JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2009, 57, 3989–3994 3989
DOI:10.1021/jf900136x

Microwave-Assisted Extraction Coupled Online with Derivatization, Restricted Access Material Cleanup, and High-Performance Liquid Chromatography for Determination of Formaldehyde in Aquatic Products

Ligang Chen, Haiyan Jin, Haoyan Xu, Lei Sun, Aimin Yu, Hanqi Zhang, and Lan Ding*

College of Chemistry, Jilin University, 2699 Qianjin Street, Changchun 130012, China

A rapid technique based on microwave-assisted extraction (MAE) coupled online with derivatization, restricted access material cleanup, and high-performance liquid chromatography (HPLC) was developed for the determination of formaldehyde in aquatic products. Formaldehyde was first extracted with water under the action of microwaves and then directly introduced into a derivatization reservoir containing 2,4-dinitrophenylhydrazine (DNPH). The formaldehyde−DNPH derivative (100 μL) was loaded into a restricted access material (RAM) precolumn for online cleanup. Subsequently, the analyte was transferred from the precolumn to an analytical column and determined by UV absorption spectrum at 352 nm. The limit of detection (LOD) was 0.27 mg kg^{-1}. The intraday and interday precisions expressed as RSDs were 3.5% and 5.0%, respectively. This method was applied to determine the presence of formaldehyde in various aquatic products. The results were in agreement with those obtained by the state standard method (steam-distillation and offline HPLC analysis) used in China and higher than those obtained by the online ultrasound-assisted extraction (UAE) method. The recoveries obtained by analyzing 11 spiked aquatic products were in the range of 70.0%−105.0%. The online technique was demonstrated to be rapid with little consumption of samples and reagents.

KEYWORDS: Microwave-assisted extraction; restricted access material cleanup; online detection; formaldehyde; aquatic products

INTRODUCTION

Formaldehyde is a potential mutagen and carcinogen, which also can result in muscle toughening and water loss in aquatic products (*1*, *2*). It is forbidden to be used as a food additive in China (*3*). However, it is often used illegally in the food processing industry because the addition of formaldehyde can prolong the storage life of some foodstuffs and give a face-lift by changing their color and smell. Moreover, enzymatic degradation of trimethylamine oxide in aquatic products during postmortem storage also can generate dimethylamine and formaldehyde (*4*−*6*). The United States Environmental Protection Agency has set an acceptable daily intake (ADI) of 0.2 mg/kg body weight for this chemical (*4*). The limit amounts of formaldehyde proposed by the Italian Ministry of Health are 60 and 10 mg kg^{-1} for gadidae and crustanceans, respectively (*2*). A rapid method for the determination of formaldehyde in aquatic products at low concentration level is required.

Online analysis technique has received a great deal of attention in recent years. The advantages of the technique are reduction of analysis time, reagent and sample consumption, and analyte loss (*7*). An online method based on dynamic ultrasound-assisted extraction (UAE) coupled with solid support derivatization and high-performance liquid chromatography (HPLC) has been developed and successfully applied to the determination of formaldehyde in textile samples (*8*). However, the method was not suitable for analysis of the food samples, such as aquatic products. The most important reason is that the matrices of the aquatic products are complex, consisting of a great amount of protein and fat (*5*). A cleanup step must be included for the separation of the formaldehyde derivative from the matrix before chromatographic analysis. The extract containing protein and fat was also not suitable for solid support derivatization, which would block the derivatization column. Another reason is that some formaldehyde in aquatic products were bonded with protein (*4*); the ultrasound technique was not effective enough for the extraction of formaldehyde from aquatic products in a short time.

Steam distillation was used as the standard method in China for extracting formaldehyde from aquatic products (*9*). Its disadvantages are the consumption of a large amount of solvent and the long extraction time. Microwave-assisted extraction (MAE) has received increasing attention as a potential alternative to traditional solid−liquid extraction methods (*10*, *11*). Over the past few decades, MAE has been used in an offline mode for

*Corresponding author. Phone: +86-431-85168399. Fax: +86-431-85112355. E-mail: dinglan@ jlu.edu.cn.

 Published on Web 4/8/2009

accelerating the sample preparation process. Online MAE has also been used in recent years, allowing the automation of the preliminary step of the analytical process (*12*−*21*).

In the determination of formaldehyde, the cleanup of the complex sample extract was usually carried out by liquid−liquid extraction (LLE) (*22*), solid-phase extraction (SPE) (*23*), or solid-phase microextraction (SPME) (*4*, *24*). The SPE coupled to HPLC has been widely used in online analysis with column-switching (*25*). Development of special and selective sorbents is very important for SPE. Among different sorbents, restricted access material (RAM) is designed specifically for the removal of macromolecules partially based on a size-exclusion mechanism. Only small molecules are able to penetrate into the pores of RAM and interact with a stationary phase bonded on their inner surface, while large molecules are eluted with the washing solvent. The RAM was mainly used in the biological area for the removal of protein (*26*, *27*). It also can be used for analyzing environmental samples for the removal of humic substances (*28*, *29*). However, its applications in analyzing food samples were still scarce (*30*, *31*).

