Int. J. Chem. Sci.: 13(3), 2015, 1085-1092
ISSN 0972-768X
www.sadgurupublications.com

COMPARATIVE STUDY OF SPME METHOD PERFORMANCE ON THE ANALYSIS OF MERCURY SPECIES IN WATER : INTEGRATED ON-FIBER VS IN-MATRIX DERIVATIZATION

WAN MOHD AFIQ WAN MOHD KHALIK[a], MD PAUZI ABDULLAH[a,b*] and MOHAMED ROZALI OTHMAN[a,b]

[a]School of Chemical Sciences and Food Technology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 BANGI, MALAYSIA
[b]Centre for Water Research and Analysis (ALIR), Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 BANGI, MALAYSIA

ABSTRACT

A comparison of derivatization step influence on the method extraction of mercury species has been carried out. Experimental design generated by using central composite design was used to optimize the extraction parameters. Signal response of targeted analytes, which accounts as sum of peak areas has shown maximum response (y = 276920, in-matrix derivatization) slightly higher compared to on-fiber derivatization (y = 274883). In both cases, two variables namely the concentration of solution and exposure time contribute significantly (ANOVA, $p < 0.05$). The time required for in-matrix derivatization to reach equilibrium is 0.9 min, slightly lower than on-fiber but similar value required for concentration of solution (1.4% m/v) and depth of fiber (49 mm) placed during extraction. Recovery for all species detected varied from 76-84% for in-matrix derivatization and 80-84% for on-fiber derivatization. For inorganic mercury trace level, analytical figure of merit namely recovery, repeatability and reproducibility better for on-fiber derivatization in the method extraction, as compared to in-matrix derivatization.

Key words: SPME method, Mercurry species, On-fiber, In-matrix.

INTRODUCTION

Derivatization is immediate reaction in sample treatment to transform targeted analytes into derivative form by using derivatizing agents. The derivative product must enhance selectivity, sensitivity and also provide better signal response during chromatographic

*Author for correspondence; E-mail: mpauzi@ukm.edu.my

separation in gas chromatography or other instrumental analysis[1,2]. Derivatizing agent play a role to reduce the polarity and the ionic character or to increase hydrophobicity of analytes. Thus, it will improve their chromatographic behavior in non-polar solvents and capillary GC columns[3]. Derivatization step can be introduced into matrix samples during pre or post extraction process. Alkylation, acylation and silylation are few common derivatization reactions used in the extraction of multi species of metal element namely arsenic, mercury, lead and tin. The use of borate reagent ($NaB(Et)_4$, $NaB(C_6H_5)_4$, $NaBH_4$) are suitable and convenient as alkylating reagent in the extraction of mercury.

An advantage of using borate reagent is that derivatization can be accomplished in matrix of samples, favored in buffered conditions[3,4]. In the presence of ethyl ligand containing species, ethylating reaction seem to be unfavorable. Alternatively, phenylation or propylation may also be used to distinguish between ethyl and inorganic species in the same matrix sample[3,5,6]. The efficacy of derivatization can still be limited by several factors such as more pre equilibrium time required for reaction, unsuitable functional group, excess usage of reagent and potential for matrix interferences[2,7,8].

In most of the recent study for determination of metal species, the derivatization and extraction steps are simultaneously carried out, this is known as in-matrix derivatization. However, the degree of substitution can be different in both direction and magnitude, thus can reduce method sensitivity and precision. In this light of concern, introduction of derivatization step in headspace (on-fiber) perhaps can lead to improvement on the resulting better signal response of chromatographic detection. The aim of this work was to compare the method performance (signal response) between two approaches of the derivatization step with the aid of response surface methodology.

EXPERIMENTAL

Chemical and materials

Mercuric salt (methylmercury (II) chloride, ethylmercury (II) chloride, mercury (II) chloride) and sodium tetraphenylborate with > 99.5% purity were purchased from Sigma Aldrich (St. Louis, USA). Methanol (liquid chromatography grade), sodium chloride, sodium acetate (analytical grade reagent) and acetic acid solution were purchased from Merck (Darmstadt, Germany). Polydimethylsiloxane fiber with the thickness 100 μm was purchased from Supelco (Bellefonte, USA). Fiber was conditioned according to the instructions provided by the manufacturer before the analysis. Ultrapure water was obtained from Milli-Q Easypure Rodi system (Barnstead, USA). Working solution was subsequently

diluted from stock solution (100 mg/L) in ultrapure water. Derivatizing agent, sodium tetraphenylborate was prepared fresh daily by dissolving appropriate amount in ultrapure water.

