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Research Note

Comparative Study of Trace Level of Extracted Mercury in Different Water Samples with Aided Multivariate Statistical Analysis

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A B S T R A C T

Comparative study of trace level of extracted mercury in different types of water was successfully carried out. Data sets of batch samples were grouped in two clusters (C I; 4, C II; 4) to represent similarity of data structure under optimized and direct extraction procedure respectively. Similarity level for inter batch samples (optimized procedure) was obtained in the range of 96.7 – 99.2 %; which was better than by direct extraction (67.2 – 92.5 %) with mean distance from centroid was calculated at 0.462. The first two components (PC1 and PC2) on score plot explained about 86.2 % (ultrapure) and 73.9 % (salt water) of the total variance in signal data sets. In discriminant analysis, latent variables namely pH, extraction time and temperature were able to enhance the correctness of inter batch sample variations accounted to be 80 and 91.7 %. A fitted model expressed by multiple linear regressions obtained with two organomercury species (methyl and ethyl) were recognized as independent variables explained about 90.04 % (ultrapure) and 90.85 % (salt water) traceability from sum of peak areas. Analysis of real samples gave relative standard deviation value of less than < 0.33 % indicating that good performance in terms of repeatability. Recovery was found to range from 75.62 – 95.46% (river water) and 73.44 - 91.14 % (sea water).

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INTRODUCTION

Multivariate analysis or known as chemometric method is the step of processing data with numerous statistical techniques in order to extract latent variable at minimum loss of information. It can be implemented to various subject fields not only in chemistry but also in food analysis, microbiology, pharmaceutical and environmental monitoring studies. In analytical method development, this technique was used to design optimum procedure, validate the experimental procedure and discriminate the chromatograms signal response of targeted compounds in complex matrices [1, 2]. Cluster analysis (CA), factor analysis (FA) and discriminant analysis (DA) is a few common chemometric methods used for data processing. Cluster analysis was applied only to observe the similarity measured characteristic between different batches of samples (clusters) and not to discriminate latent factors in determining the difference between clusters; thus, this technique is called as unsupervised pattern recognition method [3]. The graphical output generated from CA shows how variables are merged on one axis, whereas the other axis gives the distance at which any two clusters are joined [4].

Principle component analysis (PCA) is a pattern recognition method used to manipulate the complex data that are often inter related variables. It also provides visual aid (score and loading plot) for the identification of homogeneity and inhomogeneity in the data sets [5, 6]. The first principal component must represent the highest variation in the data sets, then the second principal component is orthogonal to the first and the remaining variation will decrease consecutively till cumulative variance reaches to 100%. Each variance is equal to the eigenvalue of correlation matrixes [5, 7]. To ascertain the relationships between independent and dependent variables, multiple linear regression was always performed. High agreement between the

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experimental and predicted values should be obtained to indicate the good quality of the models [8].

Numerous microextraction techniques have been employed by researchers namely solid phase (SPME), stir bar (SBME), single drop (SDME), liquid liquid (LLME), liquid phase (LPME) and etc. in order to extend the analytical procedure towards green chemistry approach. One of the most applied techniques in analytical methods is solid phase microextraction, which is simple, fast and solvent free. This technique is a combination of pre concentrate, derivatization, extraction and clean up steps into a single device [9-11].

In complex matrices with high molecular weight of analytes, the extraction time could be shortened by increasing the temperature level, thus would enhance the evaporation mass transfer rate [12]. Pre concentrate is a necessary step for tracing pollutant like mercury because organometallic species usually present at low level of concentrations and there are high chances to lose through volatility.

This technique (SPME) has been successfully applied to extract mercury species present in river or marine water[9, 13-16]. To the best of our knowledge, there is no report to ascertain the detection of mercury species by using one SPME technique for different types of water. In this study, multivariate analysis was applied to construct the classification group of extracted mercury based on their detection (signal response) and discriminate the latent variables under the optimum conditions. The significance of this work is to understand the applicability of the developed technique to trace mercury species composition at low concentrations level in different sources of water.

