# Simultaneous determination of multiple amino acids and their occurrence in drinking water systems in Zhejiang Province, China

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**ABSTRACT**

A simultaneous analytical method was proposed for the determination of 13 kinds of free amino acids (FAAs) in the aqueous environment by high performance liquid chromatography (HPLC) combined with pre-column derivatization and solid-phase extraction technology. The recoveries for FAAs were between 8.9% and 87.6%, the detection limits of FAAs were in the range of 0.4–2.09 mg/L without enrichment pretreatment, the relative standard deviations were less than 0.6%. The method has been successfully applied to determine low concentrations of FAAs in real water samples. The occurrence of total FAAs in the source water of Hangzhou City ranged from 17.4 to 39.0 μg/L. Tyr, Lys, and Asp were the dominant species and found with the highest concentrations of 13.4, 13.3, and 6.9 μg/L, respectively. The conventional water treatment and advanced water treatment with ozonation and activated carbon were not ideal for the removal of FAAs, thus harmful disinfection byproducts might originated in the subsequent chlorination process, including regulated carbonaceous disinfection by-products (C-DBPs) and nitrogenous disinfection by-products (N-DBPs).

*Keywords:* Solid phase extraction; Pre-column derivatization; HPLC; Free amino acids; Source water; Drinking water system

## 1. Introduction

In recent years, the occurrence and control of dissolved organic nitrogen (DON) in water have gradually attracted researcher's attention [1,2] along with new identification of the nitrogenous disinfection by-products (N-DBPs) which have much more toxicity than the currently regulated halogenated carbonaceous disinfection by-products (C-DBPs) [3]. Besides generating C-DBPs, DON is the main precursor deriving N-DBPs such as haloacetonitriles (HANs), *n*-nitrosodimethylamine (NDMA), and specify, etc. [4]. These potential carcinogens will be formed during chlorine or chloramine disinfection process [5]. N-DBPs are generally found in drinking water at lower

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concentrations than trihalomethanes (THMs) and haloacetic acids (HAAs) since the occurrence of DON is typically lower than dissolved organic carbon (DOC), and the later is the important precursor of C-DBPs in source water [6]. DON compounds are difficult to remove in conventional drinking water treatment processes because most of them have functional groups prone to generating hydrogen bonds around the water molecules, which enhance their hydrophilicity [7].

Free amino acids (FAAs) comprise an important portion of the DON in source water, which have been highlighted as key precursors in the formation of a variety of DBPs [8,9]. FAAs cannot be effectively removed by traditional drinking water treatment processes unless advanced treatments are adapted [10–13]. During chlorination, their presence leads to the formation of aldehydes, nitriles, and other DBPs resulting in potential hazards to ecosystem or human health [8,14–16].

The DBPs formation potentials are directly related to the structure and concentration of FAAs [17]. Therefore, to have knowledge of FAAs occurrence in source water and treated water is meaningful and important. Some scholars have investigated the distribution of FAAs in various types of water, and found that FAAs concentration ranged from 20 to 720 μg/L in Yellow River [18]. Brosillon et al. [19] performed a year-long study on the raw water of three water plants in Paris and concluded that FAAs varied greatly with the season. However, the source, distribution, and fate of FAAs in each process stage of the DWTPs are not well-described, especially for single FAAs since they may have various transformation characteristics. Therefore, it is significant to determine the concentration of each FAA in DWTPs to have the basic information of DBPs precursors.

Diverse analytical methods have been proposed for analyzing FAAs including thin layer chromatography, capillary electrophoresis, gas chromatography, and high performance liquid chromatography (HPLC) [20–23]. HPLC is widely used and accepted due to its high resolution, sensitivity, great versatility, and simple sample treatment [24]. To increase the sensitivity of detection, FAAs are usually transformed into easily separated and detected derivatives before analysis. Since HPLC combined with pre-column derivatization still has limitations for detecting micrograms of FAAs in complex matrices pretreatment like solid phase extraction (SPE) is required to make water samples purified and enriched [19,25]. The goal of this paper is to propose an effective analytical method for the determination of micropollutant FAAs in water bodies using SPE, pre-colum derivatization, and HPLC determination. By this method, the occurrence of single FAAs in raw water and engineered water of DWTPs were obtained.

## 2. Experimental

### 2.1. Instrumentations

Sample extraction was performed by using a Visiprep DL SPE apparatus (Sigma-Aldrich, USA). SCX SPE columns (60 mg/3 mL, CNW Technologies GmbH, Shanghai) and nitrogen blowing apparatus (OA-SXS, USA) were used.

Liquid chromatography analyses were performed using a LC-20AD liquid chromatograph (Shimadzu, Japan) equipped with four pumps (LC-20AD), an autosampler (SIL-20A), and a UV-vis detector (SPD-20A). The separation was carried out by a reversed-phase C18 column (CNW, GER, 250 mm × 4.6 mm, 5 μm).

