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Development and validation of a hydrophilic interaction liquid chromatographic method for determination of aromatic amines in environmental water

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ABSTRACT

A simple, precise, and accurate hydrophilic interaction liquid chromatographic (HILIC) method has been developed for the determination of five aromatic amines in environmental water samples. Chromatography was carried out on a bare silica column, using a mixture of acetonitrile and a buffer of NaH_2PO_4–H_3PO_4 (pH 1.5, containing 10 mM NaH_2PO_4) (85:15, v/v) as a mobile phase at a flow rate of 1 mL min^{-1}. Aromatic amines were detected by UV absorbance at 254 nm. The linear range of amines was good ($r^2 > 0.998$) and limit of detection (LOD) within 0.02–0.2 mg L^{-1} (S/N = 3). The retention mechanism for the analytes under the optimum conditions was determined to be a combination of adsorption, partition and ionic interactions. The proposed method was applied to the environmental water samples. Aromatic amines were isolated from aqueous samples using solid-phase extraction (SPE) with Oasis HLB cartridges. Recoveries of greater than 75% with precision (RSD) less than 12% were obtained at amine concentrations of 5–50 μg L^{-1} from 100 mL river water and influents from a wastewater treatment plant (WWTP). The present HILIC technique proved to be a viable method for the analysis of aromatic amines in the environmental water samples.

1. Introduction

Toxic aromatic amines, such as aniline and other substituted derivatives, are important industrial chemicals that are used to make dyes, synthetic polymers, rubbers, pesticides, cosmetics, medicines, and many other chemicals. They may be released both from these manufacturing processes and power generators, such as coal-conversion waste facilities [1,2]. As a result, these residues have become significant contaminants in environmental waters and are especially problematic given their known toxicity and biological activity [3–4]. These chemicals have been classified as priority pollutants by US Environmental Protection Agency (EPA), and their use is extensively regulated [5,6]. Given the increasing use of these compounds in various industries, monitoring of their levels in environmental waters is of critical importance to the protection of human health and the environment. This has increased the demand for the development of simple, reliable, sensitive and rapid analytical methods.

Analytical techniques, including gas chromatography (GC) coupled with different detectors [1,6–13], capillary electrophoresis (CE) [14–20], and high-performance liquid chromatography (HPLC) [21–32], have been used for determining aromatic amines in water samples. GC has previously been reported to be a suitable method for trace level determination of amines in water. However, a derivatization step is generally necessary to improve the gas chromatographic properties of the amines because of their polarity [1,7,9–11]. Moreover, aromatic amines are more difficult than aliphatic amines to be derivatized. Capillary electrophoresis (CE) is a fast and efficient tool for chemical analysis, and several analytical methods for aromatic amines using CE have been reported [14–20]. The drawback of CE for environmental analysis is the low concentration sensitivity due to its limited optical path length. Reversed phase HPLC (RP-HPLC) is still regarded as the most convenient technique presently available for the analysis of aromatic amines in water, but the retention and separation of amines are often challenging with RP-HPLC because of their high polarity and basicity [33]. In some cases, the addition of ion-pair reagents such as alkylammonium salts in the mobile phase is necessary for RP-HPLC separations [21]. Recently, hydrophilic interaction liquid chromatography (HILIC) has emerged as a useful analytical tool that rivals RP-HPLC in many applications dealing with polar or ionized compounds in complex matrices [33–37]. To our knowledge, little work has been reported about studies on the analysis of amines with HILIC.

HILIC is a technique in which the analytes interact with a hydrophilic stationary phase and are eluted with a high ratio of organic:aqueous mobile phase [38]. A bare silica stationary phase, commonly used in normal phase liquid chromatography (NPLC), is

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also applicable to HILIC mode separation. Naidong [34] and Li and Huang [35] reviewed the use of bare silica in HILIC mode for bioanalysis and basic compounds separations, respectively. Hemström and Irgum [33] devoted parts of their 2006 review to various silica materials, separation mechanisms, and their HILIC applications. Ikegami et al. [36] discussed the separation efficiencies of columns packed with bare silica in HILIC. HILIC offers a different separation mechanism and can be used as an alternative method to complement traditional RP-HPLC. The considerable advantage of HILIC is the higher sensitivity for the compounds analyzed by electrospray ionization MS (ESI-MS), together with the decreased column backpressure, caused by the high organic content of the mobile phase which greatly enhances the ESI signal [33–36,39]. These features dramatically increase the range of HILIC applications by allowing analysis of highly polar substances including biologically active compounds, such as pharmaceutical drugs [33–36,40–44], peptides and proteins [33,36,38,45,46]. However, there have only been a few reports of HILIC applied to the analysis of environmental samples [47–51], and thus more information is needed to better define the potential utility of HILIC in this area.

