Accepted Manuscript

Trace determination and occurrence of eight chlorophenylacetonitriles: An emerging class of aromatic nitrogenous disinfection byproducts in drinking water

Di Zhang, Tom Bond, Stuart W. Krasner, Wenhai Chu, Yang Pan, Bin Xu, Daqiang Yin

PII: S0045-6535(18)32460-3

DOI: <https://doi.org/10.1016/j.chemosphere.2018.12.127>

Reference: CHEM 22826

To appear in: *ECSN*

Received Date: 11 October 2018

Revised Date: 16 December 2018

Accepted Date: 18 December 2018

Please cite this article as: Zhang, D., Bond, T., Krasner, S.W., Chu, W., Pan, Y., Xu, B., Yin, D., Trace determination and occurrence of eight chlorophenylacetonitriles: An emerging class of aromatic nitrogenous disinfection byproducts in drinking water, *Chemosphere* (2019), doi: <https://doi.org/10.1016/j.chemosphere.2018.12.127>.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Drinking Water Treatment Plant

Solid Phase Extraction

Trace determination and occurrence of eight chlorophenylacetonitriles: an emerging class of aromatic nitrogenous disinfection byproducts in drinking water

Di Zhang^{a,b}, Tom Bond^c, Stuart W. Krasner^d, Wenhai Chu^{a,b,*}, Yang Pan^e, Bin Xu^{a,b}, Daqiang Yin^{a,b}

^a State Key Laboratory of Pollution Control and Resources Reuse, Key Laboratory of Yangtze River Water Environment, Ministry of Education, International Joint Research Center for Sustainable Urban Water System, College of Environmental Science and Engineering, Tongji University, Shanghai, 200092, China

^b Shanghai Institute of Pollution Control and Ecological Security, Shanghai, 200092, China

^c Department of Civil and Environmental Engineering, University of Surrey, Guildford, GU2 7XH, UK

^d La Verne, California 91750, United States

^e State Key Laboratory of Pollution Control and Resource Reuse, School of the Environment, Nanjing University, Nanjing 210023, Jiangsu, China

* Corresponding author.

Address: Room 308 Mingjing Building, College of Environmental Science and Engineering, Tongji University, 1239 Siping Road, Yangpu District, Shanghai, 200092, China

Tel: +86 021 65982691; Fax: +86 021 65986313;

E-mail address: feedwater@126.com; waterman@tongji.edu.cn.

Phone: 0086-18721871983; 0086-21-65982691

Abstract

Two chlorophenylacetonitriles (CPANs) (2-chloro- and 3,4-dichlorophenylacetonitrile), representatives of an emerging class of aromatic nitrogenous disinfection byproducts, were recently identified in chlor(am)inated drinking water with liquid/liquid extraction and gas chromatography/mass spectrometry (GC/MS). Due to their high cytotoxicity, they are potentially significant drinking water contaminants. The detection limit for these two CPANs with the previous method was 100 ng L⁻¹. To search for additional CPAN isomers, a more sensitive method for the simultaneous determination of eight CPANs was developed using solid-phase extraction (SPE)-GC/MS. GC/MS parameters and SPE pre-concentration conditions, including SPE cartridge, eluent type, eluent volume, and sample pH, were optimized. Under optimized conditions, the new method had method detection limits, method quantification limits, and precision ranging from 0.15 to 0.37 ng L⁻¹, 0.50 to 0.95 ng L⁻¹, and 5.8% to 11%, respectively. The recoveries of the eight CPANs ranged from 92% to 102%. The concentrations of the eight CPANs in nine finished drinking waters were determined to be at concentrations ranging from 0.5 to 155 ng L⁻¹. Seven CPANs were detectable in all samples. CPANs were detected at concentrations between 0.8 and 155 ng L⁻¹ in chlorinated waters, and from 0.5 to 15 ng L⁻¹ in chloraminated waters. Across all waters, the sum of all CPANs in chloraminated waters was 13% of that in chlorinated systems.

Keywords: Disinfection byproducts; Chlorophenylacetonitriles; Solid-phase extraction; Identification; Occurrence; Drinking water;

38 **Abbreviations:**

39 CPANs, Chlorophenylacetonitriles; GC/MS, Gas chromatography/mass spectrometry; SPE,
40 Solid-phase extraction; DBPs, Disinfection byproducts; TOX, Total organic halogen; THMs,
41 Trihalomethanes; HAAs, Haloacetic acids; HANs, Haloacetonitriles; HAcAms, Haloacetamides;
42 2-CPAN, 2-Chlorophenylacetonitrile; 3-CPAN, 3-Chlorophenylacetonitrile; 4-CPAN,
43 4-Chlorophenylacetonitrile; 2,3-DCPAN, 2,3-Dichlorophenylacetonitrile; 2,4-DCPAN,
44 2,4-Dichlorophenylacetonitrile; 2,5-DCPAN, 2,5-Dichlorophenylacetonitrile; 2,6-DCPAN,
45 2,6-Dichlorophenylacetonitrile; 3,4-DCPAN, 3,4-Dichlorophenylacetonitrile; MDLs, Method
46 detection limits; N-DBPs, Nitrogenous DBPs; DWTPs, Drinking water treatment plants; DOC,
47 Dissolved organic carbon; TN, Total nitrogen; EI, Electron ionization; SIM, Selected ion mode;
48 MCPANs, Monochlorophenylacetonitriles; DCPANs, Dichlorophenylacetonitriles; MQLs, Method
49 quantitation limits; RSD, Relative standard deviation; CV, Coefficient of variation;