### 2.2. Reagents

All the left enantiomeric free amino acids (L-FAAs) standards including aspartic acid (Asp), glutamic acid (Glu), histidine (His), threonine (Thr), serine (Ser), alanine (Ala), proline (Pro), valine (Val), methionine (Met), phenylalanine (Phe), isoleucine (Ile), leucine (Leu), and lysine (Lys), hydrochloric acid and sodium acetate anhydrous (99.7%) were supplied by Sinopharm Chemical Reagent Co., Ltd., (Shanghai, China).

FAAs (1 g/L) were prepared in hydrochloric acid (0.1 mol/L) and stored at 4°C. Phenyl isothiocyanate (PITC) and triethylamine (TEA) were obtained from Sigma-Aldrich (St. Louis, MO, USA). HPLC-grade acetonitrile, methanol and *n*-hexane were purchased from CNW Technologies GmbH (USA). One mole per liter acetonitrile-TEA (43:7, v/v) and 0.1 mol/L acetonitrile-PITC (247:3, v/v) were made into derivatization reagents *A* and *B*. Ultrapure water was obtained from Ulupure (Sichuan Super Pure Technology Co., Ltd., China).

### 2.3. Pretreatment of water samples

All the water samples were filtered through a 0.45 μm glass fiber membrane and adjusted to pH 1.3. The SCX columns were pre-conditioned with 4.5 mL of methanol, followed by 9 mL of 0.1 M hydrochloric acid, and dried under vacuum. Then 1,000 mL water sample was loaded at a flow rate of 5 mL/min and target compounds were eluted with 9 mL of methanol (with 5% ammonia). The extracts were evaporated to dryness under gentle flow of nitrogen and reconstituted with 1 mL of 0.1 M hydrochloric acid before derivatization.

Two hundred microliters of each derivative reagent *A* and *B* were mixed completely with 100 μL of water sample by vortex oscillator and incubated at 25°C for 40 min. Then 400 μL of *n*-hexane and 1,000 μL of pure water was added for liquid–liquid extraction. The supernatant was removed and another 400 μL of *n*-hexane was added for further extraction. The water phase was filtered with a hydrophilic polytetrafluoroethylene (PTFE) membrane before analysis.

### 2.4. Dectection methods

The suitable chromatographic conditions were 0.1 mmol/L sodium acetate (*A* phase) and acetonitrile (*B* phase) as the mobile phase, flow rate of 1.0 mL/min, and a column temperature of 40°C. The injection volume was 80 μL. The UV detector (254 nm) was employed. The mobile phase composition during gradient elution is showed in Table 1.

Measurement of DCAN (dichloroacetonitrile) and TCNM (trichloronitromethane) were performed by the standard method (USEPA). Methyl tert-butyl ether (containing 1,2-dibromopropane as internal standard, 2 mL) and $Na_2SO_4$ solution (4 g/25 mL water) was added into samples immediately. After shaking the water samples for 2 min, the

Table 1
Mobile phase composition of gradient elution

| Time (min) | A % | B % |
|---|---|---|
| 0 | 95 | 5 |
| 10 | 91 | 9 |
| 20 | 89 | 11 |
| 21 | 79 | 21 |
| 34 | 77 | 23 |
| 35 | 75 | 25 |
| 43 | 68 | 32 |
| 44 | 20 | 80 |
| 49 | 10 | 90 |
| 50 | 95 | 5 |
| 53 | 95 | 5 |

supernatant was withdrawn into a GC-vial, and DBPs determined with GC-ECD system.

### 2.5. Sample method

Samples were collected from drinking water treatment plants (DWTPs) and water supply system in cities H1 and H2, Zhejiang Province. Q River (SW1) and T Stream (SW2) are the main sources of city H1. For city H2, the XT Stream (SW3), LaoHuTan Reservoir (Reservoir 1), and T Lake (Lake 1) are the main sources.

Table 2 showed the treatment process and water quality parameters in DWTPs and WWTP. XF (H1_DWTP1), QT (H1_DWTP2), NXQ (H1_DWTP3), and JX (H1_DWTP4) DWTPs are located in H1 City. DWTP1 and DWTP4 adopt conventional treatment processes, while advanced treatment processes including ozonation and membrane filtration were employed in DWTP2 and DWTP3. DWTP2, DWTP3, and

DWTP4 take source water from SW1, DWTP1 from SW2. TH (H2_DWTP5) and CX (H2_DWTP6) DWTPs are located in H2 City, adopt conventional treatment and advanced treatment process, respectively. DWTP5 draws source water from Lake 1, DWTP6 from SW3 and Reservoir 1. The water network is from the water supply network of DWTP5 and DWTP6 in H2 City. BL WWTP (H2_WWTP1) treats residential sewage in H2 City. Sampling sites were shown in Fig. 1.