The aim of this study was to develop a method for the determination of five aromatic amines (listed in Table 1) by a simple, rapid, and accurate HILIC protocol employing a bare silica column, and to demonstrate the applicability of the method to analysis of environmental water samples. Various parameters, including mobile phase pH, type and content of organic modifier, were tested to determine their impact and relative contribution to HILIC separation. These results provide additional insight regarding the use of silica column in HILIC separation as well as guidance for separation of similar compounds in the future.

2. Experimental

2.1. Reagents and materials

All aromatic amines investigated were of analytical-reagent grade. 1-Naphthylamine (1-NA, ≥99.5%) was purchased from Shanghai Silian Industrial Co. (Shanghai, China), aniline (AL, ≥99.5%) from Tianjin Dongliqu Tianda Chemical Reagent Factory (Tianjin, China), N,N-dimethylaniline (N,N-DMA, ≥99%) from Hongsheng Chemical Industry (Nanjing, China), N,N-diethylaniline (N,N-DEA, ≥99%) from Shanghai Chemical Reagent Factory (Shanghai, China), and benzidine (BZ, ≥99.5%) from Chongming Yuxi Reagent Factory (Shanghai, China). HPLC-grade acetonitrile (ACN), isopropanol (IPA), tetrahydrofuran (THF) and methanol were obtained from Tedia Company, USA. Solutions of 1 mg mL^{-1} of each analyte were prepared in methanol and stored at 4 °C. Composite solutions were prepared by combining an aliquot of each stock solution and diluting the mixture with deionized water. River water was taken from the Qiusuo River at the campus of China Three Gorges University (CTGU). Influent and effluent samples were collected from one wastewater treatment plant (WWTP) in Yichang city. Cleanert PEP cartridges (3 mL/60 mg) were available from Agela Technologies (Beijing, China). Oasis HLB cartridges (3 mL/60 mg) were purchased from Waters (Milford, MA, USA).

2.2. Instrumentation

The HPLC system (Waters, Millipore Co., Milford, MA, USA) consisted of a model 600E pump and 2996 photodiode array detector. All separations were carried out on a 5 μm Kromasil 100-5SIL column (25 cm × 4.6 mm, Eka Chemicals AB, Bohus, Sweden). The mobile phases were pump-mixed dynamically from NaH_2PO_4–H_3PO_4 buffers and acetonitrile at specified compositions. Note that buffer concentrations and pH values refer to the aqueous portion alone. The buffers were prepared by adjusting a 10 mM NaH_2PO_4 solution to the required pH with phosphoric acid (specific density, 1.834 g mL^{-1}). The flow rate was 1.0 mL min^{-1} and the injection volume was 20 μL. The UV detection was at 254 nm, and the column temperature was maintained at 35 °C.

2.3. Environmental water sample preparation and analysis

SPE Vacuum Manifolds (Mediwax Company, USA) were employed for the pre-concentration/elution of amines from water. All water samples were collected in 1 L opaque PTFE bottles and double filtered through Whatman No. 42 paper (2.5 μm) and 0.45 μm membranes, and then adjusted to pH 7.0 with an appropriate amount of diluted NaOH or HCl, and then extracted by SPE using Oasis HLB cartridges. Prior to extraction, the SPE cartridge was conditioned with 3 mL methanol followed by 3 mL of distilled water. The sample solution (100 mL) was loaded onto the cartridge at room temperature. After washing with 2 mL 2% methanol aqueous solution, the cartridge was dried under nitrogen for 5 min, and eluted with 3 mL of methanol. An identical procedure was followed with Cleanert PEP cartridge. The extracts were evaporated to dryness under a gentle stream of nitrogen and reconstituted in 0.5 mL of mobile phase to obtain 200-fold pre-concentration. A 20 μL aliquot was injected into the HPLC–UV system.

The SPE recovery of analytes was determined in river water and WWTP influent sample matrices. Samples were spiked with a mixed standard and extracted using HLB cartridges in triplicate. The concentration recovered was compared to the initial spiking concentration. The concentrations of each amine were calculated by measuring peak areas, and these areas were compared with the calibration graphs of the standards.