50

1. Introduction

In order to prevent waterborne disease, disinfectants are used to inactivate microorganisms. Unfortunately, unintentional reactions between disinfectants, natural organic matter, and halides (i.e., Br^- and I^-) result in the formation of a wide range of disinfection byproducts (DBPs) of health concern (Richardson and Postigo, 2010; Karanfil, 2008; Plewa et al., 2004; Li and Mitch, 2018). DBPs are significant because a number of epidemiological studies show an association between the long-term consumption of chlorinated drinking water and the risk of bladder cancer, rectal cancer, and adverse reproductive and developmental health effects (Bove et al., 2002; Bove et al., 2007; Villanueva et al., 2007; Swan et al., 1998). Yet only a fraction of the total organic halogen (TOX) produced during disinfection (e.g., trihalomethanes [THMs], haloacetic acids [HAAs], haloacetoneitriles [HANs], and haloacetamides [HAcAms]) has been intensively investigated with respect to their formation, occurrence, cytotoxicity, and genotoxicity (Krasner et al., 2006; Richardson et al., 2007; Muellner et al., 2007; Plewa et al., 2008; Chuang et al., 2015; Yan et al., 2018). In contrast, a large portion of the TOX (approximately 50% or more) has not been chemically identified (Krasner et al., 2006). The carcinogenic potency of the regulated THMs and HAAs is at least two orders of magnitude too low to explain the enhanced bladder cancer risk at typical drinking water concentrations (Bull et al., 2011). It is therefore important to explore the unknown part of TOX, which potentially contains plausible bladder carcinogens. Amongst emerging DBPs, aromatic DBPs have received increasing attention (Qin et al., 2010; Zhao et al., 2010; Pan et al., 2013; Zhai et al., 2014). Toxicity tests, either based on T24 bladder cancer cells or marine polychaete *Platynereis dumerilii*, showed that aromatic DBPs are typically several orders of magnitude more toxic than aliphatic ones (Yang and Zhang, 2013; Du et al., 2013; Wagner and Plewa, 2017).

In our preliminary study, 2-chlorophenylacetonitrile (2-CPAN) and 3,4-dichlorophenylacetonitrile (3,4-DCPAN), were identified as aromatic nitrogenous DBPs (N-DBPs) in drinking water using liquid/liquid extraction and gas chromatography/mass spectrometry (GC/MS). Their concentrations ranged from under the method detection limit ([MDL], 100 ng L⁻¹) to 530 ng L⁻¹. The toxicological evaluation of CPANs based on Chinese hamster ovary cells indicated that CPANs induced cell apoptosis at relatively low concentrations (10⁻⁵ M), approximately three order of magnitudes lower than the regulated DBP chloroform (one of the THMs). Additionally, the presence of the benzene ring enhanced the electron density of the nitrile carbon, leading to the high stability of the CPANs regardless of the presence of a disinfectant (i.e., chlorine or chloramine) (Zhang et al., 2018). CPANs may be important contributors to the toxicity of chlor(am)inated water. However, their isomers were absent in all of the drinking water samples tested in the previous study, likely due to their low level of occurrence and use of an insensitive method (MDL of 100 ng L⁻¹ each) (Zhang et al., 2018). Drinking water matrices are complicated, thus an appropriate pre-concentration method with a high recovery, high enrichment, and high selectivity is imperative for the simultaneous determination of CPANs at low concentrations. Solid-phase extraction (SPE) is a commonly used technique for pre-concentration of trace compounds in water via adsorption on selective cartridges (Camel, 2003a; Loos and Barceló, 2001). It is extensively used for the detection of multiple trace compounds due to its high operability and repeatability, low MDLs, and low solvent consumption (Wang et al., 2016; Kubáň et al., 2015). More importantly, the availability of diverse sorbent materials with modified surface functionalities significantly improves the selectivity for target analytes (Hennion, 1999; Hu et al.,

2018). In addition, SPE can easily achieve a 1000-fold pre-concentration of CPANs by optimizing the ratio of sample volume to elution volume.

Thus, the aim of this study was to set up a sensitive and precise approach using SPE-GC/MS to confirm whether or not six other unidentified CPANs (3-chlorophenylacetonitrile [3-CPAN], 4-chlorophenylacetonitrile [4-CPAN], 2,3-dichlorophenylacetonitrile [2,3-DCPAN], 2,4-dichlorophenylacetonitrile [2,4-DCPAN], 2,5-dichlorophenylacetonitrile [2,5-DCPAN] and 2,6-dichlorophenylacetonitrile [2,6-DCPAN]) are formed in actual drinking water during disinfection (chlorination and chloramination), and to simultaneously determine the concentration of eight CPANs, including two identified CPANs (2-CPAN, 3,4-DCPAN) and six unidentified CPANs. The paper describes the optimization of multiple SPE-GC/MS parameters. The developed method was utilized for monitoring the concentrations of eight CPANs in drinking water. Establishment of a more sensitive method to determine CPAN concentrations will facilitate the understanding of the occurrence and control of this toxic class of DBPs in drinking water.

2. Materials and methods

2.1. Chemical and materials

2-CPAN (98%), 3-CPAN (98%), 4-CPAN (98%), 2,6-DCPAN (98%), and 3,4-DCPAN (98%) standards were purchased from Aladdin Industrial Inc. (Shanghai, China). 2,3-DCPAN (98%) standard was provided by TCI Industrial Inc. (Shanghai, China). 2,4-DCPAN (99%) standard was obtained from Macklin Industrial Inc. (Shanghai, China). 2,5-DCPAN (98%) standard was supplied by Bide Pharmatech Ltd. (Shanghai, China). Physical and chemical characteristics of CPANs are listed in Table S1. Oasis HLB (hydrophilic–lipophilic balance) cartridges (30 mg, 3 mL) and Oasis WAX (weak anion exchanger) cartridges (500 mg, 6 mL) were purchased from

Waters Industrial Inc. (USA). ENVI-18 cartridges (30 mg, 3 mL) were obtained from Supelco (USA). Bond Elut-PH (500 mg, 3 mL) cartridges were provided by Agilent (USA). Other chemicals, including phosphate, sodium hydroxide (NaOH), and hydrochloric acid (HCl), were supplied by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Chemicals used in this study were of analytical grade unless otherwise noted.

