Food Chemistry 119 (2010) 364–367

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Determination of total tin and organotin compounds in shellfish by ICP-MS

Zhen-hua Yu [a,b,c,*], Jun-qing Sun [b], Miao Jing [b], Xuan Cao [b], Frank Lee [b], Xiao-ru Wang [b,*]

[a] Institute of Oceanology, Chinese Academy of Sciences, Qingdao, China
[b] First Institute Oceanography of SOA, Qingdao, China
[c] Graduate University of Chinese Academy of Sciences, Beijing, China

ARTICLE INFO

Article history:
Received 17 December 2008
Received in revised form 14 April 2009
Accepted 24 May 2009

Keywords:
HPLC-ICP-MS
Organotin compounds
Speciation
Shellfish

ABSTRACT

A method based on microwave digestion and inductively coupled plasma-mass spectrometric (ICP-MS) analysis was established for the determination of total tin in shellfish samples. Good linearity of the calibration curves was obtained for tin elements (r = 1.0000). Detection limit for Sn was 34.6 ng/g. Total tin concentrations in these samples ranged from non-detectable to 0.45 μg/g. High-performance liquid chromatography hyphenated with inductively coupled plasma-mass spectrometry (HPLC-ICP-MS) was applied to the simultaneous determination of five organotin compounds in the shellfish samples. The fresh and freeze-dried shellfish samples were treated by ultrasonic extraction with two different extraction solvents. Four organotin compounds including dibutyltin (DBT), tributyltin (TBT), diphenyltin (DPhT) and triphenyltin (TPhT) in shellfish samples were detected. It was found that the dominate species in the samples were tributyltin (TBT) and triphenyltin (TPhT).

1. Introduction

Organotin compounds (OTs) have been widely used in the production of polyvinylchloride (PVC) materials as heat and light stabilisers and as anti-bacterial and anti-fungal agents in pesticides and anti-fouling applications. Their toxicity, especially tributyltin (TBT) and triphenyltin (TPhT), in coastal environment has resulted in deleterious effects on non-target organisms such as shellfish. OTs can be cumulated in shellfish, which can then impact on human health because of bioaccumulation through the food chains. Generally, the determination of organotin compounds in shellfish has been carried out by gas chromatography (GC) (Munoz, Gallego, & Valcarcel, 2005a, 2005b). However, the derivation step required for GC analysis often results in yield irreproducibility because of matrix interference. High-performance liquid chromatography (HPLC) (Vinas, Lopez-Garcia, Merino-Merono, Campillo, & Hernandez-Cordoba, 2004) does not involve a derivation step, which can eliminate a potential source of uncertainty in the final result. The determination of OTs is usually performed by means of HPLC coupled with various detectors, such as AAS (Minganti, Capelli, & Pellegrini, 1995), AES (Munoz et al., 2005a, 2005b) and MS (Chiron, Roy, Cottier, & Jeannot, 2000). Among the detection techniques used in recent years, ICP-MS offers unique advantages including element specificity, wide dynamic linear range and low detection limits. In this study, a method was developed to determine simultaneously five OTs in fresh and freeze-dried shellfish samples.

2. Experiment

2.1. Reagents

HPLC separations for organotin applications: glacial acetic acid (HAC) was obtained from Fluka; acetonitrile (HPLC grade) and methanol (HPLC grade) were obtained from Merck, Germany; and sodium acetate (NaAC) was obtained from Tianzhi chemical company of Zibo, China. Triethylamine (TEA, HPLC grade) was obtained from ACROS. De-ionised water was obtained from a water purification unit at 18 MΩ (Millipore, USA). Trimetyltinchloride (TMTCl), dibutyltinchloride (DBTCl), tributyltinchloride (TBTCl), diphenyltinchloride (DPhTCl) and triphenyltinchloride (TPhTCl) were all obtained from Aldrich.

2.2. ICP-MS and HPLC-ICP-MS conditions

An Agilent 7500a ICP-MS was used for total Sn and organotin detection. Speed wave MW-3$^+$ microwave digestion system (Berghof, Germany) was employed to digest the samples. An Agilent Technologies 1100 HPLC system was used for HPLC separations. When total Sn was determined, Optimisation of the ICP-MS conditions was achieved by adjusting the torch position and tuning for reduced oxide and doubly charged ion formation with a standard tuning solution containing Li, Y, Ce and Tl in 2% HNO_3. The ICP-MS condition was shown as Table 1. When HPLC was coupled with ICP-MS for organotin separation, there was about 80% organic solvent in HPLC mobile phase, which may cause carbon build-up on the ICP plasma cone. In order to reduce the solvent loading to the plasma, the double-pass spray-chamber was peltier cooled to

* Corresponding authors. Address: Institute of Oceanology, Chinese Academy of Science, Qingdao, China. Tel./fax: +86 0532 88963253.
E-mail address: yzhhgm607@163.com (Z.-h. Yu).