The samples were collected in December 2017, January, and May 2018. A certain amount of water samples were collected in each process section of the DWTPs and water samples from distribution system (DS1,2,3,4,5,6,7,8,9,10,11, 12,13) were collected in May, 2018. The water samples collected shall not be less than 5 L. On arrival at the laboratory, all natural water samples were filtered through 0.45 μm polyethersulfone membrane filters and stored at 4°C until extraction to prevent analyte degradation.

## 3. Results and discussion

### 3.1. Liquid chromatography separation

Fig. 2 showed the chromatograms of FAAs standards a real water sample. All standards were well-separated with the highest intensity of Val and the lowest of His. Isomers of Ile and Leu appeared closely and showed similar intensity.

### 3.2. Validation of the method

#### 3.2.1. Linearity, LOD, and LOQ

Standards solutions of 13 kinds of FAAs ranging from 1 to 40 mg/L were derivatized and analyzed as shown in Table 3. Good linearities were obtained for all FAAs with good coefficients of determination ($R^2$). The LOD and LOQ were obtained by multiplying the standard deviation of the signal-to-noise ratio by 3.0 and 10.0. The LODs for the method were
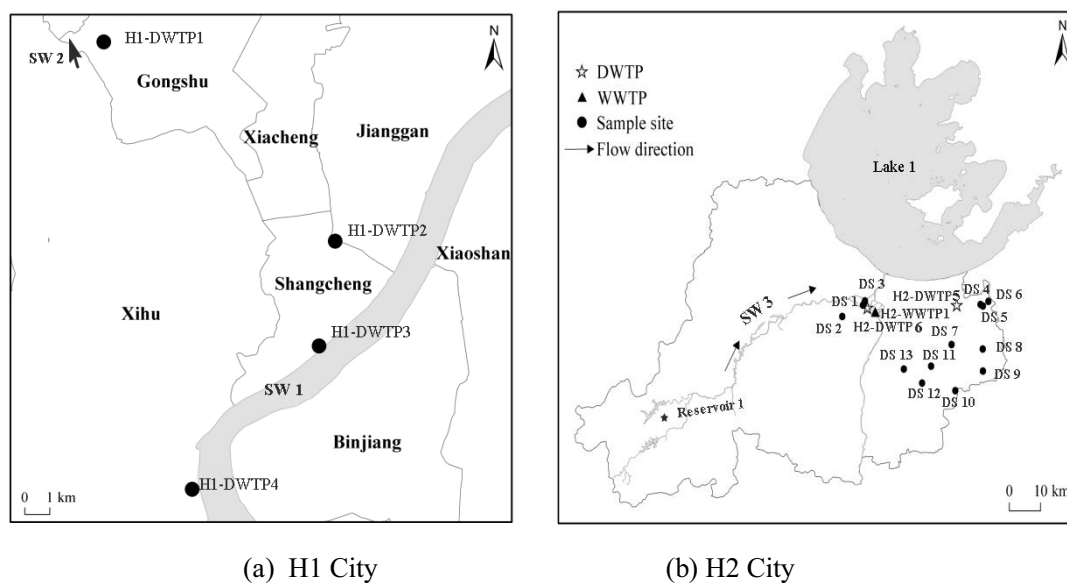


(a) H1 City

(b) H2 City

Fig. 1. Map of the sampling sites (a) H1 City and (b) H2 City.

Table 2
Treatment process and water quality information of DWTPs and WWTP

| Water sample | TOC mg/L | pH | Conductivity (µs/cm) | Water treatment processes | Location |
|---|---|---|---|---|---|
| BL WWTP | 22.5 | 7.13 | 1,256 | Urban sewage, grit, activated sludge method (SBR-CASS), trickling filter, chlorination | ZJ, China |
| CX DWTP | 1.81 | 7.22 | 150 | Surface water-flocculation, sedimentation, filtration, chlorination | ZJ, China |
| TH DWTP | 2.64 | 7.19 | 315 | Surface water-preozonation, coagulation, flocculation, sedimentation, ozonation-bioactive carbon, filtration, chlorination | ZJ, China |
| JX DWTP | 2.38 | 7.08 | 399 | Surface water-coagulation, flocculation, sedimentation, filtration, chlorination | ZJ, China |
| NXQ DWTP | 2.12 | 7.05 | 221 | Surface water-coagulation, flocculation, sedimentation, ozonation-bioactive carbon, filtration, chlorination | ZJ, China |
| QT DWTP | 1.96 | 6.94 | 229 | Surface water-flocculation, sedimentation, filtration, micromembrane, chlorination | ZJ, China |
| XF DWTP | 2.95 | 6.86 | 281 | Surface water-coagulation, flocculation, sedimentation, filtration, chlorination | ZJ, China |