2.2. SPE procedures

Unidentified CPAN isomers were suspected to be present in drinking water at ng L^{-1} concentrations, so it was hypothesized that SPE pretreatment was necessary, in order to increase the method sensitivity. The recovery and MDLs of SPE-GC/MS methods are related to type of cartridges, eluent, sample pH, and eluent volume; thus, these parameters were optimized in the current study. The SPE was operated using a 12-port SPE vacuum manifold (Supelco, USA) equipped with large volume samplers (Teflon [PTFE] tubes), a diaphragm vacuum pump (KNF), and a Visidry drying attachment (Supelco, USA). A Visidry drying attachment connected to a nitrogen bottle was used for drying the cartridges after adsorption and the final evaporation. First, 6 mL of methanol was loaded onto the cartridges at a flow rate of 1 mL min^{-1} for wetting cartridges, followed by 6 mL of ultrapure water at a flow rate of 1 mL min^{-1} to remove air and impurities. Then, 1 L of aqueous solution was loaded onto the cartridges at a flow rate of 4 mL min^{-1} . During conditioning and sample addition, SPE packing materials must be kept wet by allowing about 1 mm of the last used conditioning solvent to remain above the top cartridge frit to ensure efficient absorption, otherwise, the cartridges require conditioning again. After adsorption, the cartridges were dried with a constant flow of nitrogen for 30 min. Then the eluent was loaded

at a flow rate of 1 mL min^{-1} before being evaporated down to 1 mL with nitrogen. This final extract was transferred to an autosampler glass vial and analyzed by GC/MS.

1 L of a mixed eight CPAN standard solution at a concentration of 100 ng L^{-1} for each CPAN was used to evaluate optimization of the SPE process. The eight CPANs were dissolved in methanol at 1 g L^{-1} . Stock solutions were stored in amber glass bottles at 4°C and prepared fresh every week. The working solutions (100 ng L^{-1} each) were freshly prepared by diluting stock solutions with ultrapure water or buffer solutions before each use. Buffered solutions were prepared with phosphate (10 mM) and were adjusted to the desired pH (i. e., 1-12) with NaOH or HCl.

In phase 1 of tests, recoveries of samples pretreated with different type of cartridges, including ENVI-18, Oasis WAX, Bond Elut-PH, and Oasis HLB, were investigated. Since CPANs were found to be stable at neutral pH, the sample pH was adjusted to 7 before SPE. In phase 1, 5 mL of dichloromethane was used to elute the target compounds. ENVI-18 cartridges were selected for subsequent tests. Because the recoveries of target compounds are highly dependent on the eluent polarity, recoveries of the eight CPANs with ENVI-18 cartridge with 5 mL of dichloromethane, cyclohexane, n-hexane, and ethyl acetate were evaluated at pH 7 in phase 2. Dichloromethane was selected for subsequent tests. In addition, as sample pH can greatly affect extraction efficiency, so the recovery of the eight CPANs with ENVI-18 cartridges was tested at pH 1-12 in phase 3, using 5 mL of dichloromethane. For phase 4, pH 5 was selected, due to higher recovery than other pH levels. In phase 4, recoveries of the eight CPANs with ENVI-18 cartridges with different eluent volumes (i.e., 3, 5, and 10 mL) of dichloromethane were tested and the sample pH was adjusted to 5 before SPE.

2.3. Finished water samples

Drinking water samples were collected from nine drinking water treatment plants (DWTPs) applying different water treatment processes, thus their characteristics varied in parameters such as pH, dissolved organic carbon (DOC), NH_4^+ -N, total nitrogen (TN), and ultraviolet absorbance at 254 nanometer (UV_{254}) (Table S2). Upon sampling, a portable photometer (HACH Pocket Colorimeter™ II, USA) was used to measure residual disinfectant concentrations, then sodium sulfite (10 mg L^{-1}) was added to quench the residual disinfectant, at 120% of the disinfectant concentration on a stoichiometric basis (Zhang et al., 2018). Water samples were transferred to the laboratory via refrigerated transport. Upon collection, each sample was filtered through a $0.22 \mu\text{m}$ fiber membrane filter (Millipore, USA). The treatment process applied at each plant and the post disinfectant, as well as major parameters of the water samples, including pH, DOC, NH_4^+ -N, TN, and UV_{254} , are listed in Table S2. The pH was measured using a portable pH meter (pH-10, China). DOC and TN were measured using a Shimadzu TOC-VCPH analyzer equipped with a total nitrogen measurement unit, and NH_4^+ -N was measured using a HACH 6000 UV spectrophotometer (USA) based on a colorimetric method using NaOCl and salicylate reagent packages (Baethgen and Alley, 1989). UV_{254} was measured using a UV-vis spectrophotometer (UV-9000S, Metash instrument, China). Water quality parameters were measured on the day of sample collection. After that, sample pH was adjusted to 5 using phosphate buffer (10 mM), NaOH or, HCl, after which samples were stored at 4°C until analysis. Samples were analyzed within 24 h. An SPE blank and a spiked sample were analyzed in each batch of real drinking water samples. All samples were analyzed in triplicate.

2.4. Analytical methods.

A GC/MS (Shimadzu-QP2020, Japan), operated in the electron ionization (EI) mode, was used with a RTX-5MS (30 m $\times$ 0.25 mm ID, 0.25 μ m film thickness) column for analysis. The GC inlet was operated isothermally at 220 °C. Helium was used as the carrier gas at a constant flow of 2.31 mL min⁻¹. After injection, the GC oven temperature was kept at 50 °C for 3 min. Then the temperature was ramped to 110 °C at 25 °C min⁻¹ and was held for 3 min. Then the temperature was ramped to 155 °C at 25 °C min⁻¹. Finally, the temperature was raised to 220 °C at 20 °C min⁻¹ and was maintained for 3 min. The GC/MS was operated in the selected ion mode (SIM) for fast selective detection of CPANs. Under these GC conditions, all eight CPANs could be resolved with a single run. The eight main MS peaks, which were selected for EI-SIM detection, are presented in Table S1.