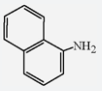
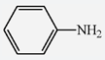
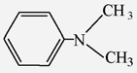
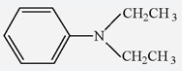
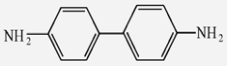
3. Results and discussion

3.1. Systematic approaches to method optimization

3.1.1. Type and amount of organic modifier

A number of experiments were conducted on the bare silica column in order to optimize the separation of the amines. First, the type and amount of organic modifier were examined. The five analytes were chromatographed using isocratic conditions with either methanol, ACN, IPA or THF in combination with a buffer of NaH_2PO_4–H_3PO_4 (pH 1.5, containing 10 mM NaH_2PO_4) at the same concentration (85:15, v/v) as mobile phases. When comparing methanol, IPA, THF with ACN as the organic modifier, marked differences were noticed (Fig. 1). Modifier elution power for eluting the amines increased in the following series: ACN < IPA < methanol < THF, which is partly inconsistent with the elution strength order of these solvents on silica, i.e. THF (ε_o = 0.48) < ACN (ε_o = 0.50) < IPA (ε_o = 0.60) < methanol (ε_o = 0.73) [55]. The elution strength of THF and ACN are approximate, however the solutes are retained more strongly with ACN than THF. The solutes eluted slowest with ACN, which also provided the best resolution of the mixture. In addition, the peak elution order with the four modifiers differed from one another. The above observations indicated that an adsorption process (e.g. hydrogen bonding) existed between the solutes and the stationary phase besides partition mechanism [33]. According to Alpert [38], the retention in HILIC is caused by the partitioning between the bulk of the mobile phase and an immobilized layer enriched with water formed close to the stationary phase. The more polar the analyte is, the more it prefers the water layer and therefore is more retained, and vice versa. On the basis of partition mechanism, one can easily understand the differences in retention behavior of the solutes with the four modifiers. It is the differences in solubility that lead to the

Table 1
Structures and physical properties of aromatic amines investigated in the present study.

Compound	Structure	Molecular formula	Molecular weight (g mol^{-1})	pK_a	log K_{ow}
1-Naphthylamine (1-NA)		$C_{10}H_7NH_2$	143.19	3.92	2.20
Aniline (AL)		C_6H_7N	93.13	4.63	0.94
N,N-Dimethylaniline (N,N-DMA)		$C_6H_5N(CH_3)_2$	121.18	5.08	2.31
N,N-Diethylaniline (N,N-DEA)		$C_6H_5N(C_2H_5)_2$	149.24	6.57	3.31
Benzidine (BZ)		$NH_2C_6H_4C_6H_4NH_2$	184.24	3.3/4.3	1.34

Note: pK_a and log K_{ow} values from Refs. [52–54].

differences in retention with the different organic modifiers. It is well known that the amines show higher solubility in THF than in methanol, IPA or ACN because of the THF amphiphilicity, thus causing the solutes to be eluted sooner. It is worth noting that, in this study we also consider the weak electrostatic interactions that can take place on silica column.

In our study, the elution order partially followed the log K_{ow} pattern. For example, according to the literature, the log K_{ow} values for 1-NA, AL and BZ are 2.2, 0.94 and 1.34, respectively. The most hydrophilic compound is AL, however, BZ was retained the longest (Fig. 1). This result indicated that adsorption played a role in analyte retention since BZ had one more –NH_2 than 1-NA and AL which resulted in longer retention [33,35,56]. However, ion exchange was also involved in the retention of the analytes. The longer retention of AL (pK_a = 4.63) relative to 1-NA (pK_a = 3.93) was ascribed to ion exchange interactions between the solutes and stationary phase, since the higher pK_a would lead to stronger interactions with the ion exchange sites [56]. The indication of the existence