This research was aimed at the design of an online approach for the determination of formaldehyde in aquatic products involving MAE, 2,4-dinitrophenylhydrazine (DNPH) derivatization, RAM cleanup, HPLC separation, and UV detection. This method was applied to an analysis of different aquatic products and compared with the state standard method (offline steam-distillation) used in China and the online UAE method.

MATERIALS AND METHODS

Chemicals and Samples. Chromatographic grade acetonitrile (ACN) was purchased from Fisher (Pittsburgh, PA, USA). Guaranteed grade formaldehyde (100 μg mL^{-1}) as a standard solution was purchased from Guangfu Fine Chemical Research Institute (Tianjin, China). Pure water was obtained by the Milli-Q water purification system (Millipore, Bedford, MA, USA). Other chemicals were of analytical grade. Eleven aquatic product samples belonging to pisces (hairtail, yellow croaker, cod, catfish, and whitebait), cephalopoda (squid and cuttlefish), holothuroidea (sea cucumber), crustacea (shrimp), gastropoda (npatunede cumingi), and scyphozoa (jellyfish) were obtained from local markets. Their producing area is Dalian (China). These products were chosen and analyzed because the formaldehyde in them was detectable according to the previous studies (*4*, *32*).

Instruments. An online system was assembled in our laboratory. In this system, a TM_{010} microwave resonance cavity built in the laboratory was applied as the microwave coupling device. The schematic diagram and some other information of the microwave device were represented in the literature (*33*, *34*). A WGY-20 Microwave generator (Letter Swan, Changchun, China) with a maximum microwave output power of 100 W was used. The extraction vessel (60 mm long, I.D. Three mm) was made of polytetrafluoroethylene. Two FI-2100 peristaltic pumps (Haiguang, Bingjing, China) and a P230 high pressure pump (Elite, Dalian, China) were used. An Agilent 1100 liquid chromatograph (Palo Alto, CA, USA) was used, which was equipped with a quaternary pump, a heated column compartment, a UV detector, and a LC workstation. A Symmetry C18 column (150 mm × 4.6 mm I.D., 5 μm) was used as the LC analytical column (Waters, Milford, MA, USA). A Capcell Pak MF Ph-1 (20 mm × 4.0 mm I.D., 5 μm) column packed with RAM was obtained from Shiseido (Tokyo, Japan). A KQ 2200E ultrasonic bath (Ultrasonic Instrument, Kunshan, China) was used.

Analytical Procedure. A 0.1 g minced sample was weighed accurately and placed between two small plugs of glass fiber, which had been pretreated by methanol. Then the extraction vessel was put in the microwave resonance cavity. Five milliliters of DNPH solution (80 μg mL^{-1}) dissolved in the NaH_2PO_4 buffer (pH 4.0) was added into the derivatization reservoir.

Step 1 (5 min): 5 mL of extraction solvent (water) was passed through the extraction vessel at the flow rate of 1.0 mL min^{-1}. When the extraction vessel was properly filled with water, microwave heating was started with a power of 50 W, and the two tuning screws were adjusted to get the lowest reflected power. The formaldehyde extracted with water under the action of microwaves was transferred from the extraction vessel to the derivatization reservoir and derivatized by DNPH at 60 °C.

Step 2 (6 min): when the extraction was completed, the derivatization reaction was delayed 5 min again to ensure complete reaction. Subsequently, the formaldehyde−DNPH derivative was cooled and driven into the 100 μL sample loop. At the same time, the RAM column was conditioned in sequence with 3 mL of ACN and 3 mL of 5% ACN aqueous solution at the flow rate of 1.0 mL min^{-1}.

Step 3 (5 min): when the sample loop was filled, the formaldehyde derivative in the sample loop was introduced into the RAM column, and the matrix was washed out by 4 mL of 5% ACN aqueous solution at the flow rate of 1.0 mL min^{-1}. At the same time, the baseline of the detector was established by the chromatographic mobile phase of 50% ACN aqueous solution.

Step 4 (10 min): the analyte trapped on the RAM column was eluted in the back-flush mode into the LC column with the mobile phase at a flow rate of 1.0 mL min^{-1}. The transfer time was 1.5 min. The analyte separated by the LC column was monitored at 352 nm.

Steam Distillation and Offline HPLC Analysis (Standard Method). The steam distillation and offline HPLC determination of formaldehyde in aquatic products were performed according to the state standard method used in China (*9*). Ten grams of the minced sample was put into a 250 mL distillation flask, and 20 mL of pure water was added into it. This mixture was stirred and laid up for 30 min. Subsequently, 10 mL of 10% phosphoric acid aqueous solution was added into it. Water vapor generated from the boiling water in a flask was introduced continuously into the distillation flask to distill the sample. The vapor from the distillation flask was condensed and collected into a 250 mL round-bottom flask, which was immersed in a water bath. The distillation process was stopped when 200 mL of the distillate was collected.