3. Results and discussion

3.1. Parameter optimization of GC/MS for eight CPANs

This study focused on eight CPANs because two were previously identified in drinking water. Thus, we anticipated that other undetected CPAN isomers could be present at ng L⁻¹ levels in drinking water. Suspected brominated and iodinated phenylacetone nitriles were not found with GC/MS in the scan mode and their standards were not commercially available, so we focused on CPANs in this study. A sensitive and accurate method was established before determining whether the target analytes were present in real drinking water. Eight commercially available CPAN standards (i.e., 2-CPAN, 3-CPAN, 4-CPAN, 2,3-DCPAN, 2,4-DCPAN, 2,5-DCPAN, 2,6-DCPAN and 3,4-DCPAN) were investigated. We first examined the GC and MS characteristics of the eight CPAN standards, obtaining accurate parent ions and product ions of eight CPANs, as shown in Fig.

S1. Three monochlorophenylacetonitriles (MCPANs) were ionized through a reduction step to generate $[M-Cl]^+$ and $[M-Cl-CN-H]^+$ ion fragments, so m/z of 151 (parent ion), 116, and 89 were naturally used for the identification of the three MCPANs when the MS was operated in the SIM mode. As for the dichlorophenylacetonitriles (DCPANs), $[M-Cl]^+$, $[M-Cl-CN-H]^+$, and $[M-2Cl-H]^+$ were generally formed; accordingly, m/z of 185, 150, 123, and 114 were used for identification. $[M-Cl]^+$ was the predominant product ion for both MCPANs and DCPANs. However, it is difficult to determine the exact CPAN structures by mass spectra because the characteristic ions of the three MCPANs are same, as are those for the five DCPANs. In addition, the physical characteristics (e.g., boiling point) of the three MCPANs and of the five DCPAN isomers are similar (Table S1). For instance, the differences in boiling points between 3-CPAN and 4-CPAN, and between 2,5-DCPAN and 2,6-DCPAN were less than 2 °C each, resulting in the same retention times for each pair, hindering their specific determination.

The optimal method for separating CPAN standards was determined by varying one parameter at a time and comparing the resolution of adjacent chromatographic peaks. The GC inlet, initial temperature, holding time, heating rate, and final temperature were optimized in this way. The GC inlet temperature was tested by varying it from 200 to 260 °C, 5 °C at a time, and the resolution was found to be optimal at 220 °C. The heating programs were executed in two phases, aiming to separate three MCPANs and five DCPANs, respectively. In the first phase, the initial temperature of the GC oven increased from 40 to 70 °C, 5 °C at a time, and 50 °C was demonstrated to be most suitable. Residence time at 50 °C was also tested. The residence time increased from 1 to 5 min, 1 min at a time; the resolution of eight CPANs was optimized when the residence time was 3 min. Three MCPANs entered the MS when the oven temperature was about 110 °C, so that the

oven temperature was first ramped to 110 °C. Different compounds have different responses to heating rate, and by constantly adjusting the heating rate, from 10 to 30 °C min⁻¹, the three MCPANs were found to be completely separated at 25 °C min⁻¹. The same optimization was conducted to achieve different retention time for the five DCPANs. The final oven temperature was also evaluated, over 200 to 280 °C. A much higher temperature will increase column loss and a lower temperature was not considered suitable for the gasification of compounds with high boiling points, causing the appearance of a “ghost peak”, which would interfere with quantification. The background interference was negligible when the final temperature was 220 °C. Once the parameters for the GC/MS were determined, optimization of the SPE conditions was undertaken.

3.2. Parameter optimization of SPE for eight CPANs

SPE parameters were optimized through testing mixed eight CPAN standards at 100 ng L⁻¹ each in buffer solution. The dominant factors, including cartridge and eluent type, eluent volume, and pH were evaluated.

3.2.1. Effect of SPE cartridges on CPAN recovery

[Fig. 1]

The main requirements for the selection of the appropriate SPE cartridge included: (1) selectivity towards the target compounds (i.e., CPANs); (2) the capability to extract target compounds over a wide pH range; and (3) a high capacity. Four different cartridges were tested. The cyano group in CPANs possesses the largest negative charge in the carbanion, contributing the polarity of phenylacetonitrile (Binev et al., 1995), and the introduction of chlorine, a group with high electronegativity (Deborde et al., 2008), increase the polarity of CPANs. ENVI-18 and HLB

cartridges are considered general purpose adsorbents for acidic, neutral, and basic compounds, and both cartridges are reported to be suitable for the adsorption of medium and polar compounds (Chu et al., 2012; Zhao et al., 2010). They are widely used in SPE owing to outstanding reproducibility and chemical stability. Bond Elut-PH cartridges are suitable for the selection of nonpolar compounds and these cartridges show different selectivity on aliphatic functionalized phases and aromatic compounds due to the electron density of the aromatic ring (Jha et al., 2013). Both reverse-phase and ion-exchange retention occur when samples flow through Oasis WAX cartridges, so this kind of cartridge has been used for pre-concentration of both short and long-chain compounds (Li et al., 2009). The recoveries of these cartridges are shown in Fig. 1a. The highest recoveries of all eight CPANs were obtained with ENVI-18 cartridges (83%-91%), whereas the recoveries of Oasis HLB and Oasis WAX cartridges were slightly lower, while relatively low recoveries for the three MCPANs pretreated with Bond Elut-PH cartridges were observed. Thus, ENVI-18 cartridges were selected for subsequent tests. The ENVI-18 cartridge is made using a silica gel base material and the matrix active group is C18 and is polymerically bonded. Target compounds were reserved on solid phase through Van der Waals forces or hydrophobic interaction (Camel, 2003b). End-capped ENVI-18 cartridges can avoid irreversible adsorption and the introduction of 17% carbon content increased its affinity. In addition, reversed polymeric phase provides additional π - π interaction when π -electrons are present in the analyte (i.e., CPANs) (Lundgren and Schilt, 1976), resulting in higher recovery of CPANs.