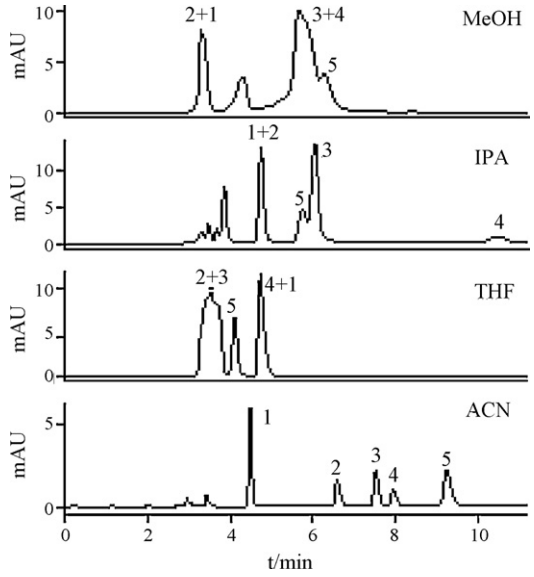


Fig. 1. Influence of organic modifier on the separation of aromatic amines. Conditions: column, Kromasil 100-5SIL (25 cm × 4.6 mm, 5 μm); mobile phase, pH 1.5 NaH_2PO_4–H_3PO_4 buffer (containing 10 mM NaH_2PO_4) modified with various organic solvents (85:15, v/v), flow rate 1 mL min^{-1}, detection at 254 nm. Peaks identification: (1) 1-NA; (2) AL; (3) N,N-DMA; (4) N,N-DEA; (5) BZ.

of ion exchange interaction was also demonstrated by the retention of N,N-DMA and N,N-DEA. With N,N-DMA and N,N-DEA, the log K_{ow} values are 2.31 and 3.31, respectively, but the more polar N,N-DMA (pK_a = 5.08) eluted earlier than N,N-DEA (pK_a = 6.57). The buffer pH 1.5 is predicted to be low enough to keep the solutes and silanols protonated; however, HILIC buffers are commonly prepared in water and later mixed with organic modifier (ACN, methanol, IPA or THF in this instance). When an aqueous buffer is diluted with organic modifier, its acid/base properties (namely pH and buffer capacity) are altered owing to both the dilution and the change of solvent [56,57]. Exact pH values for the organic modifier/water mixtures of the mobile phases are not known, and thus the exact ionization states of the analytes and silanols are unknown. Ion exchange interactions (mixed mode effect) appear to be likely based on the retention of the analytes. In other words, the organic modifier affects not only the dissociation of buffer compound, but also the pK_a of both analytes and silanols, further dominating the retention behavior of the analytes where water content is fixed.

Based on the above results, ACN was chosen as the organic modifier because of the superior resolution obtained with the mobile phase containing ACN. The effect of ACN content on amines retention was studied by using various concentrations (%, v/v) of ACN with a buffer of NaH_2PO_4–H_3PO_4 (pH 1.5, containing 10 mM NaH_2PO_4). The retention and selectivity of amines increased with elevated concentrations of ACN as predicted from the HILIC mechanism. Relatively small changes in mobile phase composition influenced both retention and selectivity of the studied amines on silica column (Fig. 2). Using a solvent with 80% ACN, 1-NA eluted with the solvent front, and N,N-DEA coeluted with N,N-DMA. When 90% ACN was used, there was excessive retention of BZ with k' value of 4.7. At 85% ACN all peaks can be separated with retention factors of 0.6–2.1 and resolution greater than 1.5 (Fig. 1). As expected, the selectivity and the degree of retention were predominantly controlled by the type and content of the organic modifier in the mobile phase. This agrees with the HILIC effects previously described by Alpert and others [33,38]. In addition, the retention of amines was shortened when the content of water, a stronger elution solvent in HILIC, was increased in the mobile phase. This indicates that the adsorption between the analytes and the silica stationary phase may also be a primary retention mechanism.

3.1.2. Buffer pH

The effect of the mobile phase pH on HILIC separation for amines on the silica was investigated by changing the pH of the buffer solutions before mixing with ACN. The pH of a 10 mM NaH_2PO_4

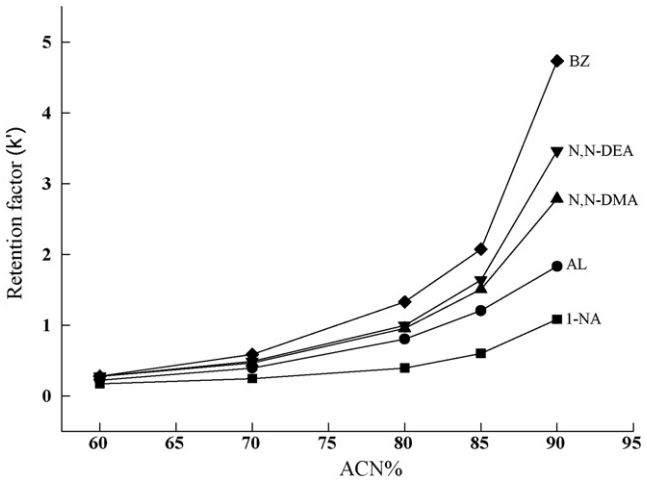


Fig. 2. Influence of ACN concentration on the retention factor (k') of aromatic amines. Conditions and peaks identification as given in Fig. 1, except only various concentrations of ACN were utilized.

