Asian Journal of Chemistry; Vol. 35, No. 7 (2023), 1692-1696

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2023.27905

Trace Detection Method for Tetramine in Drinking Water by Atmospheric Pressure Gas Chromatography-Mass Spectrometry

HEJUN DUAN, YIXING FENG and YUMIN NIU*

Beijing Key Laboratory of Diagnostic and Traceability Technologies for Food Poisoning, Beijing Center for Disease Control and Prevention, Beijing, 100013, P.R. China

*Corresponding author: E-mail: 15210904641@163.com

Received: 20 April 2023; *Accepted*: 5 June 2023; *Published online*: 6 July 2023; AJC-21293

This study reports a trace detection method for tetramine in drinking water using atmospheric pressure gas chromatography-tandem mass spectrometry (APGC-MS/MS). After dilution with acetone, the drinking water samples were directly measured using APGC-MS/MS. Separation of tetramine was achieved using a DB-5MS weak polar column (30 m × 0.25 mm, 0.25 μm) and detected with APGC ionization source in positive ion mode. Cone gas and auxiliary flow rates were optimized to 250 L/h and 350 L/h, respectively. Quantitative and qualitative analysis was conducted by monitoring two molecular ions of the target compound (*m/z* 121.6 and *m/z* 92.6) under multiple reaction monitoring mode (MRM). The linear relationship between tetramine and its concentration was good in the range of 5-100 ng/L, with a correlation coefficient of 0.9999. The detection limit of the method was 0.3 ng/mL and the quantification limit was 1.0 ng/mL. The recovery of the samples was approximately 80.63% to 86.84%, with an intra-day RSD of 1.2% to 3.5% and an inter-day RSD of 2.9% to 4.9%, which met the requirements for trace detection. This method is simple, fast and highly sensitive and it is suitable for the trace detection of tetramine in drinking water.

Keywords: APGC-MS/MS, Water, Drinking water, Tetramine.

INTRODUCTION

Food poisoning is a serious food safety problem and has great social impact. China is a country with high incidence of food poisoning. According to the monitoring report of the National Health and Family Planning Commission, food poisoning caused by chemical and toxic foods is the most important cause of death in foodborne diseases in China from 2003 to 2020, accounting for more than 80% of deaths [1-3]. Rodenticide is the most important factor of chemical poisoning and the main substance of food terror (poisoning). Tetramine is the most common rodenticide and also a highly toxic rodenticide [4-7]. The production and use of tetramine has been explicitly prohibited in the List of Prohibited and Restricted Pesticides Published by the Ministry of Agriculture of China. However, due to its significant deratization effect, there are illegal commercial law illegal production and sales. Tetramine is colourless and odourless, often used to poison or commit suicide and thousands of intentional or accidental food poisoning incidents have occurred in China, resulting in hundreds of deaths [8-11]. Kan *et al.* [12] reported 49 poisoning cases and 13 environmental pollution cases among 65 poisoning cases between 1989 and 2000, with polluted water sources and poisoning in kitchen as the main causes. Su *et al.* [13] reported that 57 of 76 cases of tetramine poisoning were rural population, mainly involving drinking water and leftover meals. Tetramine poisoning in drinking water is more serious than other food poisoning.

It has been reported that human deaths occur when administered 7-10 mg of tetramine, so there is an urgent need to establish a highly sensitive method for detecting tetramine in environmental, food and drinking water poisoning [4]. The existing research mainly uses gas chromatography for the detection of tetramine, such as the standard GA/T 205-1999 gas chromatographic qualitative and quantitative analysis method for tetramine in poisoning case samples recommended by the Ministry of Public Security of China and the minimum quantitative limit of the method is 10 ng/mL; technical specification for forensic identification SF/Z JD01077003-2010 deter-

mination of tetramine in blood and urine by gas chromatography, with the lowest limit of quantification of 0.02 μg/mL; HJ 614-2011 determination of rodenticide in soil by gas chromatography issued by Ministry of Environmental Protection, the limit of quantification of this method is 14 μg/kg.