3.2.2. Effect of different eluents on CPAN recovery

After the adsorption of CPANs, captured CPANs were eluted from the packing materials using suitable eluents. The eluent must be properly selected to ensure stronger affinity for the target

compounds. Four commonly used eluents with different polarities (dichloromethane, n-hexane, ethyl acetate and cyclohexane) were tested. The results are shown in Fig. 1b. The recoveries of the dichloromethane and ethyl acetate ranged from 83% to 131%, whereas recoveries of n-hexane and cyclohexane were much lower ($\leq 30\%$), indicating that the polarity of the CPANs was closer to dichloromethane and ethyl acetate. The recoveries were higher than 100% when eluted with ethyl acetate, possibly due to interference from material leached from the packing. Dichloromethane had the best desorption capacity, and it is the most suitable eluent for CPANs.

3.2.3. Effect of sample pH before SPE on CPAN recovery

Solution pH affects the retention of ionizable target compounds on SPE cartridges. Selecting an optimal pH can enhance adsorption during the SPE procedure. As shown in Fig. 1c, the recoveries of ENVI-18 cartridges tended to increase with increasing pH from 1 to 5 and then declined. Acceptable recoveries ($> 80\%$) were obtained when the pH was in the range of 2 to 7. Basic conditions (i.e., pH 9-11) are not conducive to the determination of CPANs, recoveries $< 60\%$ observed. This can be attributed to the instability of CPANs under basic conditions. As illustrated in Fig. 1c, pH 5 was the most suitable for CPAN detection.

3.2.4. Effect of eluent volume on CPAN recovery

As shown in Fig. 1d, recoveries of the eight CPANs did not show significant differences when the cartridges were eluted with 5 or 10 mL of dichloromethane. Recoveries under both these conditions were higher than with 3 mL of dichloromethane. A 5 mL eluent volume was adequate to obtain acceptable recoveries ($> 90\%$). To reduce the use of organic solvents, this was selected as the eluent volume.

3.3. Detection limit, quantitation limit, recovery, and precision of the new method

The MDLs, method quantitation limits (MQLs), recoveries, and precision of eight CPANs were examined for the newly developed SPE-GC/MS method. The sample pH for determination of CPANs was adjusted to 5 before SPE. For each CPAN, the MDL was determined by multiplying the appropriate one-sided 99% t-statistic (when $n = 7$, t-statistic = 3.14) by the standard deviation obtained from seven analyses of a matrix spike containing the CPANs of interest at a concentration three to five times the estimated MDL (CPANs = 1 ng L⁻¹ each) as shown in equations (1) and (2):

$$\text{MDL} = 3.14 S \quad (1)$$

$$S = \sqrt{\frac{1}{n-1} \sum_{i=1}^n (x_i - \bar{x})^2} \quad (2)$$

$$\text{RSD} = \text{CV} = 100 S / \bar{x} \quad (3)$$

$$\bar{x} = \frac{1}{n} \sum_{i=1}^n x_i$$

x_i = the measurement of the variable x

RSD = relative standard deviation

CV = coefficient of variation

The MQL was defined as the lowest concentration that can be reliably achieved within specified limits of precision and accuracy during routine laboratory operating conditions and was defined as 10 times the signal to noise ratios. The precision is estimated by means of seven replicate analyses of the MQL. The most commonly used estimation of precision is the relative RSD or the CV, which was calculated as shown in equation (3).

[Table 1]

The recoveries were tested at three different CPAN concentrations (0.5, 5, and 50 ng L⁻¹ each). As shown in Table 1, the new method was demonstrated to be sensitive and accurate with detection limits ranging from 0.15 to 0.37 ng L⁻¹, MQLs ranged from 0.50 to 0.95 ng L⁻¹ (where the MQL was typically ~ 3 x MDL), and precision was in the range of 5.8% to 11% for the eight CPANs. Acceptable recoveries (>90%) and low MDLs were observed for this newly developed method.

3.4. Identification and occurrence of eight CPANs in actual drinking water

Having optimized the analytical conditions, the concentrations of eight CPANs in nine drinking waters were determined. When samples collected from DWTP 1 were analyzed with GC/MS, chromatographic peaks appeared in retention time of the corresponding CPAN standards (Fig. 2a and Fig. 2b) and their mass spectra completely matched the corresponding CPAN standards (Fig. S1). In addition, the eight CPAN standard solution was added into the finished water sample from DWTP 1 at a concentration of 10 µg L⁻¹ each and the responses of the eight peaks were amplified (Fig. 2d). For this reason, CPANs were confirmed to be present in DWTP 1. CPANs were not observed in source water collected from DWTP 1 (Fig. 2c), indicating that they were produced during the disinfection process. Eight CPANs were also demonstrated to be present in the other eight finished water samples in various concentrations.

[Fig. 2]

[Table 2]

The eight CPANs were detected in finished drinking water samples from all nine DWTPs at concentrations ranging from 0.5 to 155 ng L⁻¹ (Table 2), and none of the CPANs were detected in source waters. The three MCPANs were detected in all nine samples, among which 2-CPAN was