solution was adjusted with phosphoric acid to 1.5, 2.5, 3.5, and 6.5. Thus, the hydrophilic interaction and elution power for ionic interactions under study were kept constant, and the retention largely depended on the charge variation of the amines and the stationary phase. There was an overall decrease in retention as the buffer pH value was increased from 1.5 to 6.5 (Figs. 3 and 4). When the buffer pH was changed from 1.5 to 2.5, only N,N-DEA showed favorable retention, while 1-NA, AL, N,N-DMA and BZ eluted close to the void volume of the column with elution bands showing nearly no separation (Fig. 4). When the buffer was adjusted to a pH higher than 3.5, all solutes were eluted with no separation and noticeable decrease in retention. This demonstrated that the ionic exchange retention mechanism was concomitant with adsorption and partition. When the pH of the mobile phase was increased, the solutes became progressively deprotonated, and thus less retained by the ion exchange mechanism, resulting in the sharp decrease in retention.

All the presented data supported the concept that ion exchange played a key role in the retention of the solutes. At low pH, the analytes were protonated and the column was partially negatively charged; thereby the adsorption as well as electrostatic interaction was presumably significant. However, at higher pH, these basic

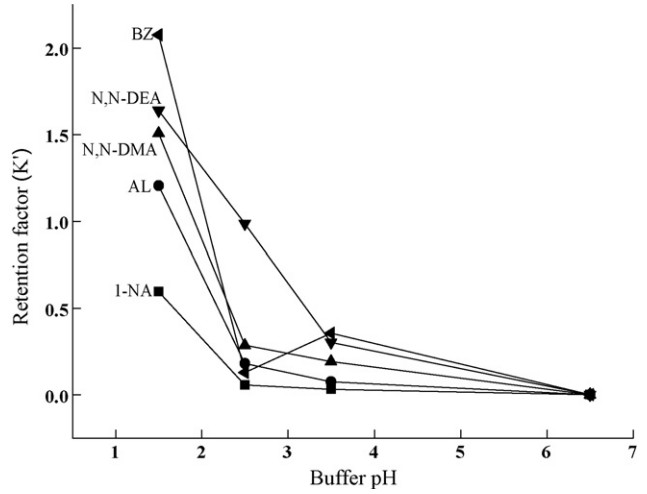


Fig. 3. Influence of mobile phase pH on the retention factor (k') of aromatic amines. Conditions and peaks identification as given in Fig. 1, except only ACN and the buffers at various pH were utilized.

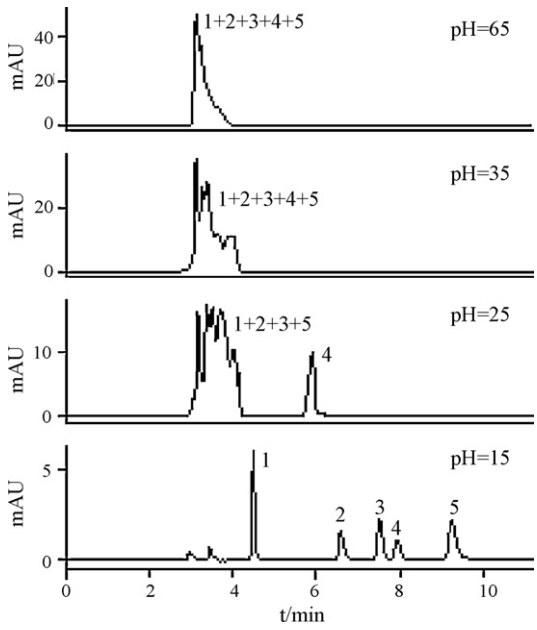


Fig. 4. Comparison of the chromatographic separation of aromatic amines at different buffer pH. Conditions as given in Fig. 3.

compounds became neutral and the ion exchange contribution was insignificant to the retention of these amines, thus leading to a much larger decrease in retention factors at pH 2.5. To further confirm this result, diphenylamine ($pK_a = 0.9$) was used as a test compound at buffer pH 1.5. Its retention pattern fit our model. Diphenylamine was not retained on the silica column because it was present as a neutral molecule under this condition, which did not offer the possibility of cation exchange mechanism.