Table 3
Linearity, LOD, LOQ of 13 kinds of FAAs

| FAAs | $R^2$ | Linear range (mg/L) | LOD (mg/L) | LOQ (mg/L) | Retention time (min) |
|---|---|---|---|---|---|
| L-Asp | 0.9987 | 5–40 | 1.90 | 6.33 | 5.06 |
| L-Glu | 0.9992 | 5–40 | 1.56 | 5.20 | 5.90 |
| L-Ser | 0.9998 | 1–40 | 0.41 | 1.37 | 13.28 |
| L-His | 0.9970 | 1–40 | 0.50 | 1.67 | 16.15 |
| L-Ala | 0.9995 | 5–40 | 2.07 | 6.90 | 20.15 |
| L-Pro | 0.9984 | 5–40 | 2.09 | 6.97 | 21.80 |
| L-Tyr | 0.9997 | 1–40 | 0.41 | 1.37 | 29.59 |
| L-Val | 0.9994 | 1–40 | 0.85 | 2.83 | 30.53 |
| L-Met | 0.9970 | 5–40 | 1.90 | 6.33 | 31.66 |
| L-Ile | 0.9997 | 5–40 | 1.15 | 3.83 | 34.99 |
| L-Leu | 0.9997 | 5–40 | 1.90 | 6.33 | 35.61 |
| L-Phe | 0.9999 | 5–40 | 1.54 | 5.13 | 40.03 |
| L-Lys | 0.9999 | 1–40 | 0.40 | 1.33 | 44.74 |

in the range of 0.4–2.09 mg/L and the LOQs were in the range of 1.33–6.97 mg/L. Lys showed the lowest LOD and LOQ values whereas the highest LOQ observed was for Pro.

### 3.2.2. Precision and accuracy (recovery)

A series of standards at different concentrations were measured to demonstrate repeatability of the method. The relative standard deviation (RSD) for the measurements was <6% and had an average of 3%. Australian researchers Zuo et al. [26] reported pH had influence on the FAAs recoveries. Spike experiments were carried out to determine the precision of SPE pretreatment. The target FAAs standards were prepared in pure water at concentrations of 20 and 40 mg/L, after column elution, blow-dry, and volume determination, the concentration was analyzed by LC, and the recovery was calculated. As shown in Fig. 3 under two concentrations of 20 and 40 mg/L, recoveries for FAAs ranged from 8.9% to 87.6%. Three replicates were performed for each experiment. Thirteen spiked water samples were analyzed and mean recoveries were in the range of 50%–80%, except for Ala, Ile, and Leu (8.9%, 21.6%, and 15.8%, respectively). Lys, Phe, Tyr, and Asp can be well-extracted and the recoveries were above 70%. Therefore, HPLC combined with precolumn derivatization and SPE is feasible for this experiment.

### 3.3. Occurrence of FAAs in source and treated water

The occurence of FAAs in real water samples collected from two cities in Zhejiang were determined. Fig. 4 shows the concentration levels and each sample contained at least seven kinds of FAAs. The dominant FAAs were Asp, Ser, Tyr, Met, Ile, Leu, Phe, and Lys of which tthe concentrations ranged from 0.28 to 13.39 µg/L.
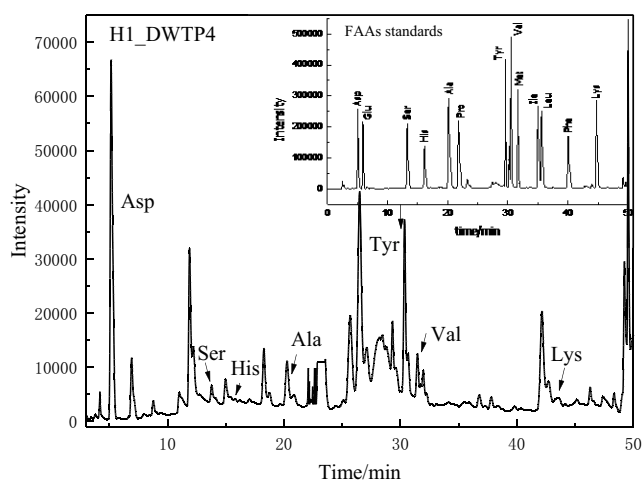
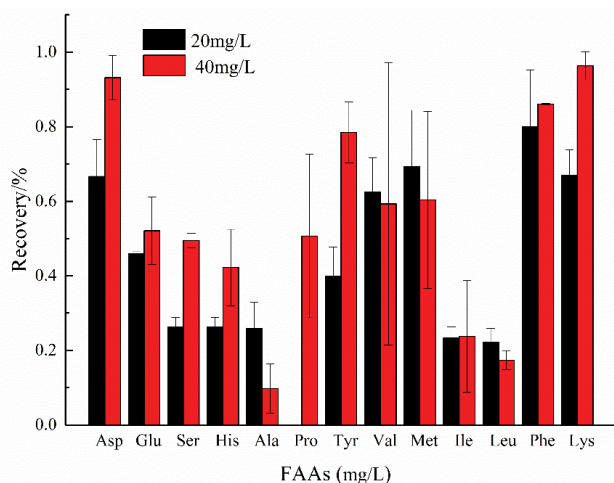Fig. 2. HPLC chromatograms of FAAs standard samples and FAAs in H1_DWTP4.