detected with the highest concentration, with a median concentration of 15 ng L^{-1} and maximum at 155 ng L^{-1} . The concentrations of 3-CPAN and 4-CPAN were lower, with a median concentration of 2.5 and 8.2 ng L^{-1} , respectively. Compared with MCPANs, lower concentrations of DCPANs were detected in finished water. Except for 2,4-DCPAN, median concentrations of the other kinds of DCPANs were lower than 5.0 ng L^{-1} each. It may be explained that both cyano group and chlorine were electron withdrawing groups, resulting in the decrease of the electron density of the benzene ring, so the introduction of the second chlorine substituent is usually more difficult than the first to insert (Yildirim et al., 2006). 2,3-DCPAN was detected in seven of nine samples, whereas the other seven CPANs were detected in the nine finished waters. The DOC levels of the finished water samples were about 3 mg L^{-1} ($2.41 - 3.95 \text{ mg L}^{-1}$), but the waters varied in CPAN concentrations. Water samples from five DWTPs that had been disinfected with chlorine had higher CPAN concentrations than that from the four DWTPs using chloramine. Eight CPANs ranged from 0.8 to 155 ng L^{-1} in chlorinated drinking waters and the median concentrations were $75, 11, 26, 12, 12, 16, 5.3,$ and 9.0 ng L^{-1} of 2-CPAN, 3-CPAN, 4-CPAN, 2,3-DCPAN, 2,4-DCPAN, 2,5-DCPAN, 2,6-DCPAN, and 3,4-DCPAN, respectively, whereas CPANs ranged from 0.5 to 15 ng L^{-1} in chloraminated waters and corresponding median concentration of the eight CPANs were $10.6, 1.9, 2.7, 2.1, 6.5, 1.7, 4.1,$ and 2.1 ng L^{-1} . Overall, the sum of all CPANs generated in chloraminated waters was 13% of that in chlorinated systems. In addition, water samples from DWTP 6 and 8 had the lowest concentrations of all eight CPANs, from 0.5 to 7.3 ng L^{-1} . The impacts of different treatment processes on the concentration of CPANs are worthy of further study.

4. Conclusions

In this study, a new method with SPE-GC/MS was developed for the simultaneous determination of eight CPANs in drinking water. The effect of SPE pretreatment parameters, including SPE cartridge type, eluent type, eluent volume, and sample pH before SPE, on the recovery of eight CPANs was evaluated. The optimized method was demonstrated to be sensitive and accurate with MDLs of the eight CPANs ranging from 0.15 to 0.37 ng L⁻¹. The precisions of the eight CPANs are in the range of 5.8%-11% and the recoveries of the eight CPANs ranged from 92% to 102%. Using the new method, all eight CPANs, excluding 2,3-DCPAN, were detected in all nine finished drinking water samples at concentrations ranging from 0.5 to 155 ng L⁻¹. On a median basis, chloramine resulted in less CPAN formation than chlorine. 2-CPAN was the most abundant individual CPAN species in both chlorinated and chloraminated drinking waters. More research into the impact of treatment processes and disinfectants on CPAN concentrations is needed.

Appendix A. Supplementary data

Supplementary data related to this article is available in this appendix.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (NO. 51822808; 51578389; 51778445), the National Major Science and Technology Project of China (NO. 2017ZX07201005), the Shanghai City Youth Science and Technology Star Project (NO. 17QA1404400), and State Key Laboratory of Pollution Control and Resource Reuse Foundation (NO. PCRRE16009) and Fundamental Research Funds for the Central Universities.

References

Baethgen, W. E., Alley, M. M., 1989. A manual colorimetric procedure for measuring ammonium

- nitrogen in soil and plant kjeldahl digests. *Commun. Soil Sci. Plan.*, 20, 961-969.
- Binev, I. G., Tsenov, J. A., Velcheva, E. A., Juchnovski, I. N., 1995. Infrared spectra and structure of phenylacetonitrile and of its carbanion: an ab initio force field treatment. *J. Mol. Struct.*, 344, 205-215.
- Bove, F., Shim, Y., Zeitz, P., 2002. Drinking water contaminants and adverse pregnancy outcomes: a review. *Environ. Health Persp.*, 110, 61-74.
- Bove, G. E., Rogerson, P. A., Vena, J. E., 2007. Case control study of the geographic variability of exposure to disinfectant byproducts and risk for rectal cancer. *Int. J. Health Geogr.*, 6, 18.
- Bull, R. J., Reckhow, D. A., Li, X. F., Humpage, A. R., Joll, C., Hrudey, S. E., 2011. Potential carcinogenic hazards of non-regulated disinfection by-products: haloquinones, halo-cyclopentene and cyclohexene derivatives, N-halamines, halonitriles, and heterocyclic amines. *Toxicology*, 286, 1-19.
- Camel, V., 2003. Solid phase extraction of trace elements. *Spectrochim. Acta, Part B*, 58, 1177-1233.
- Chu, W. H., Gao, N. Y., Yin, D. Q., Krasner, S. W., Templeton, M. R., 2012. Trace determination of 13 haloacetamides in drinking water using liquid chromatography triple quadrupole mass spectrometry with atmospheric pressure chemical ionization. *J. Chromatogr. A*, 1235, 178-181.
- Chuang, Y. H., McCurry, D. L., Tung, H.-h., Mitch, W. A., 2015. Formation pathways and trade-offs between haloacetamides and haloacetaldehydes during combined chlorination and chloramination of lignin phenols and natural waters. *Environ. Sci. Technol.*, 49, 14432-14440.
- Deborde, M., von Gunten, U., 2008. Reactions of chlorine with inorganic and organic compounds during water treatment-Kinetics and mechanisms: a critical review. *Water Res.*, 42, 13-51.
- Du, H., Li, J., Moe, B., McGuigan, C. F., Shen, S., Li, X. F., 2013. Cytotoxicity and oxidative damage