Given these results, it was concluded that manipulation of the mobile phase pH was a technique that worked well for ionizable analytes since the retention characteristics of ionizable compounds were greatly influenced by the pH of the mobile phase. It was determined that when the buffer pH was 1.5, the analysis was complete within 10 min with good peak shapes and enough resolution for all the tested amines.

3.2. HILIC method validation

An important part of method validation is the system suitability test (SST). The SST was performed under optimized chromatographic conditions. Theoretical plates, peak asymmetry and resolution of individual compounds were established (Table 2). The resultant values showed that the method performance met the criteria for method validation of the recent EU guidelines [58].

The linearity of the method used for each amine assay was evaluated on a calibration curve of the peak area versus the concentration of the analyte (x, mg L^{-1}). The calibration curves were linear ($r^2 > 0.998$) over the range of 0.2–20 mg L^{-1} for 1-NA and N,N-DEA, 0.05–20 mg L^{-1} for AL, 0.5–20 mg L^{-1} for N,N-DMA, and

Table 2
Results of system suitability test.

Compound	Theoretical plates	Asymmetry factor	Resolution between the two adjacent compounds (Rs)
1-NA	1.02×10^4	1.01	
AL	1.16×10^4	1.07	9.9
N,N-DMA	1.43×10^4	1.02	3.8
N,N-DEA	1.49×10^4	1.05	1.6
BZ	1.11×10^4	1.10	4.3

0.4–40 mg L^{-1} for BZ, with detection limits (S/N = 3) on the order of 0.02–0.2 mg L^{-1}.

Accuracy and precision were determined at three amine concentrations: 0.5, 5 and 20 mg L^{-1}. The repeatability of the method was estimated from five consecutive injections of a standard mixture of five amines at the three concentrations (retention times, peak areas and peak heights were determined). The same mixture of amines was injected for 3 consecutive days to determine reproducibility. Repeatability studies gave RSD within 0.3–0.5% for retention time, 0.5–1.5% for peak height and 0.3–1.0% for peak area, respectively. The RSD values for reproducibility studies were 0.5–0.8% for retention time, 0.6–1.5% for peak height and 0.5–1.1% for peak area over the three concentrations (i.e., 0.5, 5 and 20 mg L^{-1}) evaluated during 3 days. These results indicated that the proposed method has excellent precision as evidenced by very stable t_R and peak area for the analytes. In addition, under these working conditions, the column did not need to be conditioned for a long period, and no additional conditioning of the column was required between the runs. Thus, the low pH value did not affect the column stability during the course of the study which included at least 702 injections of standard and environmental water samples without obvious deterioration of the stationary phase, e.g. shifts of retention times and loss of performance.

3.3. Applications to real water sample analysis

3.3.1. Optimization of SPE method

To achieve effective sample cleanup prior to HILIC, SPE was utilized in this study. The advantages of SPE are the reduction of solvent requirement, the removal of matrix components and the facilitation of automation [9,10,13,14,32,48]. SPE sorbent and extraction pH value were optimized. An exhaustive examination was performed using two sorbent materials that can interact with amines: Cleanert PEP and Oasis HLB [13,59]. Oasis HLB material is a polymer with lipophilic divinylbenzene and hydrophilic N-vinylpyrrolidine groups. Cleanert PEP is Polar Enhanced polymer sorbent, which is an alternative to the Waters Oasis HLB. Both materials can extract a variety of polar and non-polar compounds via their aromatic rings and hydrophilic groups. Based on these characteristics, the above two sorbents were selected for extracting aromatic amines from water samples.

To investigate the optimum pH for extraction of amines from water, distilled water samples (100 mL each, adjusted to pH 5.0, 7.0 and 9.0, respectively) were spiked with amines to a final concentration of 5 μg L^{-1} of each. The results showed that the Cleanert PEP and Oasis HLB generated similar profiles. Both cartridges produced higher rates of recovery for target compounds at pH 7.0 than at pH 5.0 and 9.0 (Table 3). At pH 7.0 good recoveries (79.46–104.3%) for all of the compounds were observed on Oasis HLB copolymer cartridges. PEP showed lower recoveries than HLB for all the compounds. Both sorbents demonstrated better recoveries at pH 5.0 compared to pH 9.0. However, when the elution volume of methanol was increased, recoveries from the two sorbents were improved at pH 9.0 but not at pH 5.0 (data not shown). Polymeric sorbents have a broad pH stability range. The mechanism of retention with polymeric sorbents are dependent on Van der Waals forces, hydrogen bonds or dipole–dipole interactions as well as selective π interaction with analytes containing aromatic rings [60]. The interactions between the amines and the sorbent cannot be explained only with Van der Waals forces between the aromatic and lipophilic divinylbenzene groups or possible hydrogen bonding between amino groups and hydrophilic N-vinylpyrrolidone groups. We believe that dipole–dipole interactions play an important role in the retention of these amines. The most important chemical property of the carbonyl group is its tendency to undergo nucleophilic addition reactions. The hydration of the carbonyl group is a rapid reaction in which water acts as a nucleophile and adds to the C=O group, and this process occurs faster in acid or base than in neutral solution [61]. The big differences in recoveries at various pH values in our study can be attributed to the differences in the hydration of the carbonyl group of N-vinylpyrrolidine from the sorbent in acid, base and neutral solutions, which results in the differences in the retention mechanism of the solutes on the sorbents (Fig. 5).