Fig. 3. Recoveries of FAAs in simulated solution pretreated by solid-phase extraction.

### 3.3.1. Occurrence of FAAs in WWTP

The information were obtained by measuring FAAs in WWTP as one of the sources to surface water. Ten kinds of FAAs were found in both source water and effluent of H2_WWTP1, among which Phe, Met, and Tyr were mainly detected. In sewage, the concentrations of total FAAs ranged from 16.63 to 48.3 µg/L. Tyr and Phe were reported enriched in cell contents, thus the quantitative dominance of these FAAs may occur from human excretion, high phytoplankton release, or by protein degradation [27,28]. These FAAs were conserved, possibly due to the selectivity of FAAs utilization by heterotrophic bacteria. In addition, the reason why Ala was not detected might be that it was more available to bacteria and/or phytoplankton during the bloom succession [29].

### 3.3.2. Occurrence of FAAs in DWTP

The concentrations of FAAs in the four DWTPs of H1 City ranged from 17.4 to 37.5 µg/L (Fig. 4). The FAAs detected in both source water and effluent of DWTPs including Asp, Glu, Ser, His, Tyr, Val, Met, Ile, Leu, Phe, and Lys. Tyr was much higher than the other FAAs with a maximum of 13.4 µg/L. The average concentrations of total FAAs in the source waters of four DWTPs were 27.4, 26.2, 32.3, and 32.1 µg/L, respectively, while the corresponding concentrations in finished water were 27.6, 25.8, 33.1, and 31.9 µg/L. FAAs were not significantly eliminated in water treatment plants, which was similar to Zuo et al. [30].

### 3.4. FAAs removal in different treatment processes

By sampling in treatment processes of DWTPs, the degradation and transfer of four FAAs with high occurrences (Asp, Tyr, Met, and Phe) were analyzed, to understand the possible amount that can reach the disinfection process.

### 3.4.1. Removal of FAAs by conventional processes

As is shown in Fig. 5, usually the removal efficiencies of FAAs in conventional process were low. The concentrations of Met and Phe decreased slightly during the flow of the process. The Asp was removed significantly by 38.1%. However, the concentration of Tyr increased.

After the coagulation process, the concentration of Tyr, Met, and Phe still remained the same since small, non-humic molecules such as FAAs are not amenable to coagulation [31]. However, the coagulation and sedimentation worked well with Asp and Met whose removal efficiencies were 42.8% and 57%, respectively. The concentration of Asp and Met in the water was increased after the filter unit, which might be due to the desorption of the adsorbate on the filter material [32].

In finished water, most of the FAAs concentrations increased, which may be caused by disinfection. During chlorine (chloramine) disinfection, the algal cells damage induced by elevated oxidant dose could promote amino acid release [33,34].

The concentration of FAAs in the distribution systems showed irregular variation due to complicated factors such as residual chlorine oxidation and bacterial decomposition [35,36]. Bacterial growth is adversely affected by low water temperature and low nutrient in winter, so FAAs are relatively more affected by residual chlorine. The residual chlorine declined with distribution distance, while organic macromolecules were likely to be decomposed into small molecules like FAAs, which may explain the increased of Met, Tyr, and Phe in DS2 [36,37]. According to the total amount of FAAs, the concentrations in DS1 and DS3 were about 68.1% and 74.3% less than those in finished water. Brosillon et al. [19] proposed that the amount of by-products depends on the amount of FAAs, which means DBPs might be produced.