- 398 induced by halobenzoquinones to T24 bladder cancer cells. *Environ. Sci. Technol.*, 47,
399 2823-2830.
- 400 Hennion, M. C., 1999. Solid-phase extraction: method development, sorbents, and coupling with liquid
401 chromatography. *J. Chromatogr. A*, 856, 3-54.
- 402 Hu, S., Gong, T., Jian, M., Tao, Y., Xian, Q., 2018. Simultaneous determination of iodinated haloacetic
403 acids and aromatic iodinated disinfection byproducts in waters with a new SPE-HPLC-MS/MS
404 method. *Chemosphere*, 198, 147-153.
- 405 Jha, B., Kavita, K., Westphal, J., Hartmann, A., Schmitt-Kopplin, Ph. 2013. Quorum sensing inhibition
406 by *asparagopsis taxiformis*, separation of the compound that interrupts bacterial communication.
407 *Mar. Drugs*, 11, 253-265.
- 408 Krasner, S. W., Weinberg, H. S., Richardson, S. D., Pastor, S. J., Chinn, R., Scilimenti, M. J., Onstad, G.
409 D., Jr, T. A., 2006. Occurrence of a new generation of disinfection byproducts. *Environ. Sci.*
410 *Technol.*, 40, 7175-7185.
- 411 Karanfil, T., 2008. Occurrence, formation, health effects and control of disinfection by-products in
412 drinking water.
- 413 Kubáň, P., Makarůtševa, N., Kiplagat, I. K., Kaljurand, M., 2015. Determination of five priority
414 haloacetic acids by capillary electrophoresis with contactless conductivity detection and solid
415 phase extraction preconcentration. *J. Sep. Sci.*, 35, 666-673.
- 416 Lundgren, J. L., Schilt, A. A., 1976. Analytical studies and applications of ferroin type chromogens
417 immobilized by adsorption on a styrene-divinylbenzene copolymer. *Anal. Chem.*, 49, 974-980.
- 418 Loos, R., Barceló, D., 2001. Determination of haloacetic acids in aqueous environments by solid-phase
419 extraction followed by ion-pair liquid chromatography–electrospray ionization mass

- 420 spectrometric detection. *J. Chromatogr. A*, 938, 45-55.
- 421 Li, F., Zhang, C., Qu, Y., Chen, J., Zhou, Q., 2009. In breakthrough volumes of perfluorocarboxylates
422 in using WAX cartridges for analysis of sewage, ICBBE., pp. 1-4.
- 423 Li, X. F., Mitch, W. A., 2018. Drinking water disinfection byproducts (DBPs) and human health effects:
424 multidisciplinary challenges and opportunities. *Environ. Sci. Technol.*, 52, 1681-1689.
- 425 Mark, G. M., Elizabeth, D. W., Kristin M., Richardson, S. D., Woo, Y. T., Plewa, M. J., 2007.
426 Haloacetonitriles vs. regulated haloacetic acids: are nitrogen-containing DBPs more toxic?
427 *Environ. Sci. Technol.*, 41, 645-651.
- 428 Plewa, M. J., Wagner, E. D., Richardson, S. D., Thruston, A. D., Woo, Y.-T., McKague, A. B., 2004.
429 Chemical and biological characterization of newly discovered iodoacid drinking water
430 disinfection byproducts. *Environ. Sci. Technol.*, 38, 4713-4722.
- 431 Plewa, M. J., Wagner, E. D., Muellner, M. G., Hsu, K. M., Richardson, S. D., 2008. In comparative
432 mammalian cell toxicity of N-DBPs and C-DBPs. *ACS Symp. Ser.*, 995, 36-50.
- 433 Pan, Y., Zhang, X. R., 2013. Four groups of new aromatic halogenated disinfection byproducts: effect
434 of bromide concentration on their formation and speciation in chlorinated drinking water.
435 *Environ. Sci. Technol.*, 47, 1265-1273.
- 436 Qin, F., Zhao, Y. Y., Qin, F., Zhao, Y. Y., Zhao, Y., Boyd, J. M., Zhou, W., Li, X. F., 2010. A toxic
437 disinfection by-product, 2,6-dichloro-1,4-benzoquinone, identified in drinking water. *Angew.*
438 *Chem.*, 49, 790-792.
- 439 Richardson, S. D., Plewa, M. J., Wagner, E. D., Schoeny, R., Demarini, D. M., 2007. Occurrence,
440 genotoxicity, and carcinogenicity of regulated and emerging disinfection by-products in
441 drinking water: A review and roadmap for research. *Mutat. Res-Rev. Mutat.*, 636, 178-242.

- Richardson, S. D., Postigo, C., 2010. Drinking water disinfection by-products. pp. 93-137.
- Swan, S. H., Waller, K., Hopkins, B., Windham, G., Fenster, L., Schaefer, C., Neutra, R. R., 1998. A prospective study of spontaneous abortion: relation to amount and source of drinking water consumed in early pregnancy. *Epidemiology*, 9, 126-33.
- Villanueva, C. M., Cantor, K. P., Grimalt, J. O., Malats, N., Silverman, D., Tardon, A., Garcia-Closas, R., Serra, C., Carrato, A., Castaño-Vinyals, G., Marcos, R., Rothman, N., Real, F. X., Dosemeci, M., Kogevinas, M., 2007. Bladder cancer and exposure to water disinfection by-products through ingestion, bathing, showering, and swimming in pools. *Am. J. Epidemiol.*, 165, 148-156.
- Wang, W., Moe, B., Li, J., Qian, Y., Zheng, Q., Li, X. F., 2016. Analytical characterization, occurrence, transformation, and removal of the emerging disinfection byproducts halobenzoquinones in water. *TrAC, Trends Anal. Chem.*, 85, 97-110.
- Wagner, E. D., Plewa, M. J., 2017. CHO cell cytotoxicity and genotoxicity analyses of disinfection by-products: an updated review. *J. Environ. Sci.*, 58, 64-76.
- Yildirim, S., Ruinatscha, R., Gross, R., Wohlgemuth, R., Kohler, H. P. E., Witholt, B., Schmid, A., 2006. Selective hydrolysis of the nitrile group of cis -dihydrodiols from aromatic nitriles. *J. Mol. B-Enzym.*, 38, 76-83.
- Yang, M. T., Zhang, X. R., 2013. Comparative developmental toxicity of new aromatic halogenated DBPs in a chlorinated saline sewage effluent to the marine polychaete *Platynereis dumerilii*. *Environ. Sci. Technol.*, 47, 10868-10876.
- Yan, M. Q., Roccaro, P., Fabbicino, M., Korshin, G. V., 2018. Comparison of the effects of chloramine and chlorine on the aromaticity of dissolved organic matter and yields of disinfection

