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Validation of a Solid Phase Extraction Technique for the Determination of Halogenated Acetic Acids in Drinking Water

(Validasi Satu Teknik Pengekstrakan Fasa Pepejal bagi Penentuan Asid Asetik Terhalogen dalam Air Minum)

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ABSTRACT

Haloacetic acids (HAAs) are one of the most common disinfection by-products formed during chlorination of drinking water. An analytical method involving solid phase extraction (SPE) followed by gas-chromatograph mass-spectrometry (GC-MS) was developed and optimized using experimental design to determine the HAAs in water. Selectivity, percent recovery, and detection limit studies were carried out on a Silia-SAX (Trimethyl ammonium chloride) SPE. Under optimized conditions, average recoveries for nine HAAs spiked in drinking water samples range from 69.2% to 108.2%. The relative standard deviation (RSD) data were found to range from 2.5% to 12.5% based upon five repeat recovery experiments and detection limit range of 0.16 to 0.009 µg/l were obtained. On this basis, SPE was studied as a possible alternative to liquid-liquid extraction (LLE) for the analysis of HAAs in water. The performance of the SPE-GC-MS with actual water samples was tested.

Keywords: Gas-chromatography mass-spectrometry; haloacetic acids; SPE

ABSTRAK

Asid haloasetik (HAA) merupakan salah satu hasil sampingan utama disinfeksi yang terbentuk semasa proses pengklorinan air minum. Satu kaedah analisis yang melibatkan pengekstrakan fasa pejal (SPE) diikuti oleh kromatografi gas-spektrometri jisim (GC-MS) telah dibangunkan dan dioptimumkan berdasarkan teknik reka bentuk eksperimen bagi menentukan HAA di dalam air minum. Faktor kepilihan, peratusan pemulihan dan had pengesanan telah dikaji menggunakan Silia-SAX (penukar anion ammonium kuatnari) SPE. Di bawah keadaan optimum, purata perolehan bagi sembilan komponen HAA dalam sampel air minum adalah dalam julat 69.2% sehingga 108.2%. Sisihan piawai relatif (RSD) bagi lima ulangan eksperimen didapati mempunyai julat antara 2.5% hingga 12.5% dan had pengesanan mempunyai julat 0.16 to 0.009 µg/l. Hasil ini menunjukkan SPE yang dibangunkan boleh digunakan sebagai kaedah alternatif menggantikan kaedah ekstraksi cecair-cecair (LLE) bagi analisis HAA di dalam air. Prestasi kaedah SPE-GC-MS yang dibangunkan diuji terhadap sampel air sebenar.

Kata kunci: Asid haloasetik; kromatografi gas-spektrometri jisim; SPE

INTRODUCTION

Chlorination is commonly used for treating drinking water and it is the main chemical disinfection measure applied worldwide, including in Malaysia. However, a number of animal studies and epidemiological investigations have shown that many disinfection by-products (DBPs) could cause cancers or have adverse effects on the urinary organs, digestive system and reproductive system (Klinefelter et al. 1995; Kronberg et al. 1989; Singer 1999).

An SPE-GC-MS was developed and optimized using experimental design version 7 (Sadia & Pauzi 2009). The experimental design was used to investigate and subsequently optimise the elution volume of derivatizing agent (10% acidic methanol), methyl-tertiary butyl ether (MTBE) volume and derivatisation time for haloacetic acids (HAAs) extraction in the water sample. Regression models and desirability functions were applied to

determine an experimental set up for acquiring the highest global extraction yield of HAAs. The elution volume and derivatisation time were the only statistically significant factors found from the study.

The purpose of this study was to validate the optimised protocol of SPE-GC-MS for the analysis of haloacetic acids in water. The validated method was then applied to the analysis of drinking water samples collected at different locations of Selangor, Malaysia.