### 3.4.2. Removal of FAAs by advanced treatment process characterized with ozonation and activated carbon

As is shown in Fig. 5, the concentration of Met and Phe increased significantly, especially after the pre-oxidation. The increase may be caused by the release from microorganisms. Xu et al. [38] reported that pre-oxidation enhanced the rupture of algal cells and excretion of intracellular
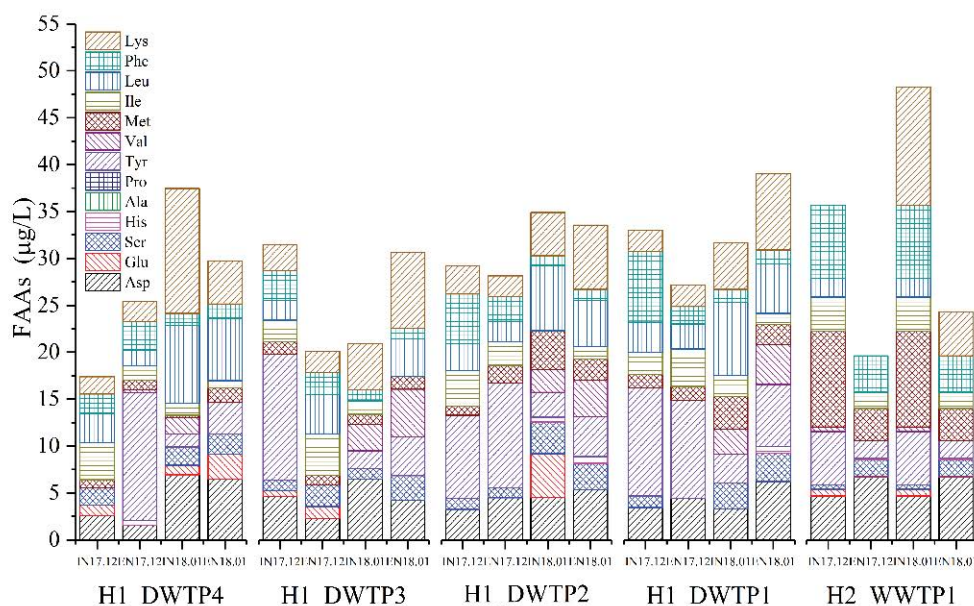
Fig. 4. Occurrence of FAAs in influent and effluent of four DWTPs and a WWTP (IN represents influent; EN represents effluent; 17.12 represents sampling time year–month).

substances including FAAs as the main components [39]. Coagulation and sedimentation had an ideal removal effect of FAAs of high concentration after pre-ozonation, as similar to which reported by Liu et al. [32]. The post-oxidation stage had little influence to Asp and Phe while Lys and Met increased, which was inconsistent with the previous studies that most of the particulate matter had been removed after the conventional process, so the interaction FAAs obtained more collision with ozone and were broke downdegraded FAAs effectively [32]. So, additional experiments are necessary to investigate if it is related to algae which vary seasonally in Lake 1. The effect of activated carbon filter on FAAs depends on type of carbon applied, the polarity of FAAs and also other substrate, etc. [10]. The microorganisms on the AC might be washed out and killed, leading to the increase of FAAs in treated water.

### 3.5. FAAs in distribution system

The FAAs cannot be removed thoroughly by drinking water treatment and the residual FAAs enter in water supply system. The occurrence of FAAs in the distribution system is shown in Fig. 6.

As shown in Fig. 6, the total FAAs in tap water ranged from 13.7 to 32.2 µg/L, with little variation and at the same level as in DWTPs. As the distance increased, the total FAAs concentration decreased and eventually remained at 13.8 µg/L. The dominant FAAs remained Tyr, Asp, Lys, and Phe in those sample sites nearby DWTPs, as in the water treatment system. However, Tyr and Asp decreased to no detection as the pipe network extended. Dominant FAAs became Lys and Phe, in the range of 3.84–5.08 µg/L. The decrease of total FAAs and the variation of dominant FAAs may be affected by complicated factors in the network including digestion by bacteria, intracellular compounds released from microrganisms, or oxidation by residual chlorine.

### 3.6. Correlation between FAAs and DBPs

FAAs and aliphatic amines have been reported to be the precursors of some N-DBPs [40,41]. The correlation between N-DBPs formed in the DWTPs and FAAs in the raw water were analyzed. DCAN and TCNM in finished water have a high correlation with Val, Phe, Ser, and Met in raw water, the correlation analysis is shown in Fig. 7.