MATERIALS AND METHODS

Chemical and reagent

Three mercury standards namely methylmercury (II) chloride, ethylmercury (II) chloride, mercury (II) chloride and sodium tetraphenylborate of purity above 99% were purchased from Sigma-Aldrich (St. Louis, USA). Sodium chloride, sodium acetate (analytical reagent grade) and methanol of liquid chromatography grade 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 a Milli-Q EasypureRodi system (Barnstead, USA).

Stock solutions of individual mercury standards were prepared in methanol at 1000 mgL^{-1} level concentrations and stored at 4°C. The primary mixture stock solutions were then subsequently diluted in ultrapure water for comparative study. The derivative reagent, sodium tetraphenylborate solution (1% $NaPh_4B$) was prepared daily in ultrapure water. Buffer solution (sodium acetic solution) used to adjust pH values was prepared by dissolving an appropriate amount of sodium acetate in acetic acid, while salt (sodium chloride dissolved in deionized water) was used to enhance the ionic strength of the required solutions. Salt water used for comparative study was prepared at 100 mgL^{-1} concentration level.

Extraction procedure and instrument analysis

In general, 25 mL an aliquot samples with spiking level at 10 μgL^{-1} (adjusted pH to 4) was transferred into 40 mL amber vials. Sodium tetraphenylborate (1mL) was added into vial, capped and were then left for 5 minutes to reach the pre-equilibrium phase. An optimum working condition was performed at temperature (22.5°C), time (20 min), pH (4) and stirring rate (200 rpm). Varian CP3800 Gas Chromatography – Electron Captured Detector (GC-ECD) equipped with HP-5ms capillary column (30 m×250 µm × 0.25 µm thickness) was used for chromatographic separation of targeted species. The description of the instrument setting in detail is summarized in Table 1.

TABLE 1. Instrument setting for the analysis of targeted compounds

Aspect	Setting
Desorption time	1.2 min
Injection temperature	200°C (splitless mode)
Detector temperature	300°C
Oven program	Initial 100°C (held 1 minute) to 300°C (ramped with 20°C/min), then held for 1 minutes. Total runtime is 13.5 minutes
Flow rate	1.5 mLmin-1 (99% purified nitrogen)
Make up flow	4 mL

Comparative analysis

In this study, the chromatographic signal data sets were being patterned subject to multivariate statistical analysis namely cluster analysis (CA), principle component analysis with factor analysis (PCA/FA), discriminant analysis (DA) and multiple linear regression (MLR). All data processing and statistical analysis was done by using Minitab Software ver. 17 (Minitab Inc., State College, USA). To ascertain the capability of developed method on mercury extraction, an analysis of four batch samples (ultrapure water, tap water, salt water and wastewater, n = 40) with spiked level at 10 μgL^{-1} was carried out. Direct extraction was also performed without adjusting the original of pH, temperature and salting effect (Table 2). Signal response as the sum of peaks obtained in both modes was then clustered using Ward method.

TABLE 2. An experimental procedure for mercury extraction

Variables	Experimental design	
	Optimum condition	Direct extraction
pH	4	6 – 8
Temperature	22.5	Room temp.
Salt addition (ppm)	8.5	No
Filtration	Yes	No
Extraction time (min)	20	20
Volume (mL)	25	25
Stirring rate (rpm)	200	200

For the recognition of homogeneity and inhomogeneity of mercury species, the principle component analysis (eigenvalue decomposition method) was carried out. The signal response data sets were standardized prior to statistical analysis. The score plot was generated to elucidate clustering tendencies in order to visualize the relationship among species. Factor analysis using Varimax rotation method was performed to evaluate what dominant species was loaded in batch experiment in order to understand their pattern correlation. Linear discriminant analysis (LDA) in standard mode was performed in order to discriminate latent criteria as a function of the optimized variables used in mercury extraction.

Multiple linear regression (MLR) was performed to select significant models for predicting the trace level of the extracted mercury in different types of water. Linear regression expressions were generated using a training set of ultrapure and salt water samples (n = 30) and the predictive ability of the resulting models was evaluated against a test set of real samples (n = 20). In this case, real samples were collected from Johor Straits (represent marine water) and Tangkas River, Malaysia (represent freshwater). In laboratory, real samples were spiked with mixture mercury standards at the level concentration of 25 μgL^{-1} before being tested following the developed method procedure.