One milliliter of the distillate was filtered and transferred into a test tube in which 0.2 mL of DNPH reagent was added. The test tube was tightly capped and placed in a 60 °C water bath for 15 min to form the derivative. The derivative was mixed with 2 mL of dichloromethane and centrifuged for 2 min at 3000 rpm. The supernatant was isolated and extracted with 1.0 mL of dichloromethane twice again. The lower layer solutions were combined and dehydrated by anhydrous sodium sulfate. Subsequently, the solution was evaporated to dryness at 60 °C, and the residue was reconstituted with 1.0 mL methanol and filtered with a 0.45-μm filter. An aliquot (20 μL) was injected into the HPLC for offline analysis.

RESULTS AND DISCUSSION

Online Cleanup Conditions. A Capcell pak MF Ph-1 column packed with RAM was used as the precolumn for the cleanup of the extract. The material in this column possesses long hydrophilic polyoxyethylene chains and hydrophobic phenyl groups on the surface of 80 Å silica in order to limit the access of large molecules such as proteins and retain small molecules longer (*35*). This column has been widely used in the analysis of biological samples (*36*−*38*). In this work, 4.0 mL of 5% aqueous ACN was selected as washing solvent in order to efficiently wash the sample matrix. The flow rate was set at 1.0 mL min^{-1}.

When the cleanup step was completed, the transfer of the analyte from the precolumn to the analytical column is a critical step. If the time is too short, a poor recovery of the analyte is observed. If the time is too long, many endogenous compounds can be transferred to the analytical column, which will affect the long-term performance of the assay. A transfer time of 1.5 min in the back-flush mode was found to be ideal for the assay.

Online Derivatization Conditions. In the determination of formaldehyde, the most often used derivatizing reagent before chromatographic analysis is DNPH, which reacted with formaldehyde to form the corresponding hydrazone (*39*, *40*). In this work, DNPH was also used as the derivatizing reagent.

The extraction and derivatization was performed simultaneously in the study, but when the MAE was completed, the derivatization was delayed a few minutes to ensure complete reaction. Several parameters affecting the performance of the online derivatization, such as DNPH concentration, pH value of buffer solution, derivatization temperature, and delay time for derivatization, were optimized by introducing 5 mL of formaldehyde standard solution (2.0 μg mL^{-1}) into the derivatization reservoir (**Figure 1**). The followed procedures were the same as those described in the analytical procedure in the section of Materials and Methods. When one parameter was changed, other parameters were fixed at their optimized values.

The effect of concentration of DNPH dissolved in NaH_2PO_4 buffer solution (0.1 mol L^{-1}, pH 4.0) from 13.2 to 132 μg mL^{-1}, which was equivalent to the molar ratio of DNPH/formaldehyde from 1 to 10, was studied (**Figure 1a**). The peak area increased with the increase of DNPH concentration up to 66 μg mL^{-1}, which was equivalent to the molar ratio 5:1 of DNPH/formaldehyde. In this work, 80 μg mL^{-1} of DNPH was adopted as derivatizing reagent in the following studies for achieving the complete reaction. The pH values of the NaH_2PO_4 buffer (0.1 mol L^{-1}) used for the derivatization were varied from 2 to 6 (**Figure 1b**). The result indicated that the peak area was not significantly different at pH values ranging from 3 to 5. In this study, a pH 4.0 NaH_2PO_4 buffer was selected.

The effect of temperatures from 20 to 80 °C on the derivatization was evaluated (**Figure 1c**). The peak area increased with the increase of the temperature from 20 to 60 °C, and no significant difference was observed above 60 °C. The derivatization temperature of 60 °C was chosen in this study.

The effect of the delay time from 1 to 10 min on the derivatization was investigated (**Figure 1d**). The peak area increased with the increase of the delay time from 1 to 4 min and did not change from 4 to 10 min. In this work, the delay time of 5 min was chosen.

Online MAE Conditions. A TM_{010} microwave resonance cavity, which has been used as a microwave coupling device, was also applied to concentrate the microwave energy in this study. Pure water was selected as extractant because it has been widely used for extracting formaldehyde from food samples (*40*, *41*). Other parameters affecting the performance of the online MAE, such as the microwave power, extractant volume, and extractant flow rate, were investigated (**Figure 2**). All experiments were performed using one squid sample. When one parameter was changed, the other parameters were fixed at their optimized values. The extraction yield of formaldehyde was defined as follows: extraction yield of formaldehyde (mg/kg, w/w) = mass of formaldehyde extracted from aquatic products /mass of aquatic products.