DON such as FAAs and proteinaceous compounds in source waters are important halogenated N-DBP precursors [4,42]. Among FAAs detected in raw water, Val, Phe, Ser, and Met showed good linear relationship with DCAN and TCNM in finished water of H2_DWTP6. The formation of DCAN in H2_DWTP6 increased significantly as the FAAs (Val and Phe) concentrations increased which was consistent with the results of Zuo et al. [30]. The increase in DCAN concentration observed for the finish water with increased FAAs might be due to the degradation of proteins and peptides in the raw water [30]. The formation of TCNM in H2_DWTP6 increased significantly as the Ser concentration increased. However, the formation of TCNM in H2_DWTP6 decreased significantly as the Met concentration increased. In H2_DWTP5, Val, Phe, Ser, and Met also showed good linear relationship with DCAN and TCNM in finished water. The formation of DCAN in H2_DWTP5 was consistent with H2_DWTP6. The formation of TCNM in H2_DWTP5 increased significantly as the Met concentration increased. However, the formation of TCNM in H2_DWTP5 decreased significantly as the Ser concentration
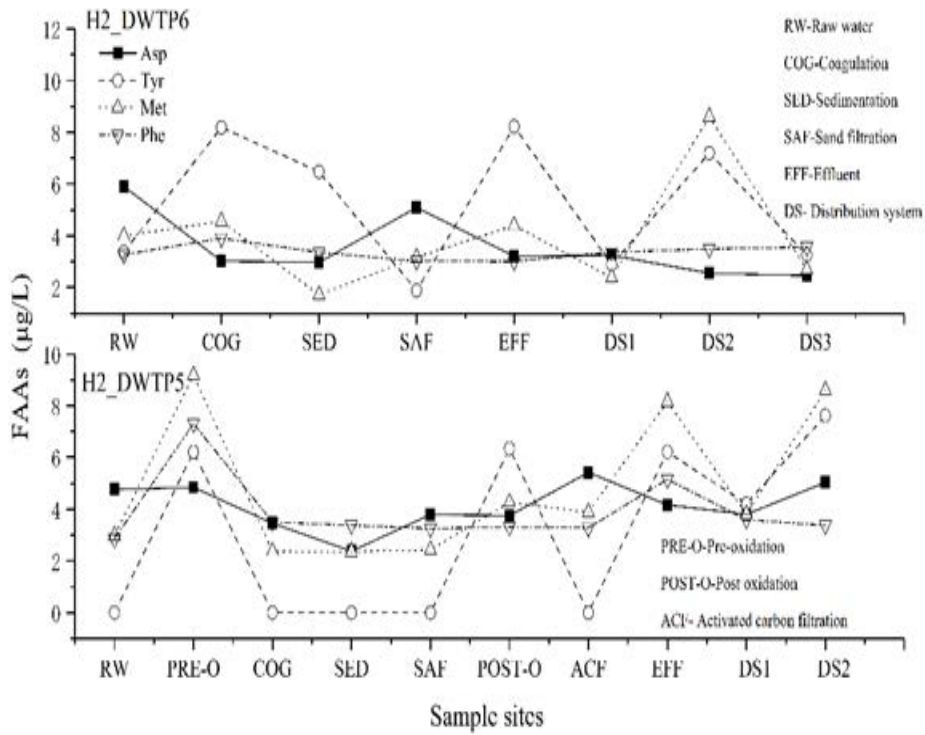
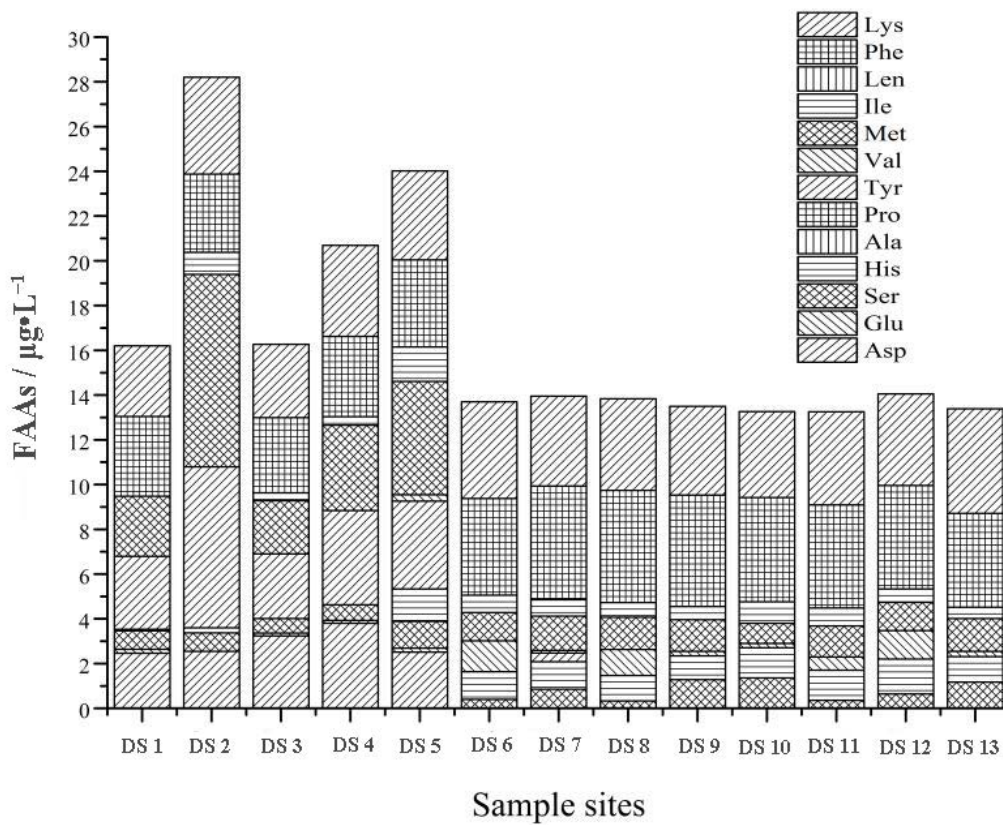Fig. 5. Concentration variation of FAAs in the drinking water system.