RESULT AND DISCUSSION

Eight sample batches were grouped into two clusters at the linkage distance, $(D_{link}/D_{max}) \times 100 < 32$. The dendogram of cluster analysis as rendered by Ward method is depicted in Figure 1. Cluster I represented the batch samples performed under optimized conditions of the ultrapure water; UPW1, tap water; TPW1, salt water; SW1 and waste water; WW1) with mean distance from centroid of 0.462. In cluster II (UPW2 – SW2) the mean distance from centroid was calculated at 0.855. Similarity level for inter batch samples were obtained in the range of 96.7 – 99.2 %, better than by the direct extraction with similarity level only achieved at 67.2 – 92.5 %. Relative standard deviation (% RSD) of batch samples under optimized conditions was obtained with value less than 1.06 %, which is much lower than performed by direct extraction procedure (< 2.49 %). This result was successfully explicated that the optimum procedure not only enhances the detection of mercury species from water samples but also assists to obtain good repeatability for routine analysis work. In the next stage of subsequent analysis, only ultrapure and salt water batch samples were used for comparative studies.

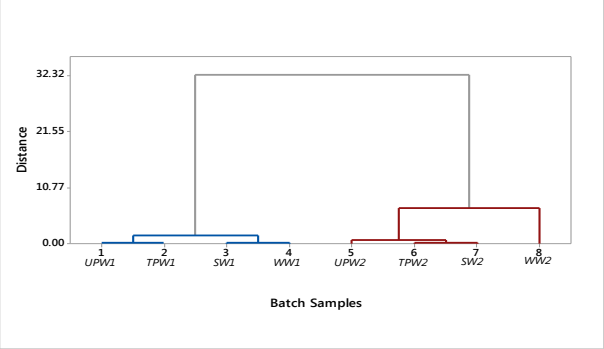

Figure 1. The dendogram of cluster analysis

In the ultrapure water samples set, the first two principle components (PC1 and PC2) explained about 86.2 % of variance from the total variances in signal response data sets. The score plot of these two components is presented in Figure 2. The first component had a strong loading on methymercury with 33.7 % variance from the total variance, with varifactor value, VF of 0.96. The second component was accounted for 33.7 % of the variance, with highly negative loading (VF =-0.97) on inorganic mercury. The third component had a strong positive loading on ethylmercury, which is associated at 32.5 % variance (VF = 0.93). Communalities of variance were high (> 0.9) indicating that extracted factor in each species fits well with the factor solution. Matrix effects in water samples to be low in ultrapure because no additional compounds presence to interfere during extractions. Furthermore, mercury analytes are known as volatile compounds, which mean that they can easily transfer from donor (in-matrix) to acceptor phase (fiber surface) in headspace area. Thus, with minimal interference in matrix samples, analytes were highly extracted during pre-concentration process.

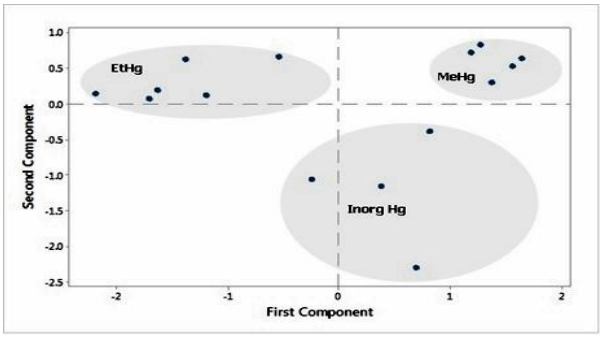

Figure 2. The score plot of PCA for detected mercury species

In the salt water samples set, the first two principle components (PC1 and PC2) represent 73.9 % of variance from the total variance in signal response data sets. A strong loading with varifactor, VF = 0.97 was obtained in the first principle component corresponding to inorganic mercury species. The second component was weighed by negative loading of methymercury species with varifactor value is -0.93. The third component had shown strong loading by ethylmercury species with varifactor value of 0.93. In this batch, the trace level of each species was equivalent with 33 % of loading factors compared to ultrapure samples. This phenomenon may due to the higher ionic strength presence in salt water higher than in ultrapure water.