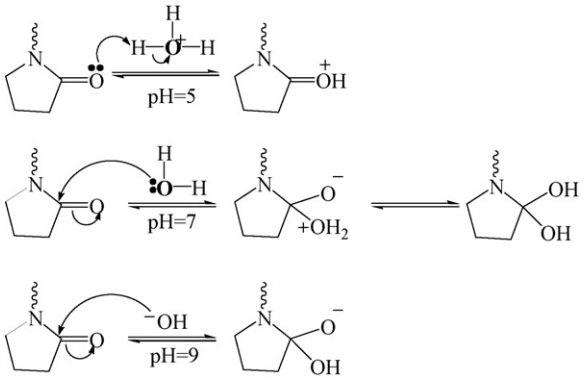


Fig. 5. The hydration mechanism of N-vinylpyrrolidone group of sorbent in acidic, neutral and basic solutions.

At pH 5.0, the tested amines partially exist in protonized form. The acid catalyst activates the carbonyl group of the sorbent toward attack by the weakly nucleophilic water molecule and forms the positively polarized conjugate acid [61]. Thus, the retention of the solutes is weakened by the charge-to-charge repulsion between the solutes and the sorbent (Fig. 5), and this reduces the recoveries for all solutes. At pH 9.0, the solutes are in their neutral form, and the sorbent forms the negatively alkoxide ion through the hydration [61], which strengthens the hydrogen bonding as well as dipole–dipole interactions between amino groups and carbonyl groups of the sorbent, leading to the desorption difficulties of the amino compounds. At pH 7.0, the hydration of the carbonyl group is slower than at pH 5.0 or pH 9.0 [61]. The carbonyl group forms into the geminal diol through the hydration, strengthening the hydrogen bonding between the solutes and the sorbent. A possible explanation for the higher recoveries of the analytes at pH 7.0 is the light hydration of the sorbent matrix, in which the dipole–dipole interactions are weaker compared with those at pH 9.0. Based on these results, the Oasis cartridge was chosen as the SPE sorbent, and the extraction pH of the method was selected to be 7.0 for further testing.

3.3.2. Environmental water analysis

In order to evaluate the practical applications of the SPE method, river water and influent wastewater from a local WWTP were analyzed for amines, the results of which are given in Table 4. 1-NA, AL and BZ were successfully detected in the wastewater of WWTP. The concentrations of 1-NA, AL and BZ were found to be 2.4, 1.1 and 3.6 μg L^{-1}, respectively. No target compounds were detected in river water. Additionally, the wastewater after treatment was also analyzed. No aromatic amines were found in the effluent of WWTP. To further validate the precision and accuracy of the method, recovery testing was carried out by spiking a known amount of the standard mixture into the river water and influent wastewater samples (also shown in Table 4). The recovery of analytes was at least 75% for all spiked concentrations with RSD less than 12%, showing that the overall amines determination method including the extraction procedure was a repeatable method. The recoveries of most of the amines were comparable from both the river and wastewa-

Table 3
Extraction recoveries of aromatic amines from water at various pH on Cleanert PEP and Oasis HLB.