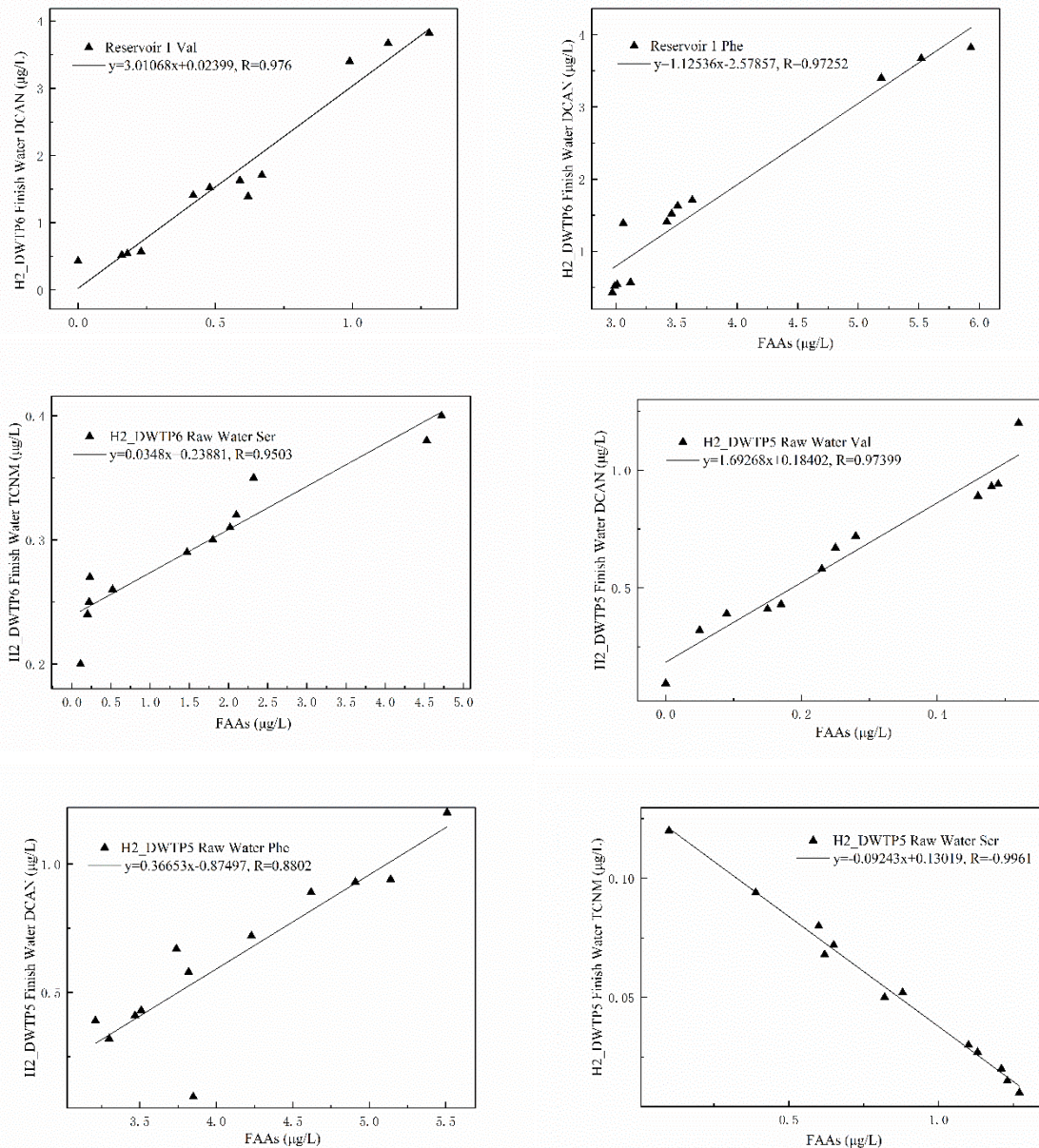Fig. 6. Occurrence of FAAs in the water distribution system.

Fig. 7. Correlation analysis of DBPs in finished water and FAAs in raw water.

increased, which is opposite of the situation in H2_DWTP6. For Ser, it was probably because of the water quality of Lake 1 which had complex microbial species capable of biodegradation [43].

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