MATERIAL AND METHODS

CHEMICALS AND REAGENTS

The acids studied were the nine HAA components namely monochloroacetic acids (MCAA), monobromoacetic acids (MBAA), dichloroacetic acids (DCAA), trichloroacetic acids

(TCAA), bromochloroacetic acids (BCAA), dibromoacetic acids (DBAA), dichlorobromoacetic acids (DCBAA), dibromochloroacetic acids (DBCBA) and tribromoacetic acids (TBAA). Individual standards were obtained from Supleco (Darmstadt, Germany). An individual standard solution of 1000 mg/L of each compound was prepared with methyl tert-butyl ether (MTBE, Supleco). Standard working solutions were prepared weekly or daily, depending on their concentration. All solutions were stored at 4°C in the refrigerator. A standard solution of 1000 mg/L of each HAA was used in acid form to study the recovery of the process. Ultrapure water was prepared with a Milli-Q water purification system (Millipore, Bedford, MA, USA). Anion exchanger Silia-SAX SPE cartridge was used.

INSTRUMENTATION

GC/MS ANALYSIS

A Hewlett-Packard (Palo Alto, CA, USA) 5890 gas chromatograph equipped with an HP5972 mass spectrometer and an HP7673 automatic injector was used. The GC system was equipped with a split/splitless injector. The fused silica capillary column DB-5.625 30 m × 0.25 mm I.D. × 0.25 µm film thickness fused silica was used. The column was inserted directly into the ion source of the mass spectrometer. The data were acquired with an HP Chemstation equipped with a Wiley 257 mass spectral library which was used to compare the experimental spectra obtained. The chromatographic conditions were as follows: the initial column temperature was 40°C which was held for 10 min, and finally it was raised to 150°C at 10°C / min. The injector was set at 210°C and the transfer line was maintained at 280°C. A 3 µL aliquot of the sample was injected in the split mode. Helium was the carrier gas used at a flow rate of 0.4 mL/ min. The electron impact (EI) ionization conditions were: ion energy 70 eV and mass range 10 to 500 in the full scan mode. Chromatograms were also recorded under time-scheduled selected ion monitoring (SIM). The MS was tuned to m/z 69, 219 and 502 with perfluorobutylamine (PFTBA).

SAMPLE PREPARATION

Silia-SAX- Trimethyl ammonium chloride (TMA-Cl) is mainly used as a strong anion exchanger (SAX) in ion exchange SPE. The function bears a positive charge across the whole pH range as well as in organic solvents. Since the chloride ion is bound relatively strongly to the ammonium, it may be suited to activate the ion exchanger by changing the chloride for an acetate counter ion.

SPE is more efficient than LLE, yields quantitative extraction that is easy to perform, is rapid and can be automated. Solvent use and experiment time are reduced. In the SPE process the sorbent used was Trimethyl ammonium chloride (TMA-Cl) as the functional group.

SPE cartridges (3 mL) were conditioned by adding two 10 mL aliquots of methanol to the cartridge and allowing

it to drain under vacuum, followed by 10 mL aliquots of deionized water. The samples were then attached to the vacuum manifold using the Teflon tubing and tube adapters. The flow rates were 1.5 mL/minute for all samples for complete passage of the water through the sorbent. In the whole process, constant flow rate was to be addressed specially in sample application and elution step. The vacuum lines were closed individually upon completion of the water transfer. To avoid loss of compounds, the vacuum was not applied to the sorbents any longer than necessary once the water had eluted. A clean up step was made using 10 mL of methanol to remove possible contaminants sample in sorbent. The teflon tubing from each sample cartridge was removed and the vial rack inserted with collection vials. A 10% H₂SO₄/MeOH solution was used as the elution solvent and placed at the top of the sorbent. Finally, the collected extracts were quantitatively methylated with MTBE at 50°C for 2 hours to produce ester derivatives. After methylation, 7 mL of Na₂SO₄ solution was added to increase the extraction efficiency. The extracted samples were placed in amber vials prior to GC analysis. In the above sample preparation technique, elution volume, MTBE volume and derivatisation time were selected as a design factors.