Generally, the higher temperature is profitable for accelerating extraction and reducing extraction time. The temperature of the extraction medium increased with the increase of microwave power. The experimental results demonstrated that the extraction yield of formaldehyde increased with the increase of microwave power when the power is in the range of 20−40 W and that the extraction yield did not significantly change from 40 to 80 W (**Figure 2a**). In this work, the microwave power of 50 W was used. The sample temperature cannot be measured in real time in the online system. The temperature of the extract can be measured only offline when it just flowed from the extraction vessel. It was 72 ± 1 °C when the microwave power was set at 50 W. The temperature of the extract before cleanup was also measured offline and was 21 ± 1 °C. The real time temperature measurement device for the online MAE will be studied in our future work. Moreover, the extraction was performed at atmospheric pressure.

The online MAE was evaluated by varying the extractant volume passing through the extraction vessel (**Figure 2b**). The experimental results demonstrated that the extraction yield of formaldehyde increased with the increase of the extractant volume from 2 to 4 mL and then was not significantly changed. The small extractant volume is not enough to completely extract the formaldehyde from the aquatic products. Five milliliters of extractant was chosen in this work.

The extraction was investigated at the extractant flow rate from 0.25 to 1.0 mL min^{-1}. The results indicated that the

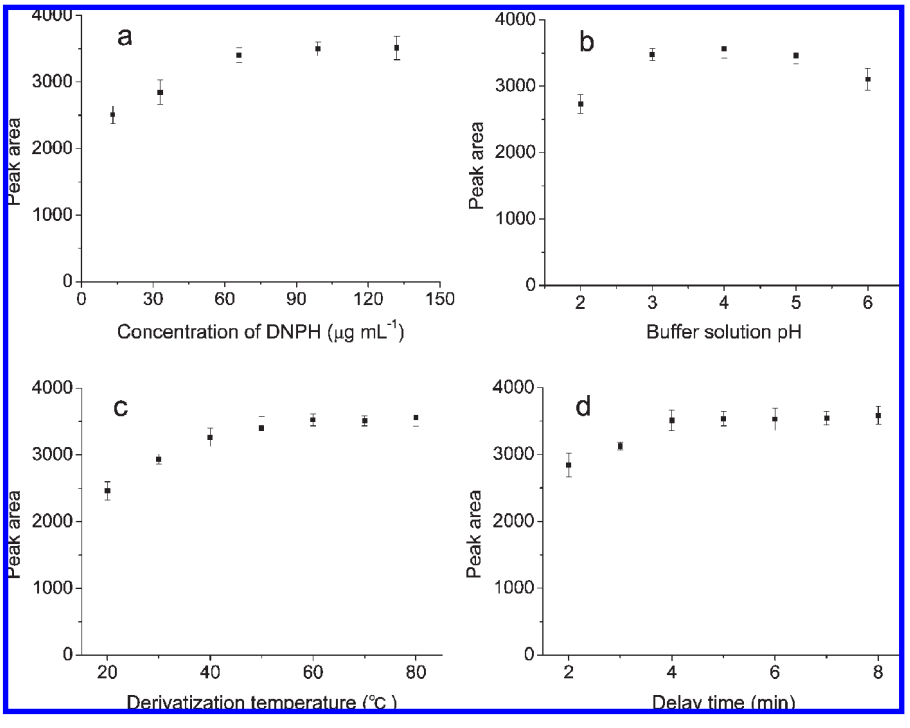

Figure 1. Influence of online derivatization parameters on the determination of formaldehyde, mean ± SD (n = 3).

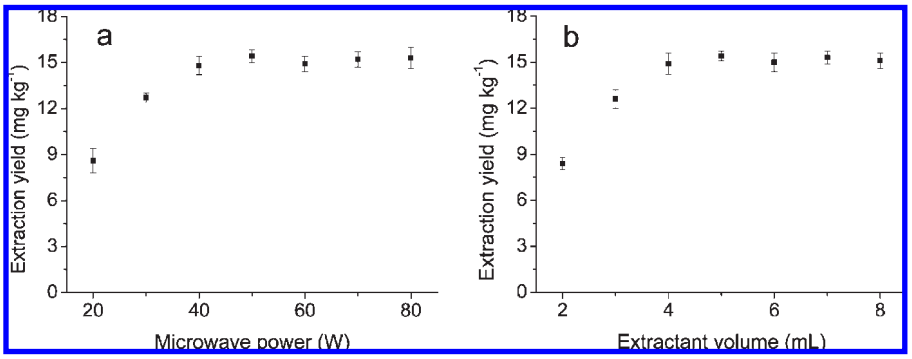

Figure 2. Influence of online MAE parameters on the extraction yield of formaldehyde, mean ± SD (n = 3).

extractant flow rate had no significant effect on the extraction yield ranging from 14.8 to 15.6 mg kg^{-1}. But the slow extractant flow rate would prolong the extraction time. In this study, 1.0 mL min^{-1} of the extractant flow rate was chosen.