An increasing ionic strength was known to be able to lead the decrease analytes solubility thus more analytes will be released into the headspace [17]. However, an addition of high salt more than the required quantity may cause an increase in the viscosity of solution, which leads to decrease the mass transfer rate [18]. In the case of ultrapure batch samples, although an addition of salt occurred, it may not be strong enough to enhance the ionic strength of analytes during the extraction process.

The dissimilarity of the mercury species loading between the batch samples is shown in the marginal plot, for example the signal response of methylmercury species against sum of peak areas (Figure 3). Box and Whisker plot of methymercury detected in salt water samples showed lesser margin as compared to ultrapure water. Small margin of scatterplot explicated that concentration of analytes detected more precise in repetitive measurements. Determination of methyl mercury was also better in the presence of high salting in effect, as can be seen in more standardized signal response load in positive value as presented in Figure 3b.

Significant criterion of optimized variables was investigated. Analysis of ultrapure and salt water samples was performed for the following batch 1) optimize pH only 2) optimize pH and extraction temperature 3) optimize pH, extraction temperature and time and 4) optimize all variables. In this study, variables namely pH, extraction time and temperature were the most influential variables that can distinguished variation between intra batch samples. Result showed that the classification matrix was assigned at 80 % (ultrapure) and 91.7 % (salt water) correctness by using these three variables in optimized procedure and increase to 100 % when all variables were optimized. Repeatability of the sample analysis was shown to be better when more variables were optimized. Discriminant function and classification matrix for salt water samples are presented in Table 3.

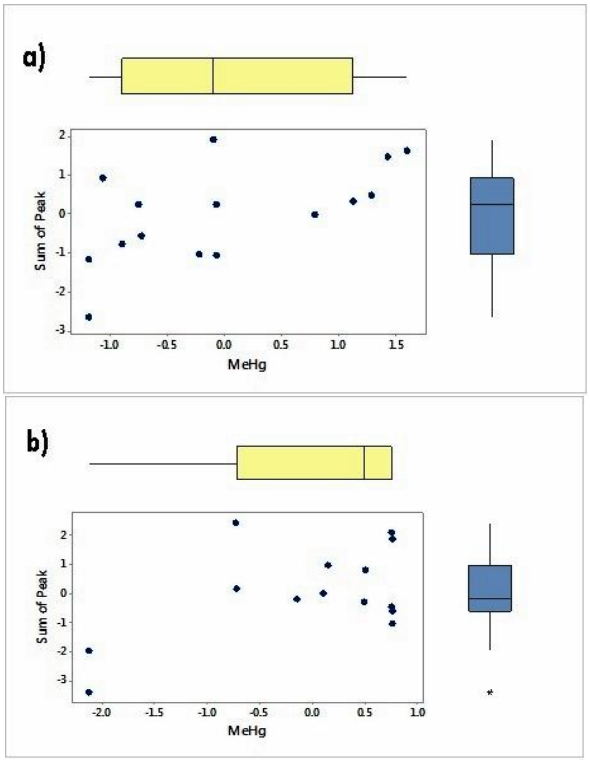

Figure 3. Marginal plot of methylmercury species loading response in a) ultrapure and b) salt water

Table 3. Discriminant function for mercury species in salt water batch samples

	Batch 1	Batch 2	Batch 3	Batch 4
Constant	1172.6	12.0	258.9	224.2
MeHg	94.0	5.0	45.5	43.5
EtHg	1362.8	130.1	639.4	593.3
Inorg Hg	73.9	8.2	34.2	31.5
N Correct	3	6	9	10
Correctness %	50	66.7	91.7	100