- 464 by-products. Chemosphere, 191, 477-484
- 465 Zhao, Y., Qin, F., Boyd, J. M., Anichina, J., Li, X. F., 2010. Characterization and determination of
- 466 chloro- and bromo-benzoquinones as new chlorination disinfection byproducts in drinking
- 467 water. Anal. Chem., 82, 4599-4605.
- 468 Zhai, H., Zhang, X. R., Zhu, X., Liu, J., Ji, M. 2014. Formation of brominated disinfection byproducts
- 469 during chloramination of drinking water: new polar species and overall kinetics. Environ. Sci.
- 470 Technol., 48, 2579-2588.
- 471 Zhang, D., Chu, W. H., Yu, Y., Krasner, S. W., Pan, Y., Shi, J., Yin, D., Gao, N. Y., 2018. Occurrence
- 472 and stability of chlorophenylacetonitriles: a new class of nitrogenous aromatic DBPs in
- 473 chlorinated and chloraminated drinking waters. Environ. Sci. Technol. Lett., 5, 394-399.

Figure captions

Fig. 1. Recoveries of eight CPANs with different cartridges (a), eluent (b), pH (c), and eluent volume (d) (initial concentration of each CPAN: 100 ng L^{-1}). The error bars in all of the figures represent the relative standard deviation of three replicates.

Fig. 2. GC chromatograms of finished (a) and source water (c) from drinking water treatment plant (DWTP) 1; 2-CPAN, 3-CPAN, 4-CPAN, 2,3-DCPAN, 2,4-DCPAN, 2,5-DCPAN, 2,6-DCPAN and 3,4-DCPAN standard compounds (b); finished water from DWTP 1 spiked with $10 \mu\text{g L}^{-1}$ of each of the eight CPANs (d).

Table 1. Method detection limits (MDLs), method quantification limits (MQLs), precision, and recoveries of eight CPANs.

CPANs	MDLs (ng/L)	MQLs (ng/L)	Precision	Recoveries		
				0.5 ng/L	5 ng/L	50 ng/L
2-CPAN	0.20	0.62	5.8%	91%	93%	94%
3-CPAN	0.15	0.50	8.1%	95%	92%	93%
4-CPAN	0.17	0.57	11%	94%	92%	92%
2,3-DCPAN	0.25	0.85	9.4%	93%	92%	91%
2,4-DCPAN	0.32	0.90	8.5%	98%	101%	99%
2,5-DCPAN	0.15	0.52	9.1%	91%	93%	91%
2,6-DCPAN	0.28	0.88	7.9%	95%	94%	95%
3,4-DCPAN	0.37	0.95	9.2%	99%	102%	99%

Table 2. Concentrations of eight CPANs in finished drinking water samples from nine drinking water treatment plants (DWTPs).

CPANs	DWTP 1 (ng/L)	DWTP 2 (ng/L)	DWTP 3 (ng/L)	DWTP 4 (ng/L)	DWTP 5 (ng/L)	DWTP 6 (ng/L)	DWTP 7 (ng/L)	DWTP 8 (ng/L)	DWTP 9 (ng/L)	Range (ng/L)	Median value (ng/L)	CPAN occurrence frequency (%)
2-CPAN	155 ± 6.2	75 ± 3.8	77 ± 1.4	14 ± 0.34	19 ± 1.2	7.3 ± 0.1	14 ± 0.2	5.0 ± 0.08	15 ± 0.3	5.0-155	15	100
3-CPAN	11 ± 0.5	69 ± 1.4	26 ± 0.5	1.7 ± 0.10	2.3 ± 0.13	0.6 ± 0.01	7.9 ± 0.1	1.3 ± 0.03	2.5 ± 0.1	0.6-69	2.5	100
4-CPAN	17 ± 0.6	56 ± 1.9	26 ± 1.2	131 ± 2.4	2.6 ± 0.10	0.6 ± 0.02	8.2 ± 0.3	2.4 ± 0.02	3.0 ± 0.2	0.6-131	8.2	100
2,3-DCPAN	12 ± 0.4	12 ± 0.3	22 ± 1.4	ND	3.7 ± 0.17	2.4 ± 0.01	10 ± 0.4	ND	1.8 ± 0.05	1.8-22	3.7	78
2,4-DCPAN	9.1 ± 0.5	12 ± 0.8	33 ± 2.2	148 ± 2.3	1.3 ± 0.10	2.5 ± 0.09	12 ± 0.09	3.0 ± 0.05	10 ± 0.3	1.3-148	10	100
2,5-DCPAN	10 ± 0.7	16 ± 1.1	6.6 ± 0.3	17 ± 0.20	0.8 ± 0.09	1.0 ± 0.04	2.4 ± 0.05	0.9 ± 0.01	4.2 ± 0.08	0.8-16	4.2	100
2,6-DCPAN	5.3 ± 0.4	5.0 ± 0.6	7.0 ± 1.1	33 ± 1.9	1.4 ± 0.12	2.4 ± 0.12	6.3 ± 0.04	0.5 ± 0.01	5.8 ± 0.08	0.5-33	5.0	100
3,4-DCPAN	9.0 ± 0.5	5.4 ± 0.7	6.0 ± 0.9	1.6 ± 0.1	12 ± 0.9	1.8 ± 0.10	1.7 ± 0.04	2.3 ± 0.01	4.7 ± 0.07	1.6-12	4.7	100

ND: Not Detected.

Highlights

A new SPE-GC/MS method was developed to determine 8 chlorophenylacetonitriles (CPANs).

6 CPANs were identified as new aromatic N-DBPs in drinking water for the first time.

CPANs were ubiquitous in finished drinking water at levels from 0.5 to 155 ng/L.

2-CPAN was the most abundant CPAN species in chlorinated and chloraminated waters.

CPAN concentrations in chlorinated waters were higher than in chloraminated waters.