Evaluation of the Online Method. The correlation coefficient obtained by analyzing six formaldehyde standard solutions in the linear range of 0.02−2.0 μg mL^{-1} was 0.9995. On the basis of the amount of sample (0.1 g) and the volume of extract (5 mL), the linear range also can be expressed as formaldehyde concentration in aquatic products in the range of 1.0−100 mg/kg aquatic products.

The blank signal present in the retention time of formaldehyde in the HPLC chromatogram is low and reproducible. This phenomenon was also reported by Liu et al. (*38*). The limit of detection (LOD), defined as the concentration corresponding to a signal equal to three times the standard deviation of the blank, was 0.27 mg kg^{-1}.

The intraday precision was evaluated by assaying one squid sample under the optimal conditions six times in one day. The extraction yields of formaldehyde obtained were 15.0, 14.6, 15.7, 15.3, 14.9, and 14.2 mg kg^{-1}. The interday precision was evaluated by assaying this sample once a day on six consecutive days. The extraction yields of formaldehyde obtained were 14.8, 14.3, 15.6, 16.2, 15.9, and 14.7 mg kg^{-1}. The intraday and interday precisions expressed as RSD were 3.5 and 5.0%, respectively.

Application of the Method. In order to demonstrate the applicability of the proposed method, it was used for the determination of formaldehyde in various aquatic products (**Table 1**). The contents of formaldehyde in these aquatic products were in the range of 3.2−29.6 mg kg^{-1}. The results obtained with the proposed method (online method) are in agreement with those obtained by the state standard method used in China (offline method). Therefore, the proposed method with the LOD of 0.27 mg kg^{-1} was suitable for the determination of formaldehyde in aquatic products. The ADI values of these aquatic products are in the range of 6.8−62.5 g/kg body weight taking into account the ADI of formaldehyde (0.2 mg/kg body weight). If the body weight of one person is 70 kg, the amounts of these aquatic products that can be consumed daily are in the range of 0.473−4.375 kg. However, this hypothesis is not very optimistic since people usually do not only eat aquatic products containing formaldehyde.

Ultrasound-assisted extraction (UAE) was also used for the comparative study. The extraction vessel was placed in the ultrasonic bath. The distance between the bottom surface of the vessel and ultrasonic source is 10 mm. Other extraction conditions were similar to those used in MAE. 0.1 g samples were extracted with 5 mL of pure water at a flow rate of 1.0 mL min^{-1} at 70 °C. The following derivatization, cleanup, and determination were the same as those used in the proposed online MAE method. The results in **Table 1** indicated that UAE was not suitable for the extraction of formaldehyde from aquatic products. The contents of formaldehyde obtained by UAE (1.7−18.4 g kg^{-1}) were lower than those obtained by MAE (3.2−29.6 g kg^{-1}) and the standard method (3.0−27.3 g kg^{-1}).

Table 1. Comparison of the Results Obtained by the Online MAE Method, Online UAE Method, and Standard Method (Offline Method)

	contents of formaldehyde in aquatic products (mg kg^{-1}) (mean ± SD, n = 3)		
samples	online MAE method	online UAE method	offline method
hairtail	3.2 ± 0.1	1.7 ± 0.2	3.0 ± 0.1
yellow croaker	10.7 ± 0.4	8.2 ± 0.5	10.4 ± 0.5
cod	29.6 ± 1.1	18.4 ± 1.1	27.3 ± 2.8
catfish	11.3 ± 0.4	7.6 ± 0.5	12.4 ± 0.6
whitebait	15.4 ± 0.4	9.3 ± 0.7	14.2 ± 0.7
squid	15.1 ± 0.5	7.3 ± 0.4	14.6 ± 0.7
cuttlefish	7.3 ± 0.2	5.2 ± 0.3	6.5 ± 0.3
sea cucumber	20.3 ± 0.4	12.8 ± 0.5	20.7 ± 0.6
shrimp	6.7 ± 0.1	4.2 ± 0.1	7.4 ± 0.2
npatunede cumingi	14.8 ± 0.3	5.3 ± 0.3	15.1 ± 0.6
jellyfish	4.2 ± 0.2	2.7 ± 0.1	3.8 ± 0.2

The recovery of formaldehyde was studied by adding a certain amount of formaldehyde standard solution into the aquatic products and then placing the spiked sample into the extraction vessel. The vessel was sealed quickly and then left for 24 h. Three levels of formaldehyde added into the samples were 2.0, 5.0, and 20.0 mg kg^{-1}. The spiked samples were analyzed by the proposed method. The recoveries of formaldehyde were in the range of 70.0%−105.0%, 82.0%−106.0%, and 92.5%−102.0% for the three concentrations, respectively (**Table 2**).