0308-8146/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2009.05.079

Table 1
ICP-MS conditions.

Parameter	Value
ICP-MS conditions	
RF power (W)	1350
Sampling depth (mm)	6.5
Carrier gas flow (L/min)	1.1
Make-up gas flow (L/min)	0
O_2/Ar mixed gas	0
Chamber temperature	2 °C
Nebuliser	Babington
Cones	Ni

−5 °C. O_2/Ar mixed gas (30%) was mixed into the make-up gas and added post-nebulisation in order to convert organic carbon to CO_2 in the plasma and avoid carbon build-up on the cones. The HPLC-ICP-MS conditions are given in Table 2.

2.3. Sampling and pretreatment

The marine biological samples were collected from located market. The biological samples included jewfish, shrimp, scallop, oyster and others. The samples were first unshelled and the soft tissues were pooled and thoroughly rinsed with de-ionised water to remove extraneous impurities, then they were freeze-dried and powdered before digestion and extraction.

2.4. Total tin determination

Before ICP-MS determination, the samples were digested in microwave digester. A measure of 0.2 g milled powder, 5 mL of HNO_3 and 1 mL H_2O_2 were placed in a Teflon digestion vessel, predigested over night at room temperature and then digested according to the US EPA method 3052. The temperature was raised to 120 °C in 5 min, then raised to 180 °C from 5 to 10 min and held for10 min at 180 °C. The digested solution was diluted with pure water to 25 g. The standard solution and reagent blanks were digested in the same way. The total tin concentration was directly determined by ICP-MS.

2.5. Extraction procedures

2.5.1. Mobile phase extraction of freeze-dried samples

About 0.2 g of dried and powdered seafood was weighted precisely in the plastic tubes and the extraction was carried out with 3 ml of CH_3CN:H_2O:CH3COOH:TEA (65:23:12:0.05%, v/v/v/v pH 3.0), The samples were extracted by ultrasonic extraction for 30 min and then centrifuged (20 min, 8000 rpm). The supernatants were filtered by 0.45 μm membrane and analysed by HPLC-ICP-MS.

Table 2
HPLC-ICP-MS conditions.

Parameter	Value
ICP-MS conditions	
RF power (W)	1550
Sampling depth (mm)	6.5
Carrier gas flow (L/min)	0.6
Make-up gas flow (L/min)	0.2
O_2/Ar mixed gas	30%
Chamber temperature	−5 °C
Nebuliser	PFA
Cones	Pt
HPLC conditions	
Column	Agilent TC-C18 (4.6 × 250 mm, 5 μm)
Mobile phase	CH_3CN:H_2O:CH_3COOH:TEA = 65:23:12:0.05% (v/v/v/v) pH 3.0
Injection	20 μL
Flow rate	0.4 mL/min

2.5.2. Mobile phase extraction of fresh samples

About 3 g of fresh shellfish sample was weighted precisely in the plastic tubes and the extraction was carried out with 5 ml of CH_3CN:H_2O:CH_3COOH:TEA (65:23:12:0.05%, v/v/v/v pH 3.0), The samples were ultrasonically extracted for 30 min and then centrifuged (20 min, 8000 rpm). The supernatants were filtered by 0.45 μm membrane and analysed by HPLC-ICP-MS.

2.5.3. HAC-NaAC extraction of freeze-dried samples

About 0.2 g of dried and powdered shellfish was weighted precisely in the plastic tubes and the extraction was carried out with 1.5 ml of HAC-NaAC (pH 4.5) and 1.5 ml methanol. The samples were extracted for 30 min and then centrifuged (20 min, 8000 rpm). The supernatants were filtered by 0.45 μm membrane and analysed by HPLC-ICP-MS.