Extraction and analysis procedure

In general, 25 mL aliquot of samples with spiking level of mercury mixture at 10 ng/mL (adjusted pH to 4) was filled up into 40 mL amber vials. To ascertain the effect of derivatizing factors on method performance, sodium tetraphenylborate (1 mL) was added in-matrix or on-fiber based on condition set up by central composite design. Other optimum working conditions during the extraction remained constant namely extraction temperature (22.5°C), time (20 min), pH (4) stirring rate (200 rpm) and salt addition (8.5 ppm). In the case of in-matrix derivatization, exposure time was pre-equilibrium time prior to extraction. Fiber was retracted back into needle once extraction was complete before inserted into injector port.

The experimental variables and design matrix are shown in Table 1. Two level factorial, 2^3 central composite design was chosen to obtain fitted model and visualize three dimensional response surface plot. Juxtaposition in this design was setup at $\alpha = \pm 1.414$. In total, 20 numbers of experiments were performed for each mode of derivatization. All data processing, analysis and design of experiment were generated by using Minitab version 17 (Minitab Inc. USA).

Table 1: Design matrix for response surface methodology

Variables	Code	Code level		
		-1	**0**	**+1**
Concentration level (% m/v)	A	0.1	0.5	1.0
Exposure time (min)	B	2	4	6
Depth of fiber (mm)	C	15	25	35

Gas Chromatography-Electron Captured Detector (GC-ECD, Model Varian CP3800) equipped with HP-5 ms capillary column (30 m x 250 μm x 0.25 μm thickness) was used for chromatographic separation of targeted species. The GC oven temperature was programmed as follows: the initial temperature was 100°C held for 1 min and ramped to 300°C at a rate 20°C/min, held for 2 min. The injector and detector temperature were set to 200°C and 300°C, respectively. Purified nitrogen was used as the carrier gas at a flow rate of 1.5 mL/min. Desorption time was 1.2 min. Analytical figure of merit such as recovery, repeatability and

reproducibility were also tested in order to distinguish the method performance between derivatization approaches. An aliquot of water samples was spiked with mercury mixture solution at 25 ng/mL level concentration.

RESULTS AND DISCUSSION

Optimization of derivatization reaction

A central composite design was performed to determine the influence of derivatization step in method extraction under optimized conditions. The second order polynomial equation obtained in both derivatization modes are given in regression equation 1 and 2.

Response Surface (on-fiber)

$$= 157300 + 28900A + 29900B + 10000C - 81200B^2 + 30000C^2 \quad \ldots(1)$$

Response Surface (in-matrix)

$$= 153200 + 30600A + 30900B + 9600C - 78600B^2 + 31900C^2 \quad \ldots(2)$$

In both equations, variables namely the concentration of sodium tetraphenylborate (A), exposure time (B) and fiber depth (C) have shown positive linearity to the fitted model. The main effects of chosen variables in mode on-fiber derivatization are illustrated in Fig. 1.

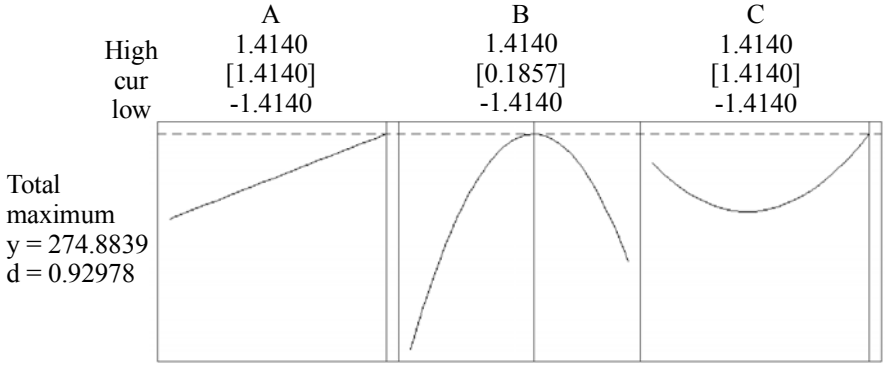

Fig. 1: Main effect on chosen variables in experimental design conditions (on-fiber mode)

