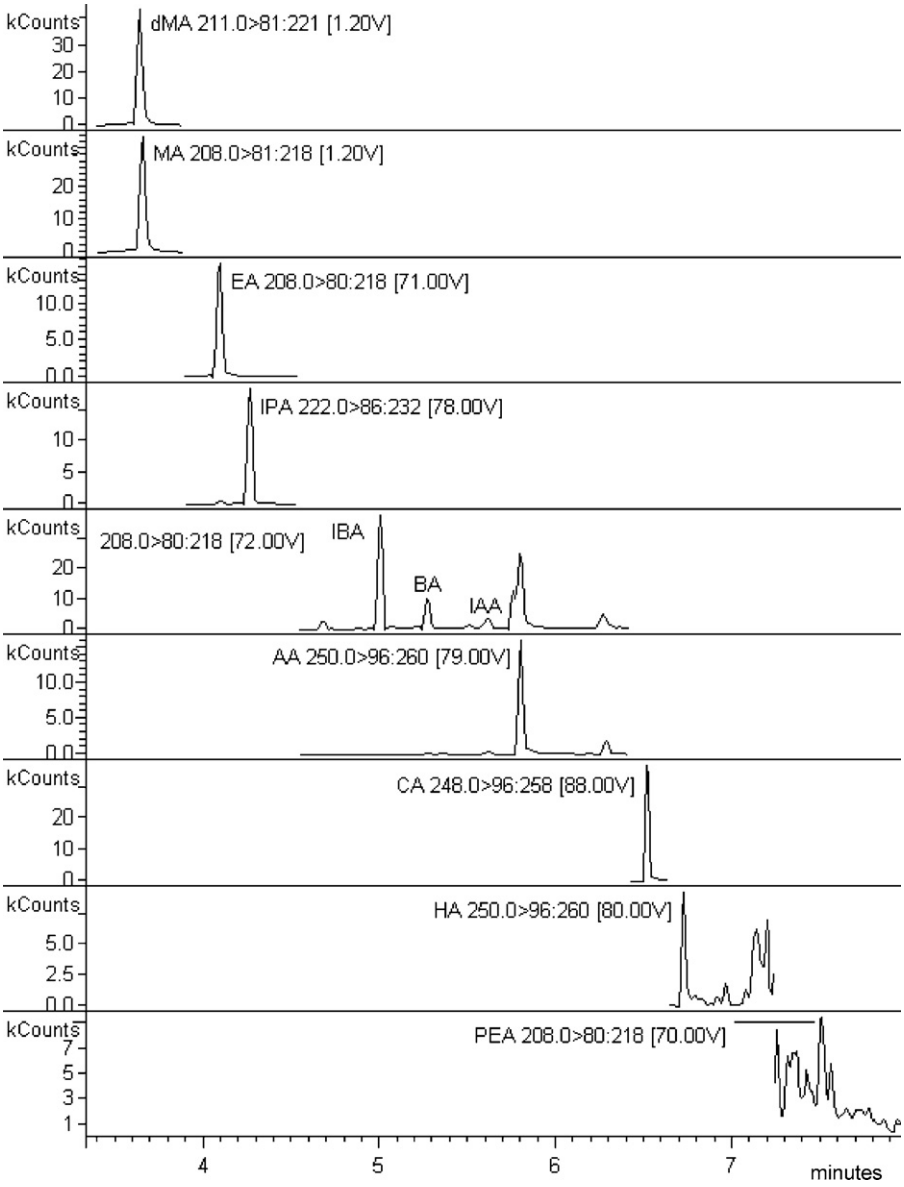

Fig. 4. Derivatization–HS-SPME–GC–MS/MS chromatograms of primary amines of a non-spiked effluent industrial WWTP A sample. Spiked dMA concentration at 20 μg/L. Extraction conditions: PA fiber, 40 °C, 15 min, 360 g/L of NaCl.

Table 4
Concentration (μg/L) of primary amines in the analysis of wastewater (n = 3, RSD <12%).

Compound	WWTP A		WWTP B		WWTP C		WWTP D			Potable water plant E	
	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent	Effluent osmosis	Influent	Effluent
Methylamine	94	25	35	29	51	192	28	1.8	0.43	1.2	0.28
Ethylamine	9.6	2.2	36	10	180	36	4.1	0.47	0.52	0.24	0.30
Isopropylamine	3.2	3.5	20	11	45	5.8	2.3	0.54	1.8	1.7	0.30
Isobutylamine	1500	0.88	5.5	1.3	66	3.4	3.5	0.61	0.18	0.57	0.12
Butylamine	5.4	0.29	0.99	1.5	97	9.6	0.43	0.07	0.14	0.95	0.33
Isoamylamine	2.1	0.20	4.7	1.9	45	2.3	4.5	0.64	0.42	n.q.	n.q.
Amylamine	1.2	2.0	n.q.	n.q.	37	2.1	n.q.	n.q.	n.q.	n.d.	n.q.
Cyclohexylamine	1500	13	17	28	43	82	12	13	n.d.	n.d.	n.d.
Heptylamine	1.2	0.32	0.37	0.26	2.2	0.98	n.q.	n.q.	n.d.	n.d.	n.d.
2-Phenylethylamine	2.9	0.40	0.90	0.60	2.6	0.45	15	0.13	n.q.	n.d.	0.03

n.d.: not detected; n.q.: not quantified.

concentration was higher in the influent than in the effluent. Thus, it seems that the treatment processes causes a partial reduction in these compounds.

Studies found in the literature have determined different levels of certain primary amines in industrial wastewater. Sacher et al. [1] determined MA, EA and BA at concentrations ranging from 1 to 30 μg/L in industrial wastewater and Pan et al. [13] detected the presence of 700 μg/L of MA in similar samples. Our results agree with those already mentioned. In river water, Akyüz et al. [6] detected MA, EA, BA and PEA concentrations ranging from 0.26 to 83.02 ng/L. No information about potable water was found in the literature.

6. Conclusions

The fully automated derivatization–HS-SPME–GC–IT-MS–MS method was shown to be fast, simple, sensitive and suitable for determining ten primary amines in wastewater at ng/L levels. Simultaneous derivatization was done with PFBAY without using any organic solvent.

The most important parameters involved in the derivatization and extraction processes were evaluated using a central composite design. Under optimized conditions, derivatization and extraction were performed with a PA fiber in headspace mode at 40 °C for 15 min in the presence of 360 g/L sodium chloride.

The proposed method avoids the use of organic solvents, achieves low LODs between 10 and 100 ng/L (except for cyclohexylamine), and offers satisfactory precision (RSD ≤11%). In addition, the entire analytical process, including sample preparation and determination, is fully automated and performed in less than 30 min, which enables high sample throughput. Moreover, the use of MS–MS rather than single MS detection provides high selectivity for the determination of primary amines in very complex matrices such as industrial wastewater samples.

Several wastewater samples, including industrial wastewater, municipal wastewater and potable water, were analysed in order to assess the applicability of the method. Although no matrix effects were observed for the less complex samples and an internal standard calibration curve was calculated, the industrial wastewater samples showed matrix effects. As a result, quantification had to be performed using standard addition. Most of the studied compounds were found in the influent and the effluent of the three industrial WWTPs at concentrations ranging from 0.20 to 1500 μg/L. In the influent and effluent municipal WWTP the amine concentrations of the detected compounds were lower, ranging from 0.07 to 28 μg/L, and even more lower in the influent and effluent of the potable water plant, ranging from 0.03 to 1.7 μg/L.

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