In summary, this work developed a rapid technique based on the online coupling of MAE with derivatization, cleanup, and HPLC determination. A TM_{010} microwave resonance cavity applied to concentrate the microwave energy was used in the MAE; thus, the extraction process was accelerated, and the extraction time for one sample is 5 min. A Capcell pak MF column packed with the restricted access material was used for cleanup of the extract. The HPLC chromatogram without interference peaks was obtained. The sample preparation and analysis took place in a closed and automated system, which avoided formaldehyde volatilization and exposure to the people. Moreover, the reliability and repeatability of the method are improved. The overall analysis time is greatly shortened in this way, and one sample can be analyzed in less than 30 min. The consumption of solvent was decreased as well. This method was used to determine the presence of formaldehyde in various aquatic products. The contents of formaldehyde in these samples were in the range of 3.2−29.6 mg kg^{-1}.

Table 2. Recoveries of Formaldehyde from Different Spiked Aquatic Products

samples	added (mg kg^{-1})	found (mg kg^{-1})	recovery (%)
hairtail	0	3.2	
	2.0	4.9	85.0
	5.0	8.0	96.0
	20.0	23.5	101.5
	0	10.7	
yellow croaker	2.0	12.4	85.0
	5.0	15.1	88.0
	20.0	30.6	99.5
	0	29.6	
cod	2.0	31.7	105.0
	5.0	34.3	94.0
	20.0	48.1	92.5
	0	11.3	
catfish	2.0	13.1	90.0
	5.0	15.9	92.0
	20.0	31.7	102.0
	0	15.4	
whitebait	2.0	17.4	100.0
	5.0	19.9	90.0
	20.0	34.7	96.5
	0	15.1	
squid	2.0	16.7	80.0
	5.0	19.8	94.0
	20.0	34.6	97.5
	0	7.3	
cuttlefish	2.0	9.2	95.0
	5.0	12.0	94.0
	20.0	26.9	98.0
	0	20.3	
sea cucumber	2.0	21.8	75.0
	5.0	24.4	82.0
	20.0	40.1	99.0
	0	6.7	
shrimp	2.0	8.3	80.0
	5.0	11.2	90.0
	20.0	26.8	100.5
	0	14.8	
npatunede cumingi	2.0	16.2	70.0
	5.0	20.1	106.0
	20.0	34.7	99.5
	0	4.2	
jellyfish	2.0	6.1	95.0
	5.0	9.1	98.0
	20.0	24.0	99.0

LITERATURE CITED

(1) Li, Z.; Ma, H.; Lu, H.; Tao, G.. Determination of formaldehyde in foodstuffs by flow injection spectrophotometry using phloroglucinol as chromogenic agent. *Talanta* **2008**, *74*, 788–792.

(2) Li, J.; Zhu, J.; Ye, L. Determination of formaldehyde in squid by high performance liquid chromatography. *Asia Pac. J. Clin. Nutr*. **2007**, *16*, 127–130.

(3) *Limited Amount of Harmful Substances in Non-Polluted Food*; NY 5073-2001; Ministry of Agriculture of the People's Republic of China: Beijing, China, 2001.

(4) Bianchi, F.; Careri, M.; Musci, M.; Mangia, A. Fish and food safety: determination of formaldehyde in 12 fish species by SPME extraction and GC−MS analysis. *Food Chem*. **2007**, *100*, 1049–1053.

(5) Bechmann, I. E. Determination of formaldehyde in frozen fish with formaldehyde dehydrogenase using a flow injection system with an incorporated gel-filtration chromatography column. *Anal. Chim. Acta* **1996**, *320*, 155–164.

(6) Nielsen, M. K.; Jørgensen, B. M. Quantitative relationship between trimethylamine oxide aldolase activity and formaldehyde accumulation in white muscle from gadiform fish during frozen storage. *J. Agric. Food Chem*. **2004**, *52*, 3814–3822.

(7) Chen, L. G.; Song, D. Q.; Tian, Y.; Ding, L.; Yu, A. M.; Zhang, H. Q. Application of on-line microwave sample-preparation techniques. *Trends Anal. Chem*. **2008**, *27*, 151–159.

(8) Chen, L. G.; Jin, H. Y.; Wang, L.G..; Sun, L.; Xu, H. Y.; Ding, L.; Yu, A. M.; Zhang, H. Q. Dynamic ultrasound-assisted extraction coupled on-line with solid support derivatization and high-performance liquid chromatography for the determination of formaldehyde in textiles. *J. Chromatogr., A* **2008**, *1192*, 89–94.

(9) *Determination of Formaldehyde in Aquatic Products*; SC/T 3025-2006; Ministry of Agriculture of the People's Republic of China, Beijing, China, 2006.

(10) Sharma, U.; Sharma, K.; Sharma, N.; Sharma, S.; Singh, H. P.; Sinha, A. K. Microwave-assisted efficient extraction of different parts of hippophae rhamnoides for the comparative evaluation of antioxidant activity and quantification of its phenolic constituents by reverse-phase high-performance liquid chromatography (RP-HPLC). *J. Agric. Food Chem*. **2008**, *56*, 374–379.