Gas chromatography-mass spectrometry has also been used for the determination of tetramine in recent years. Zhang *et al.* [14] reported the determination of tetramine in food and vomit matrix by solid phase extraction-gas chromatography combined with mass spectrometry and the detection limit of tetramine was 0.02 mg/kg. Li *et al.* [15] reported that fluoroacetamide and tetramine in fresh pork were simultaneously determined by gas chromatography-mass spectrometry and the detection limit of tetramine was 0.09 mg/kg. The above methods are mainly concentrated in soil, biological matrix and food matrix and the detection methods for tetramine in drinking water are very limited at present. Owens *et al.* [16] used solid phase extraction to enrich beverage samples and liquid chromatography tandem mass spectrometry was used to determine tetramine. The limit of quantification was 0.10 μg/mL and the limit of quantification of gas chromatography was 0.15 μg/mL. The sensitivity of the method could not meet the requirements of trace detection of tetramine [17]. Samples were analyzed for most volatile non-polar or semi-polar contaminants by gas chromatography-quadrupole-mass spectrometry (GC-Q-MS) or tandem mass spectrometry (GC-MS/MS) [18]. Due to its low sensitivity, it is usually necessary to use a solid phase extraction column for enrichment. The pretreatment process is complex and the cause of poisoning cannot be determined quickly. Atmospheric pressure gas chromatography (APGC) is a soft ionization technique that generates fewer fragments, produces multi-selective and sensitive MRM channels and improves the sensitivity and selectivity of molecular ions.

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EXPERIMENTAL

The Milli-Q ultrapure water purifier, manufactured by millipore in the United States, was used for generating ultrapure water. The cryogenic centrifuge made by Beckman Coulter in the United States was employed for separating components of samples at low temperatures. The vortex Shaker produced by Scientific Industries in the United States was utilized for mixing and stirring samples. The APGC-MS/MS system, which included a Model 7693 autosampler and a 7890A gas chromatography from Agilent located in Palo Alto, CA, USA, was used for the study. For the study, an APGC ionization source coupled with a Waters Xevo TQ-XS triple quadrupole mass spectrometer from Waters United States was used. The column was a DB-5MS weak polar column (30 m × 0.25 mm, 0.25 μm) (J&W Scientific, USA).

Ultra-pure water was obtained using a Millipore water purifier (Bedford, USA). LC/MSMS grade acetone solvent was purchased from Merck (Darmstadt, Germany) and LC/MSMS grade methanol from Fischer Chemical (Leicestershire, United Kingdom). The tap water used in the study came from Beijing City, while organic ultra-pure water was obtained from our local lab and the bottled mineral water were bought from local supermarkets. The tetramine used had a purity greater than 97% and was purchased from Toronto Research Chemicals in Canada.

Standard solution: Accurately weighed 100 mg of tetramine standard and dissolve it in a small amount of acetone in a glass beaker. Transferred the solution to a 100 mL volumetric flask, dilute with acetone up to the mark and shake well to prepare a tetramine stock solution with a concentration of 1000 mg/L. To prepare the standard curve, a series of standard samples with tetramine standard concentrations of 1, 5, 10, 20, 50 and 100 μg/L are prepared using a mixture of tetramine stock solution, ultrapure organic water and acetone (v/v = 50:50) in 2 mL glass bottles. The characteristic ion mass chromatographic peak area was plotted as the ordinate and the concentration of matrix added standard solution was plotted as the abscissa to make the standard curve.

Water samples pretreatment: Mixed well before sample treatment. Accurately pipette 1 mL sample into 4 mL glass vials, added 1 mL acetone solvent to dilute the sample, mixed well, centrifuge at 12000 rpm for 5 min and 1 mL supernatant was injection into vial for testing.

Instrument condition: Gas chromatography conditions: column DB-5MS type 5% phenyl-95% methyl polysiloxane stationary phase weak polar chromatographic column (30 m × 0.25 mm, 0.25 μm) (J&W Scientific, USA). The temperature of injection port is 280 ºC; helium was used as carrier gas at a flow rate of 3 mL/min. The gas control mode of the sample inlet is constant flow mode. The injection mode is pulse splitless injection. The injection volume was 1 μL. The initial column temperature was programmed to 40 ºC, maintained for 1min and then heated to 300 ºC at a rate of 60 ºC/min, maintained for 5 min. The interface temperature was 330 ºC, the auxiliary gas, cone gas and makeup gas were all nitrogen and the flow rates were 250 L/h, 250 L/h and 350 mL/min, respectively.