Cartridges	pH	1-NA	AL	N,N-DMA	N,N-DEA	BZ
Cleanert PEP[a]	5.0	29.56[b](11)[c]	23.13(8.4)	34.23(10)	32.07(8.9)	17.24(9.4)
	7.0	58.64(8.0)	80.21(6.8)	74.32(9.1)	70.91(13)	87.07(7.8)
	9.0	14.34(9.5)	18.34(11)	16.70(8.4)	15.31(11)	10.45(6.6)
Oasis HLB[a]	5.0	31.35(9.2)	25.31(9.3)	35.42(9.8)	31.09(7.0)	21.15(9.6)
	7.0	104.3(7.6)	83.91(7.2)	93.56(8.2)	79.46(9.0)	94.74(6.6)
	9.0	17.58(10)	20.51(8.6)	18.69(7.8)	18.05(8.3)	13.52(4.9)

[a] Distilled water 100 mL, spiked to yield final concentration for each analyte 5 μg L^{-1}.
[b] Average of six results of the spiked recoveries.
[c] The RSDs (%) are given in parentheses.

Table 4
Concentrations (μg L^{-1}) of the selected amines in various water samples and their spiked recoveries.

Samples		Analytes spiked(μg L^{-1})	1-NA	AL	N,N-DMA	N,N-DEA	BZ
River water[a] (n = 3)	Background concentration	0	n.d.	n.d.	n.d.	n.d.	n.d.
	Spike recovery (%)	5	75.02[b](11)[c]	116.5(9.4)	107.0(10)	81.61(6.8)	90.24(12)
Wastewater[d] (n = 3)	Background concentration	0	2.4(5.5)	1.1(12)	n.d.	n.d.	3.6 (6.7)
	Spike recovery (%)	5	83.33(8.1)	86.40(7.5)	100.4(5.9)	110.2(7.1)	81.20(7.6)
		20	78.32(6.8)	90.08(7.1)	91.18(6.4)	105.8(11)	111.3(8.5)
		50	84.60(9.6)	92.54(4.9)	84.23(9.6)	83.71(6.9)	82.50(9.8)

[a] River water 100 mL, spiked to yield final concentration for each analyte 5 μg L^{-1}.
[b] Average of three results of the spiked recoveries.
[c] The RSDs (%) are given in parentheses.
[d] Wastewater 100 mL, spiked to yield final concentrations for each analyte to 5, 20, and 50 μg L^{-1}.

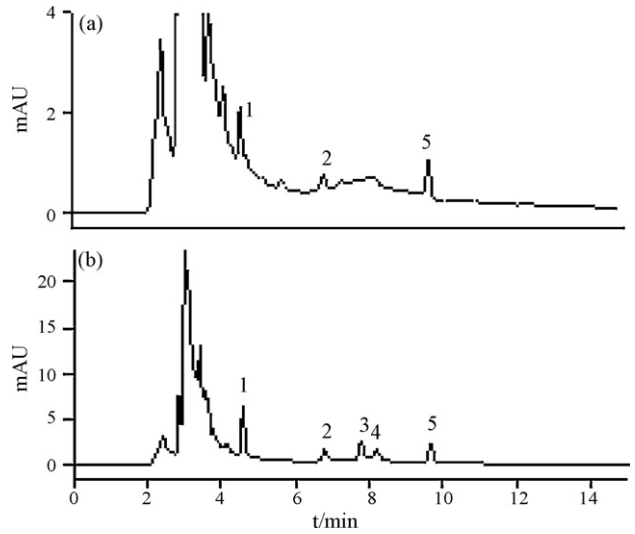


Fig. 6. Chromatogram of influent wastewater from WWTP after SPE using Oasis HLB: (a) wastewater sample and (b) the same sample spiked with 20 μg L^{-1} of aromatic amines under optimum conditions. Peaks identification as given in Fig. 1, and unmarked peaks have not been identified.

ter samples. This demonstrated that the matrix effect appeared to have little impact on the extraction efficiency. Based on the chromatograms (Fig. 6), it can be seen that the extraction procedure eliminated any interference from the environmental matrix. These results demonstrated that SPE provides a potential alternative to detect aromatic amines from environmental water samples.

4. Conclusions

In conclusion, HILIC using bare silica column with high portions of organic solvent in the mobile phase yielded excellent separation of aromatic amines in water samples. Our studies on the effect of type and amount of organic modifier and buffer pH indicated that the retention mechanism was a combination of adsorption, ion exchange and partitioning under the optimized conditions. The optimal buffer pH, type and amount of organic modifier were all equally important to achieve successful separation of the amines. The method was validated with respect to SST, linearity, precision, and accuracy for standard solutions. The validated method was successfully applied to assay amines from river water and wastewater from WWTP using SPE for sample cleanup. The SPE method optimized in this study was verified to have acceptable precision and accuracy and showed little effect from the matrix. This work demonstrates that the HILIC method we have developed will be useful for quantitative determination of aniline and its derivatives from environmental water samples.

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