Statistical model for predicting the trace level of the extracted mercury was determined by applying the multiple linear regressions. A fit model was obtained with two organomercury species recognized as the independent variables in both types of water. It explained about 90.04 and 90.85 % of the detection of signal response from sum of peak areas with $r^2 = 0.996$ (ultrapure) and 0.997 (salt water) respectively. The standard deviation of residuals was obtained only for ultrapure water samples with the value of 2.42. The regression equations of the fitted model for both ultrapure (UPW) and salt water (SW) are as follow:

$$\text{Sum of peak areas (UPW)} = 14053 + 1.0024X_a + 0.9841X_b \quad (1)$$

$$\text{Sum of peak areas (SW)} = 12240 + 1.0087X_a + 0.9965X_b \quad (2)$$

Where X_a is the peak area for methymercury signal response and X_b is the peak area for ethylmercury signal response. To predict the ability of the resulting models, it was evaluated against a test set of real samples An analysis of real samples (n = 20) with spiked concentrations (25 μgL^{-1}) was successfully performed. No significant difference was found between intra batch samples (one way ANOVA, $p > 0.05$). The mean concentration of organomercury species in river water was traced at 18.68 - 22.71 μgL^{-1} (methyl mercury) and 23.61 μgL^{-1} (ethyl mercury) which are close to the actual spiked level concentration. Recovery was calculated within the range of 75.62 – 95.46 % (river water) and 73.44 - 91.14 % (seawater). The relative standard deviation was obtained in the range of 0.06 to 0.33 % (marine water) and 0.02 to 0.31% (river water) respectively.

CONCLUSION

An assessment of trace level of the extracted mercury in different types of water was successfully carried out. An application of multivariate statistical analysis on the chromatographic signal response successfully explained about the classification data structure, homogeneity and discriminated latent variables. Cluster analysis was successfully used to distinguish the similarity data structure between optimized and direct extraction procedure. In discriminant analysis, the correctness of inter batch sample variation was improved when extraction was performed under optimized conditions A fitted model generated by linear regression showed organomercury (methyl and ethyl mercury) as good predictor to evaluate the detection of total mercury signal response (90.04 – 90.85 %). This study also verified that under optimized condition, mercury species at low level concentrations can be determined with good repeatability and recovery. An analysis of real samples with spiked standards indicated that recovery was found to range from 75.62 – 95.46% (river water) and 73.44 - 91.14 % (seawater).

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Persian Abstract

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چکیده

بررسی مقایسه ای سطح ناچیز از جیوه ی استخراج شده در انواع مختلف آب با موفقیت انجام شد. برای نشان دادن شباهت ساختار داده به ترتیب براساس روش استخراج بهینه شده و مستقیم، مجموعه داده ها از نمونه دسته ای در دو خوشه (۴ ؛CI ، ۴؛CII) قرار گرفتند. سطح تشابه برای نمونه های دسته ای درونی (روش بهینه شده) در محدوده ۹۹/۲-۹۶/۷٪ به دست آمد، که از استخراج مستقیم با میانگین فاصله محاسبه شده از مرکز ثقل در ۰/۴۶۲ بهتر بود. دو مولفه اول (PC1 و PC2) در نمودار مقدار ۸۶/۲٪(خالص) و ۷۳/۹٪(آب نمک) از کل واریانس در مجموعه داده را توضیح داده اند. در تجزیه و تحلیل مشخص، متغیر نهفته یعنی pH، زمان استخراج و دما قادر به افزایش صحت تغییرات نمونه دسته ای درونی به ۸۰ و ۹۱/۷٪ اختصاص داده شده بودند. مدل برازش بیان شده توسط رگرسیون خطی چندگانه به دست آمده با دو گونه (متیل و اتیل) ارگانو جیوه به عنوان متغیرهای مستقل در حدود ۹۰/۰۴٪ (خالص) و ۹۰/۸۵٪ (آب نمک) قابلیت ردیابی از مجموع مساحت پیک ها توضیح داده شد. تجزیه و تحلیل از نمونه واقعی مقدار انحراف معیار نسبی کمتر از ۰/۳۳%> ، نشان می دهد که عملکرد خوب در شرایط تکرار به دست آمده است. بازیابی در محدوده ۷۵/۶۲-۹۵/۴۶٪(آب رودخانه) و ۷۳/۴۴-۹۱/۱۴٪ (آب دریا) قرار گرفت.