Mass spectrometric conditions: Xevo TQ-S; Positive ion mode of APGC ionization source. The discharge current of the corona pin was 3.5 μA and the cone voltage was 15 V. The temperature of ion source was 250 ºC, the interface temperature was 320 ºC, nitrogen was used as auxiliary gas, cone gas and makeup gas and the flow rates were 250 L/h, 250 L/h and 350 mL/min, respectively. Data was collected in multi-selected reaction monitoring (MRM) mode. Quantitative analysis was performed by monitoring two molecular ions of the target compound in multiple reaction monitoring mode. Data was

processed using the quantitative application manager, including data acquisition, process and quantitative results by TargetLynx.

RESULTS AND DISCUSSION

Optimization of instrument conditions: The product ion scanning experiments were carried out in multiple reaction monitoring mode and $[M^{\bullet}]^{+}$ was used as the parent ion for the selective transition. Ionization in APGC mode was optimized by injecting a high concentration standard solution of 100 ng/mL under full scan conditions. Different collision energies (10, 20, 30, 40 and 50 eV) were analyzed respectively, while the cone voltage of tetramine was optimized and the transition with the best sensitivity was selected for subsequent MRM method development, all with a collision energy of 30 eV. Lower energies result in no ion fragmentation of the product ions, while too high energies result in excessive fragmentation and reduced sensitivity. Table-1 shows the ions and parameters that were measured. The quantitative analysis was performed in multiple reaction monitoring mode (MRM). In mass spectrometry, molecular ions formed by electron beam bombardment correspond to molecular ion peaks in mass spectra. The whole ionization process occurs in a closed environment, making it possible to control the charge transfer process. Atmospheric pressure gas chromatography (APGC) is an ionization source that allows most Waters™ universal source systems (Xevo™, SYNAPT™ and Vion™) to be coupled to the GC. It is a soft ionization technique with high ionization efficiency. Reducing the sample injection volume minimizes matrix effects and the contamination of the instrument, reducing time-consuming purification steps and increasing uptime, respectively. It is capable of detecting contaminant limits at ultra-trace levels, enabling laboratories to meet regulatory limits. The soft ionization of atmospheric pressure sources reduces the fragmentation of many compounds, providing the greater sensitivity and selectivity and simplifying the precursor ion selection in MS/MS analysis. The soft ionization is also well suited for analyzing compounds that are easily degraded. The advantage of using APGC source parameters is that excessive fragmentation of volatile compounds can be reduced by tuning the parameters. The main tuning parameters include cone gas flow and auxiliary gas flow. The cone gas flow is the essential parameter to control the ionization mechanism and optimize the ionization efficiency.

The effect of ion extraction in a closed environment is modified by the fact that the cone gas flow in an APGC ion source is in the opposite direction of the auxiliary gas flow in the source. Usually, after the confirmation of parent and daughter ions of the target compound, the tuning gas flow rates of these two streams can change the second-order optimized fragment ion kurtosis and improve the sensitivity of the target compound. It includes the usage of cone gas flow to optimize ionization and the flow rate was set at 110~350 L/h; and auxiliary gas flow, the flow rate was set to 110~400 L/h.

A response surface plot is a 3D graph (Fig. 1) displays the relationship between tetramine and a response variable. In this case, the variables being plotted are the auxiliary gas flow and cone gas flow and the response variable is the optimization of two compounds. The graph is meant to show how varying the two gas flows affects optimality of tetramine. The optimization of tetramine requires a series of experiments in which the two gas flows were varied and the results were plotted on a response surface. The *x*-axis of the plot will represent the cone gas flow, while the *y*-axis will represent the auxiliary gas flow. The *z*-axis will represent the response variable, which is the optimization of tetramine. The optimal level of optimization will be located at the highest peak in the plot. However, too high the auxiliary gas flow rate results in a lower response for the target compound. An optimum and stable region can be seen with a cone gas flow rate of 350 L/h and an auxiliary gas flow rate of 200 L/h. By using the response surface plot, the gas flows can be optimized to achieve the best results for tetramine. The response surface plot is an effective tool for visualizing the relationship between two variables and a response variable.