RESULTS AND DISCUSSION

To check the linearity of the method a range of HAAs standards at concentrations of 0, 20, 40, 60, 80, 100 µg/L were prepared. Calibration curves for each HAAs standard were obtained by plotting the concentration versus peak area counts. Coefficient of determination (R²) for all HAAs was 0.99. A linear curve fit was obtained using linear squares regression procedure. The concentration of HAAs in the SPE process were calculated using a five point calibration at concentration in the range of 20- 100 µg/l using three replicate injections at optimum method condition. The results obtained for the linearity range are shown in Table 1.

Accuracy is often calculated as recovery percentage of the analysis and determined at a known level of spiking. In order to prove the validity of the method, the halogenated acids recovery were estimated by analysing a drinking water sample water spiked with 10 µg/L, 20 µg/L, 40 µg/L and 60 µg/L. The accuracy was determined by spiking known amounts of analyte to sample across the specified range of the analytical procedure to obtain 10, 20, 40 and 60 µg/L concentrations.

Precision can be expressed in terms of relative standard deviation (RSD). It was assessed using four different spiked concentrations and five replicates for each concentration. As can be seen from the recovery results of SPE method stated in Table 2, the HAAs were recovered in the range of 69.2% to 108.2% for spiking level between 10 µg/l to 60 µg/l. The RSD from 2.5 % to 12.5 % was achieved for the above stated spiked concentrations. The RSD value is still within limit set by AOAC (Huber 2001). The decrease in recovery of MCAA at low spiking level due to low yield

TABLE 1. Calibration data for the HAAs studied

| Compounds | Range ($\mu\text{g/L}$) | Slope | Intercept | R ² | Linear regression equation |
|-----------|---------------------------|--------|-----------|----------------|----------------------------|
| MCAA | 20-100 | 1.084 | 3.9 | 0.995 | $y=1.084x+3.9$ |
| MBAA | 20-100 | 0.4747 | -0.9857 | 0.998 | $y=0.4747x-0.9857$ |
| DCAA | 20-100 | 1.0213 | -0.2143 | 0.999 | $y=1.021x-0.2143$ |
| TCAA | 20-100 | 0.939 | -1.2333 | 0.998 | $y=0.939x-1.2333$ |
| BCAA | 20-100 | 0.6244 | -1.0048 | 0.997 | $y=0.6244x-1.0048$ |
| DBAA | 20-100 | 0.2083 | 0.5857 | 0.993 | $y=0.2083x+0.5857$ |
| DCBAA | 20-100 | 0.2976 | -0.7619 | 0.995 | $y=0.2976x-0.7619$ |
| DBCAA | 20-100 | 0.0663 | -0.0476 | 0.996 | $y=0.0663x-0.0476$ |
| TBAA | 20-100 | 0.0724 | -0.0048 | 0.999 | $y=0.0724x-0.0048$ |

of the esterification reaction, photodegradation and might be pKa (2.80) value of MCAA (Anastasia 2002). The TBAA recovery was reduced due to decarboxylation and Pawlecki-Vonderheide et al. (1997) also reported the lowest response for TBAA because of partial decarboxylation of TBAA. However, these recovery levels were acceptable and within the levels recommended by the AOAC Peer Verified Methods. Similarly, the % RSD obtained were within the acceptable range of precision by the AOAC Peer Verified Methods (Huber 2001) for the analyte concentration range of 10 to 60 $\mu\text{g/l}$ (RSD from 21 % to 15 %).