Signal response of targeted analytes, which account as sum of peak areas were given better maximum response (y = 276920, in-matrix derivatization) when compared to on-fiber

derivatization (y = 274883). A good composite desirability was obtained in both mode with the desirability function, d is 0.929 (on-fiber) and 0.969 (in-matrix), respectively. The optimum working condition of derivatization step was obtained at 1.4% m/v (concentration of solution), 1.2 min (exposure time) and 49 mm (fiber depth from on top of septa cap) for on-fiber derivatization mode. The time required for in-matrix derivatization to reach the equilibrium is 0.9 min, slightly lower than on-fiber but similar value for concentration solution and depth of fiber placed during extraction. The significant of each variable was determined using ANOVA test and the p value as summarized in Table 2 and 3, respectively. In both case, variables namely concentration of solution and exposure time contribute significantly. An increase in the exposure time and concentration level of derivatizing agent prior to extraction theoretically will enhance the diffusion analytes through the fiber coating.

Table 2: Descriptive statistic of ANOVA test (on-fiber mode)

Variables	DF	Sum of square	Mean square	F Value	P Value
Model	5	83.880×10^6	16.776×10^3	8.13	**0.001**
A	1	10.021×10^6	10.021×10^3	4.86	**0.045**
B	1	10.719×10^6	10.719×10^3	5.20	**0.039**
C	1	1.118×10^6	1.118×10^3	0.58	0.460
B^2	1	57.465×10^6	57.465×10^3	27.86	**0.001**
C^2	1	7.840×10^6	7.840×10^3	3.80	0.072
Error	14	28.876×10^6	2.062×10^3	36.66	0.000
Total	19	112.756×10^6			

Bold value is significant at $p < 0.05$

Table 3: Descriptive statistic of ANOVA test (in-matrix mode)

Variables	DF	Sum of square	Mean square	F Value	P Value
Model	5	83.022×10^6	16.604×10^6	7.78	**0.001**
A	1	11.224×10^6	11.224×10^6	5.25	**0.038**
B	1	11.420×10^6	11.419×10^6	5.35	**0.036**
C	1	1.114×10^6	1.114×10^6	0.52	0.482

Cont…

Variables	DF	Sum of square	Mean square	F Value	P Value
B^2	1	53.877×10^6	53.877×10^6	25.23	**0.001**
C^2	1	8.880×10^6	8.880×10^6	4.16	0.061
Error	14	29.895×10^6	3.308×10^3	41.95	0.000
Total	19	112.916×10^6			

Bold value is significant at $p < 0.05$

It can be expected that the adsorption of inorganic mercury will increase by applying on-fiber derivatization mode. This is because inorganic mercury will form diphenylmercury, if the derivatizing agent was added in matrix sample, which is less extracted by the SPME fiber[5]. Therefore, phenylation performed better on-fiber rather than in aqueous. In this study, 1.06-fold enhancement of inorganic mercury in the extraction efficiency was obtained when applying derivatization on SPME fiber but still not significantly different. Interaction factor of fiber depth from the lowest to the highest code value has shown no significant effect in both modes.

Kinetic factor, which was assisted by the stirring effect was strong enough effect to enhance the partition coefficient of mercury species from the liquid phase. Nevertheless, signal response was higher when fiber was exposed closed to liquid phase. The interaction term between exposure time and fiber depth was illustrated in Fig. 2.