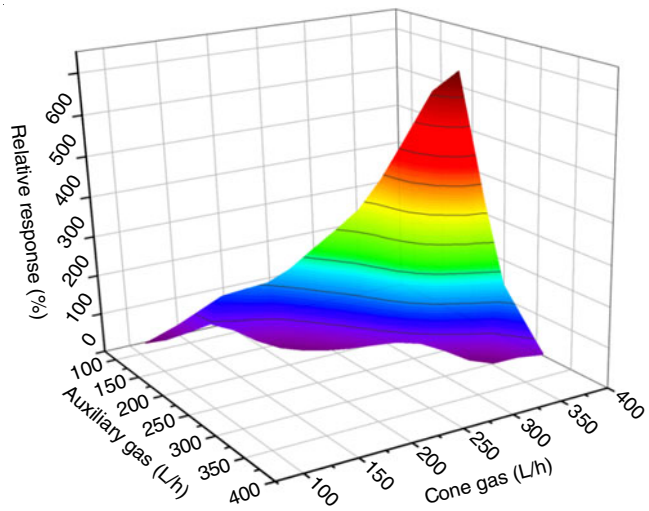

Fig. 1. Response surface of 10 µg/mL standard solution was shown to optimal relationship between auxiliary gas flow (L/h) and cone gas flow (L/h)

Methodological validation: A tetramine standard solution was prepared using a series of mixed standard samples in 1.5 mL glass bottles with LC-MS grade water and acetone (v/v = 50:50) to achieve tetramine concentrations of 1.0, 5.0, 10.0, 20.0, 50.0 and 100 µg/L. APGC-MS/MS was used to record chromatograms, plotting the quantitative ion peak area of target

TABLE-1
MASS SPECTRUM CONDITION PARAMETERS

Chemical	Molecular	Parent ion	Daughter ion	Collision energy (eV)	Cone voltage (V)
Tetramine	$C_4H_8N_4O_4S_2$	241.20	121.6*	30	15
			92.6	30	15

*The ion without interference and with the highest sensitivity is selected as the quantitative ion.

compound as the ordinate and the configured concentration as the abscissa. The resulting standard curve had a correlation coefficient of 0.9999. There is an acceptable linear relationship between peak area and concentration.The regression equation is given as:

$$y = 187.55x + 9.42$$

The quantification and the detection limit of the method are the spiked concentration values of the signal/noise ratio (S/N) ≥ 10 and ≥ 3, respectively obtained on the instrument after the standard substance is added to the blank water sample and diluted by acetone. The detection limit of this method is 0.3 μg/L. The limit of quantitation was 1.0 ng/mL. Compared with the GC-MS and LC-MS/MS detection methods reported in the literature, the sensitivity of the method is improved by 250 times and 100 times, respectively. Pang *et al.* [21] used gas chromatography-tandem mass spectrometry to detect the mass concentration curve of tetramine residues in food in the range of 25.0~250.0 ng/mL and the detection limit of method was 0.098 μg/kg.

Added 1.0 ng/mL, 5.0 ng/mL and 10.0 μg/L tetramine to the blank tap water samples, respectively. According to the method description, the six samples were pre-treated at each concentration and quantified by calibrating the standard curve of day, respectively (Fig. 2). Through quantification, the concentration was measured and calculated after spiking and then the recovery rates of the method were calculated by the ratio of the measured values of spiked samples to the true values) and continuously performed six parallel injections to examine the intra-day precision of the method. At the same time, the target compound was measured at the above spiked concentration as the spiked level for six consecutive days to examine the inter-day precision. The results are shown in Table-2. The recovery