The MDL is the lowest concentration of an analyte in sample that can be detected but not necessarily quantified, under the stated conditions of the test. LOQ, also known as the limit of reporting, is the lowest concentration of an analyte that can be quantified with acceptable precision and accuracy under the stated conditions of test (Sukiman & Pauzi 1993). For MDL, 7 replicates of a 3 $\mu\text{g/L}$ of 6 HAAs, 0.01 $\mu\text{g/L}$ of 3HAAs aqueous standard were analysed. MDL for HAAs using this method was extremely low. TCAA, DCAA, BCAA and DBAA were very sensitive in addition to determined in aqueous sample less than 0.01 $\mu\text{g/L}$. The MDL was calculated as 3.14 times the standard deviation of the 7 replicate. The value, 3.14, is the value of t for 7-1= 6 (N-1) degrees of freedom and at 99% level from the one-sided t distribution table (USEPA 1995). For this case MDL does not account for variation in sample composition and only achieve under ideal condition. Quantification limit is 3.33 times MDL. Table 2 shows the values of MDL and LOQ of each HAAs by using SPE extraction method.

For the determination of a method's robustness, a number of chromatographic parameters, for example, flow rate, column temperature, and injection volume were varied within realistic range and the quantitative influence of the analytes were determined. In the international conference of harmonisation, it is recommended to consider the evaluation of method robustness during the development, but it is not required to be included as part of a registration application (Huber 2001).

The ability of a method to determine accurately and specifically the analyte of interest in the presence of other

components in a sample matrix under the stated condition of the test (AOAC). The term qualitative selectivity is meant as the extend to which other substances interfere with the determination of a substance according to a given procedure (IUPAC 1995). Five replicates of HAAs spiked sample at four different concentrations were analysed. As can be seen from the recovery results in Table 2, the RSD range from 2.5 % to 12.5% were achieved for the four spiking levels. The overall results showed that SPE method of extraction is proved to be selective or specific.

The MDL and spiking recoveries for 9HAAs results are shown in Table 2 respectively. GC-MS method shows higher sensitivity for MCAA and offers a shorter run time. Brominated DBPs are now being recognized as toxicologically important. Many brominated DBPs have been shown to be more carcinogenic than their chlorinated analogs (Susan 2003). It is found that this developed SPE-GC-MS-SIM manages to detect both chlorinated and brominated species at the low $\mu\text{g/l}$ range in drinking water samples.

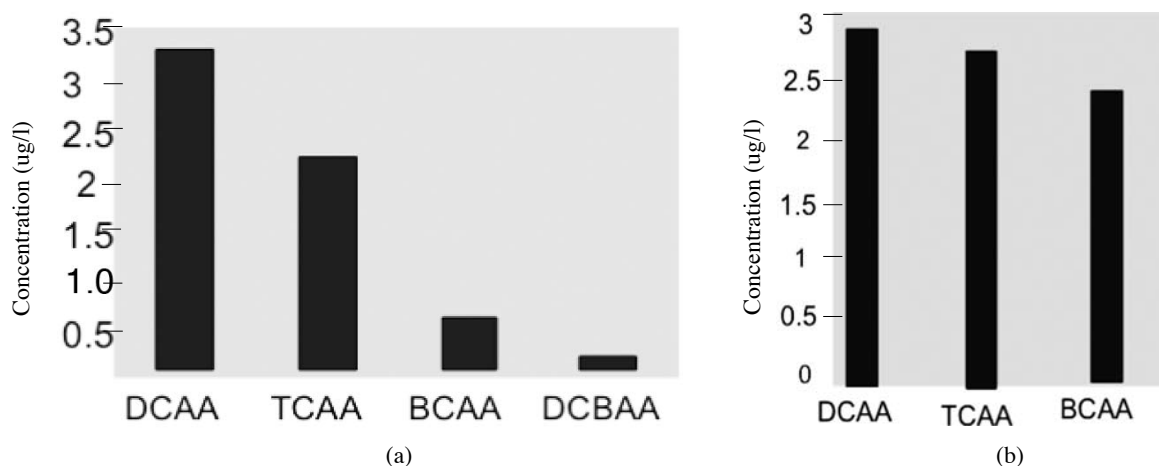
The MDL for (MBAA) and BCAA are 0.16 $\mu\text{g/L}$ and 0.009 $\mu\text{g/L}$ respectively which is lower than the typical values reported in EPA Method 552.1 (USEPA 1992), EPA Method 552.2 (USEPA 1995), and Standard Method 6251 (Clesceri 1998).