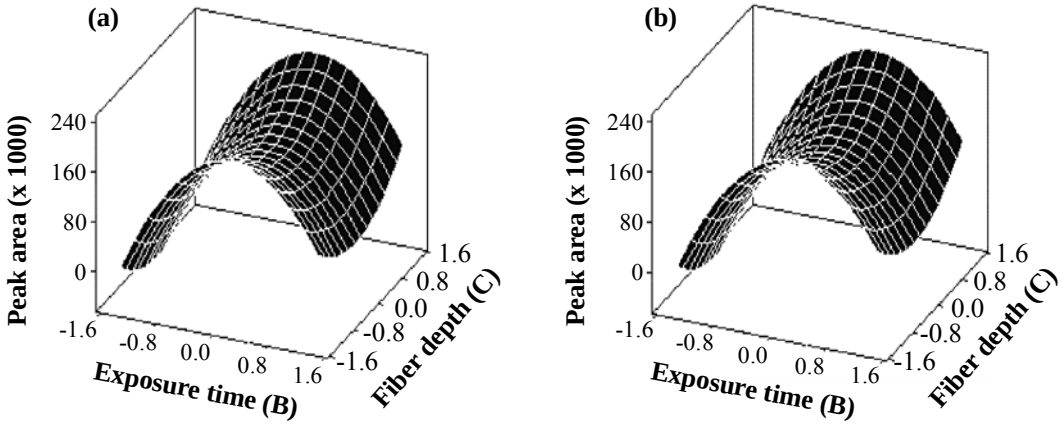

Fig. 2: Plot of response surface for interaction factor between exposure time vs fiber depth in (a) on-fiber and (b) in-matrix

Method validation

Recovery for mercury species detected was varied from 76-84% by applying in-matrix derivatization and found to be 80-84% for on-fiber derivatization. It was found that the recovery of inorganic mercury has slightly increased when phenylation reaction was expected to occur on the fiber coating surface. Good repeatability and reproducibility were obtained for all species with the mean value in the range of 17.6-20.7 ng/mL, which actual concentration was 25 ng/mL. The relative standard deviation was obtained with the percentage less than 2%. It was expected to get slightly lower value in reproducibility (n = 5) probably due to some analytes have been degraded or loss through volatility. An analytical figure of merit for method validation was summarized in Table 4 below.

Table 4: Analytical figure of merit for method validation

	Recovery (%)	**Repeatability (n=3)**		**Reproducibility (n=5)**	
	Mean	**Mean (ng/mL)**	**% RSD**	**Mean (ng/mL)**	**% RSD**
In-matrix derivatization					
MeHg	83.14	20.7	1.78	19.9	1.46
EtHg	81.83	20.4	1.97	19.5	1.62
InorgHg	76.20	18.5	1.49	17.6	1.26
On-fiber derivatization					
MeHg	82.49	20.5	1.76	20.4	0.46
EtHg	80.20	20.1	1.90	19.8	1.44
Inorg Hg	84.10	20.9	1.76	20.2	1.91

CONCLUSION

A comparative study of the influence of derivatization step in the method extraction of mercury species was successfully carried out. Variables namely the concentration of solution and exposure time contribute significantly in both cases, on-fiber and in-matrix derivatization. Signal response, accounted as the sum of peak areas for in-matrix derivatization was found to give better maximum response compared to on-fiber derivatization. However, analytical figure of merit such as recovery, precision and accuracy for inorganic mercury species show slightly better for on-fiber derivatization.

ACKNOWLEDGMENT

Authors are thankful to Ministry of Higher Education (MOHE) and Universiti Kebangsaan Malaysia for the financial research grant FRGS/1/2013/ST01/UKM/01/1. Gratitude is also extended to MOHE for the scholarship award, my Ph.D. to co-author during this study.

REFERENCES

1. F. Orata, Derivatization Reactions and Reagents for Gas Chromatography Analysis, Intech Open Access Publisher, Rijeka (2012) p. 84.
2. C. D. Stalikas and Y. C. Fiamegos, TrAC-Trends Anal. Chem., **27**, 533 (2008).
3. G. A. Zachariadis, J. Chromatogr. A, **1296**, 47 (2013).
4. A. M. Oliveira, G. A. Silva, R. J. Poppi and F. Augusto, J. Braz. Chem. Soc., **19**, 1041 (2008).
5. P. Grinberg, R. C. Campos, Z. Mester and R. E. Sturgeon, J. Anal. At. Spectrom., **18**, 902 (2003).
6. D. Gibičar, M. Logar, N. Horvat, A. Marn-Pernat, R. Ponikvar and M. Horvat, Anal. Bioanal. Chem., **388**, 329 (2007).
7. A. M. C. Ferreira, M. E. F. Laespada, J. L. P. Pavón and B. M. Cordero, J. Chromatogr. A, **1296**, 70 (2013).
8. M. Rutkowska, K. Dubalska, P. Konieczka and J. Namieśnik, Molecules, **19**, 7581 (2014).

Accepted : 30.06.2015