(11) Vryzas, Z.; Papadopoulou-Mourkidou, E. Determination of triazine and chloroacetanilide herbicides in soils by microwave-assisted extraction (MAE) coupled to gas chromatographic analysis with either GC-NPD or GC-MS. *J. Agric. Food Chem*. **2002**, *50* 5026–5033.

(12) Criado, A.; Cárdenas, S.; Gallego, M.; Valcárcel, M. Direct automatic screening of soils for polycyclic aromatic hydrocarbons based on microwave-assisted extraction/fluorescence detection and on-line liquid chromatographic confirmation. *J. Chromatogr., A* **2004**, *1050*, 111–118.

(13) Serrano, A.; Gallego, M. Continuous microwave-assisted extraction coupled on-line with liquid−liquid extraction: Determination of aliphatic hydrocarbons in soil and sediments. *J. Chromatogr., A* **2006**, *1104*, 323–330.

(14) Crespín, M. A.; Gallego, M.; Valcárcel, M. Continuous microwave-assisted extraction, solvent changeover and preconcentration of monophenols in agricultural soils. *J. Chromatogr., A* **2000**, *897*, 279–293.

(15) Morales-Muñoz, S.; Luque-García, J. L.; Luque de Castro, M. D. A continuous approach for the determination of Cr(VI) in sediment and soil based on the coupling of microwave-assisted water extraction, preconcentration, derivatization and photometric detection. *Anal. Chim. Acta* **2004**, *515*, 343–348.

(16) Ericsson, M.; Colmsjö, A. Dynamic microwave-assisted extraction coupled on-line with solid-phase extraction and large-volume injection gas chromatography: determination of organophosphate esters in air samples. *Anal. Chem*. **2003**, *75*, 1713–1719.

(17) Ericsson, M.; Colmsjö, A. Dynamic microwave-assisted extraction. *J. Chromatogr., A* **2000**, *877*, 141–151.

(18) Chen, L. G.; Ding, L.; Zhang, H. R.; Li, J.; Wang, Y. T.; Wang, X. P.; Qu, C. L.; Zhang, H. Q. Dynamic microwave-assisted extraction coupled with on-line spectrophotometric determination of safflower yellow in Flos Carthami. *Anal. Chim. Acta* **2006**, *580*, 75–82.

(19) Ericsson, M.; Colmsjö, A. Dynamic microwave-assisted extraction coupled on-line with solid-phase extraction: determination of polycyclic aromatic hydrocarbons in sediment and soil. *J. Chromatogr., A* **2002**, *964*, 11–20.

(20) Luque-García, J. L.; Luque de Castro, M. D. Water soxhlet extraction assisted by focused microwaves: a clean approach. *Anal. Chem*. **2001**, *73*, 5903–5908.

(21) Morales-Muñoz, S.; Luque-García, J. L.; Luque de Castro, M. D. Screening method for linear alkylbenzene sulfonates in sediments based on water Soxhlet extraction assisted by focused microwaves with on-line preconcentration/derivatization/detection. *J. Chromatogr., A* **2004**, *1026*, 4146.

(22) Tomkins, B. A.; McMahon, J. M.; Caldwell, W. M.; Wilson, D. Liquid chromatographic determination of total formaldehyde in drinking water. *J. Assoc. Off. Anal. Chem*. **1989**, *72*, 835–839.

(23) Oliva-Teles, M. T.; Paiga, P.; Delerue-Matos, C. M.; Alvim- Ferraz, M. C. M. Determination of free formaldehyde in foundry resins as its 2,4-dinitrophenylhydrazone by liquid chromatography. *Anal. Chim. Acta* **2002**, *467*, 97–103.

(24) Wang, Q.; Reilly, O.; Pawliszyn, J. J. Determination of low-molecular mass aldehydes by automated headspace solid-phase