The spike recovery for TBAA was acceptable (75%). This indicates that further research is required to obtain better recovery for TBAA by GC-MS method. At the low concentration the recovery efficiency of MCAA was good as compared to high concentration, whereas for TCAA, BCAA and DCBAA SPE method seems to have good recovery than the solid phase extraction membrane disks method (Martinez 1998).

The method developed enables the haloacetic compounds to be determined at low $\mu\text{g/L}$ levels, which are better to the levels obtained by the most common method used based on an LLE process. Moreover, in the SPE-GC-MS method the use of carcinogenic compounds such as diazomethane is avoided. The application of the method was evaluated with tap water sample taken from Kajang and Bangi. The presence of some components of haloacetic acids in Kajang and Bangi can be seen in Figure 1.

TABLE 2. Mean recovery, relative standard deviation and detection limits of the SPE-GC-MS method at four Spiking levels (n=5 replicates)

| Compounds | Spiking level 10 µg/L | | Spiking level 20 µg/L | | Spiking level 40 µg/L | | Spiking level 60 µg/L | | MDL (µg/L) | LOQ (µg/L) |
|-----------|--------------------------|------|--------------------------|------|--------------------------|------|--------------------------|------|---------------|---------------|
| | %R | %RSD | %R | %RSD | %R | %RSD | %R | %RSD | | |
| MCAA | 91.5 | 3.8 | 91.5 | 3.8 | 82.6 | 4.1 | 82.6 | 7.1 | 0.21 | 0.65 |
| MBAA | 69.2 | 9.9 | 69.2 | 9.9 | 77.4 | 6.2 | 70.4 | 5.1 | 0.16 | 0.48 |
| DCAA | 110.2 | 4.8 | 110.2 | 4.8 | 98.4 | 5.0 | 110.0 | 6.0 | 0.02 | 0.06 |
| TCAA | 91.2 | 7.0 | 91.2 | 7.0 | 94.2 | 4.9 | 81.4 | 5.4 | 0.03 | 0.09 |
| BCAA | 80.0 | 3.6 | 80.0 | 3.6 | 88.6 | 2.3 | 83.8 | 4.8 | 0.009 | 0.02 |
| DBAA | 108.2 | 2.5 | 108.2 | 12.5 | 100.2 | 7.9 | 114.6 | 3.6 | 0.04 | 0.12 |
| DCBAA | 88.2 | 6.9 | 88.2 | 6.9 | 91.4 | 6.8 | 83.6 | 6.6 | 0.08 | 0.26 |
| DBCBA | 97.5 | 6.3 | 97.5 | 6.3 | 107.6 | 6.9 | 98.8 | 6.5 | 0.21 | 0.63 |
| TBAA | 75.2 | 6.2 | 75.2 | 6.2 | 76.6 | 8.8 | 78.7 | 12.5 | 0.42 | 1.26 |



Dichloroacetic acids (DCAA), Trichloroacetic acids (TCAA), Bromochloroacetic acids (BCAA), Dichlorobromoacetic acids (DCBAA)

FIGURE 1. Mean concentrations of HAAs in (a) Bangi (b) Kajang tap water

CONCLUSION

An SPE-GC-MS-SIM method was validated using a strong anion exchanger SPE cartridge and 50 ml of water sample. The method was able to analyse all the 9 HAAs components with detection limit (MDL) of 0.009 to 0.42 µg/L, recovery of 69.9 to 114.6% and precision (in terms of relative standard deviation) of 2.5 to 12.5%. It has clear advantages over the USEPA method 552.1 and other previous reported methods with regards to MDL and recovery for all 9 HAAs. Analysis of drinking water samples around Bangi and Kajang using this method indicated that the concentrations of HAAs were below the maximum contaminant levels of 60 µg/L (for the sum of the five regulated HAAs) established by United State Environmental Protection Agency (USEPA) regulations.

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