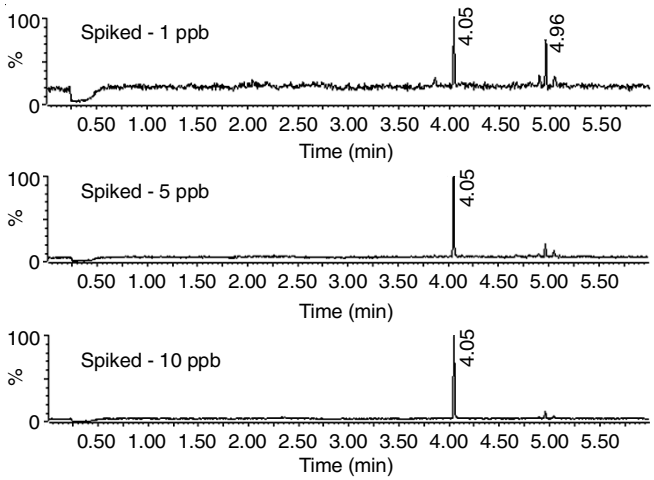

Fig. 2. TIC intensity of tetramine in water samples at different spiked levels

TABLE-2
RECOVERIES AND RSD% OF TETRAMINE BY TAP WATER IN DAYS AND BY DAYS

Spiked conc. (ng/mL)	Recovery (%)	Intra-day RSD (%) (n = 6)	Inter-day RSD (%) (n = 6)
1	80.63	2.03	4.65
5	84.46	3.53	4.92
10	86.84	1.21	2.86

was 80.63~86.84%, within-day RSD was 1.2%~3.5%, between-day RSD was 2.9%~4.9%.

Li *et al.* [15] reported that fluoroacetamide and tetramine were simultaneously determined in fresh pork by GC-MS and the detection limit of tetramine was 0.09 mg/kg. The above methods are mainly concentrated in soil, biological matrix and food matrix and the detection methods for tetramine in drinking water are currently very limited at present. Jager *et al.* [22] reported that they developed an automated SPME-GC-MS method for the determination of tetramethylene disulfotetramine in foods. The optimized method provided the limit of detection was 2.7 ng/g. Owens *et al.* [16] used solid phase extraction to enrich beverage samples and determined the tetramine by the liquid chromatography tandem mass spectrometry. The limit of quantification was 0.10 μg/mL and the limit of quantification of gas chromatography was 0.15 μg/mL. However, the sensitivity of the method could not meet the requirements of trace detection of tetramine. Now, the new method developed significantly improved the sensitivity of the determination of tetramine.

Methodological validation of other water samples: Mineral water, purified water and ultra-pure water samples were selected to prepare matrix spiked samples with low, medium and high concentrations (spiked concentrations were 1, 5 and 10 ng/L, respectively) as shown in Table-3. Six samples were analyzed for each type of water. According to the accompanying standard curve, the measured concentrations of spiked samples after deducting the background were calculated respectively and the recovery rate and precision RSD of the method were obtained.

Application in real samples: Ten samples of drinking water and commercial water were detected by the method. The results showed that tetramine was not detected in 10 water samples.

Conclusion

An atmospheric pressure gas chromatography-tandem mass spectrometry (APGC-MS/MS) method was successfully developed to determine of tetramine in drinking water. Three levels of tetramine were added to tap water. The average recovery rate was about 83.9%. The intra-day RSD was 1.21%~3.53% and the inter-day RSD was 2.86%~4.92%. The limit of detection was 0.30 ng/mL and the limit of quantification was 1.00 ng/mL. The method has the characteristics of high sensitivity,

TABLE-3
RECOVERIES AND RSD% OF TETRAMINE IN MINERAL WATER, PURIFIED WATER AND ULTRA-PURE WATER

Compounds	Spiked conc. (ng/L)	Mineral water (n = 6)		Purified water (n = 6)		Ultrapure water (n = 6)	
		Recovery	RSD (%)	Recovery	RSD (%)	Recovery	RSD (%)
Tetramine	1	81.32	3.91	82.32	2.77	83.56	2.95
	5	83.41	2.98	89.82	2.95	86.54	1.93
	10	85.62	2.45	88.28	1.93	87.48	2.01

simple sample extraction process and the like, the recovery rate and the precision of the experiment meet the requirements of monitoring residue analysis of water samples, is suitable for the determination of tetramine in water samples and the detection limit meets the requirements of the European Union standard. The established separation conditions can provide the tetramine with a suitable retention time on the chromatogram and the peak shape is good and then it avoids the interference of impurity peaks. The sensitivity of trace detection of tetramine in the water samples is defectively improved. The method has good recovery, sensitivity, reproducibility and stability.

ACKNOWLEDGEMENTS

This work was funded by Science and Technology Plan Project of Beijing Z221100007122002 and D171100008317001.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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