microextraction with in-fibre derivatisation. *J. Chromatogr., A* **2005**, *1071*, 147–154.
(25) Rodriguez-Mozaz, S.; Lopez de Alda, M. J.; Barceló, D. Advantages and limitations of on-line solid phase extraction coupled to liquid chromatography−mass spectrometry technologies versus biosensors for monitoring of emerging contaminants in water. *J. Chromatogr., A* **2007**, *1152*, 97–115.
(26) Souverain, S.; Rudaz, S.; Veuthey, J. L. Restricted access materials and large particle supports for on-line sample preparation: an attractive approach for biological fluids analysis. *J. Chromatogr., B* **2004**, *801*, 141–156.
(27) Cassiano, N. M.; Lima, V. V.; Oliveira, R. V.; Pietro, A. C.; Cass, Q. B. Development of restricted-access media supports and their application to the direct analysis of biological fluid samples via high-performance liquid chromatography. *Anal. Bioanal. Chem*. **2006**, *384*, 1462–1469.
(28) Hogendoorn, E. A.; Dijkman, E.; Baumann, R. A.; Hidalgo, C.; Sancho, J. V.; Hernandez, F. Strategies in using analytical restricted access media columns for the removal of humic acid interferences in the trace analysis of acidic herbicides in water samples by coupled column liquid chromatography with UV detection. *Anal. Chem*. **1999**, *71*, 1111–1118.
(29) Hogendoorn, E. A.; Westhuis, K.; Dijkman, E.; Heusinkveld, H. A. G.; Boer, A. C. D.; Evers, E. A. I. M.; Baumann, R. A. Semi-permeable surface analytical reversed-phase column for the improved trace analysis of acidic pesticides in water with coupled-column reversed-phase liquid chromatography with UV detection: determination of bromoxynil and bentazone in surface water. *J. Chromatogr., A* **1999**, *858*, 45–54.
(30) Bellagamba, F.; Moretti, V. M.; Mentasti, T.; Albertini, A.; Luzzana, U.; Valfre, F. High-performance liquid chromatographic determination of polyamines in milk as their 9-fluorenylmethoxycarbonyl derivatives using a column-switching technique. *J. Chromatogr., A* **1997**, *791*, 79–84.
(31) Oliveira, R. V.; Cass, Q. B. Evaluation of liquid chromatographic behavior of cephalosporin antibiotics using restricted access medium columns for on-line sample cleanup of bovine milk. *J. Agric. Food Chem*. **2006**, *54*, 1180–1187.
(32) Wang, X. H.; Li, B. H.; Xie, F.; Huang, F.; Ma, J.; Yu, A. M. A novel method of fast sample pretreatment for in situ determination of formaldehyde in water-soaked foodstuffs. *Chem. J. Chinese U*. **2005,**, *26*, 2015–2018.
(33) Chen, L. G.; Jin, H. Y.; Ding, L.; Zhang, H. R.; Wang, X. P.; Wang, Z. M.; Li, J.; Qu, C. L.; Wang, Y. T.; Zhang, H. Q. On-line coupling of dynamic microwave-assisted extraction with high-performance liquid chromatography for determination of andrographolide and dehydroandrographolide in *Andrographis paniculata* Nees. *J. Chromatogr., A* **2007**, *1140*, 71–77.
(34) Chen, L. G.; Ding, L.; Jin, H. Y.; Song, D. Q.; Zhang, H. R.; Li, J. T.; Zhang, K.; Wang, Y. T.; Zhang, H. Q. The determination of organochlorine pesticides based on dynamic microwave-assisted extraction coupled with on-line solid-phase extraction of high-performance liquid chromatography. *Anal. Chim. Acta* **2007**, *589*, 239–246.
(35) Lee, H. M.; Choi, S. J.; Jeong, C. K.; Kim, Y. S.; Lee, K. C.; Microbore, H. S. L. High-performance liquid chromatographic determination of cisapride in rat serum samples using column switching. *J. Chromatogr., B* **1999**, *727*, 213–217.
(36) Kanda, T.; Kutsuna, H.; Ohtsu, Y.; Yamaguchi, M. Synthesis of polymer-coated mixed-functional packing materials for direct analysis of drug-containing serum and plasma by high-performance liquid chromatography. *J. Chromatogr., A* **1994**, *672*, 51–57.
(37) Baek, S. K.; Lee, S. S.; Park, E. J.; Sohn, D. H.; Lee, H. S. Semi-micro high-performance liquid chromatographic analysis of tiropramide in human plasma using column-switching. *J. Pharm. Biomed. Anal*. **2003**, *31*, 185–189.
(38) Jeong, C. K.; Lee, H.-Y.; Jang, M.-S.; Kim, W. B.; Lee, H. S. Narrowbore high-performance liquid chromatography for the simultaneous determination of sildenafil and its metabolite UK-103,320 in human plasma using column switching. *J. Chromatogr., B* **2001**, *752*, 141–147.
(39) Heyden, Y. V.; Nguyet, A. N. M.; Detaevernier, M. R.; Massart, D. L.; Plaizier- Vercammen, C. J. Simultaneous determination of ketoconazole and formaldehyde in a shampoo: liquid chromatography method development and validation. *J. Chromatogr., A* **2002**, *958*, 191–201.
(40) Liu, J.; Peng, J.; Chi, Y.; Jiang, G.. Determination of formaldehyde in shiitake mushroom by ionic liquid-based liquid-phase microextraction coupled with liquid chromatography. *Talanta* **2005**, *65*, 705–709.
(41) Wang, S.; Cui, X.; Fang, G.. Rapid determination of formaldehyde and sulfur dioxide in food products and Chinese herbals. *Food Chem*. **2007**, *103*, 1487–1493.

Received for Review September 25, 2008. Revised manuscript received March 22, 2009. Accepted March 29, 2009. This work was supported by the Natural Science Foundation of China (grant number: 20875037).