3. Results and discussion

3.1. Total tin determination

3.1.1. Selection of isotopes of tin

The total tin in shellfish samples was determined by Agilent 7500a ICP-MS. The detector of Agilent 7500a ICP-MS was a quadrupole MS. The major disruptions in analysis were isobars interference, oxide ion interference, doublely charged ions interference and polyatomic ions interference from aqueous solution and carrier gas. There are ten tin isotopes, and studies were carried out to select the isotope with the smallest analytical interference and maximum sensitivity. Among these isotopes, the natural abundances of ^{112}Sn, ^{114}Sn and ^{15}Sn are lower than 1%. At the same time, ^{115}Sn was disturbed by In while ^{124}Sn was disturbed by YCl. The abundances of ^{118}Sn and ^{120}Sn are the highest. The two isotopes were less disturbed by oxides, polyatomic ions and hydride. ^{118}Sn was selected for analysis. Because ^{118}Sn was less disturbed than ^{120}Sn by chloride and argon.

3.1.2. The calibration study, detection limits and method precision

A series of Sn standard solutions of 0 μg/L, 0.5 μg/L, 1 μg/L, 10 μg/L, 100 μg/L were used to construct the calibration curve, for which a good linear relationship was observed. The linear equation was $Y = 0.1936X + 0.0087$ with a correlation coefficient of 1.0000.

Under the optimal conditions of the apparatus, replica blank samples were measured for seven times. Detection limit was set at three times of the relative standard deviation, i.e., 34.6 μg/kg.

The Oyster sample was measured for five times and the RSD was 1.78%, which showed that the method was very precise.

3.1.3. The accuracy of method

To establish the accuracy of this method, the TORT-2 standard reference material was analysed. The measured result of Sn at 0.042 mg/kg was in good agreement with the reference value of 0.040 mg/kg, which shows the high accuracy of the method.

3.1.4. Sample analysis

The results of total tin concentrations in shellfish samples are given in Table 3. All concentration values presented in this paper are calculated based on dry weight of the sample. It was shown that total tin concentrations in these samples ranged from undetectable to 0.45 mg/kg.

Table 3
The result of total tin in shellfish (mg/kg).

Element	Oyster	Shrimp	Scallop	Conch	Mussel	Squid	R.P.	Clam 2
Sn	0.39	ND[a]	0.45	0.25	ND	ND	0.37	0.13
RSD (%)	2.32	4.23	2.45	3.32	4.34	4.21	3.76	2.47

[a] ND shows that the value is lower than the detection limit.

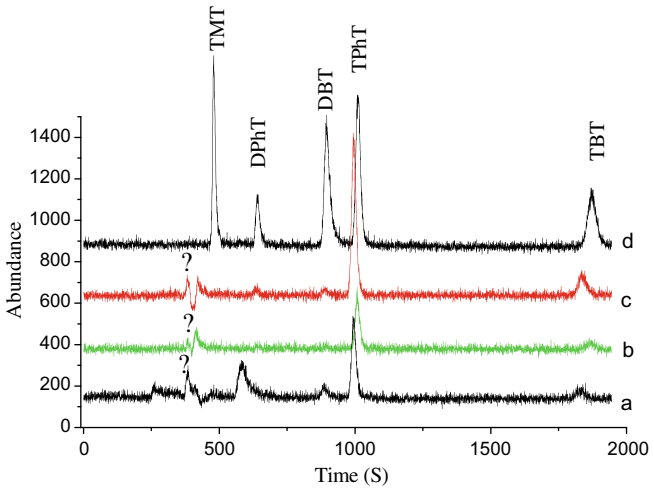

Fig. 1. The HPLC-ICP-MS chromatographic spectrum of 5 mixed OTs standards (d) and organotin compounds in R.P. (a) 3 g fresh sample extracted by 5 ml mobile phase; (b) 0.2 g freeze-dried sample extracted by 1.5 ml 1 M HAC-NaAC and 1.5 ml methanol and (c) 0.2 g freeze-dried sample extracted by 3 ml mobile phase).

3.2. Chromatographic speciation studies

3.2.1. Optimisation of HPLC conditions

Most of the OTs compounds are either non-polar or weakly polar, and thus can be well separated by reversed phase C_{18} column. In this study, an Agilent TC-C_{18} column was selected for OT analysis. The optimum mobile phase composition developed was a mixture of CH_3CN:H_2O:CH_3COOH: TEA = 65:23:12:0.05 (v/v/v/v), with pH adjusted to 3.0. Acetonitrile was preferred because it gave sharper peaks than the other commonly used organic phase methanol, a finding which was also reported by Chiron and co-workers. A composition of 65% of acetonitrile in water was found to provide the optimum separation. 0.05% TEA as ion-pair reagents was added in the solution to speed up the HPLC separation because of the excessive retention times of the OTs. Furthermore, 12% of acetic acid was added in the mobile phase to lessen the peak trailing problems due to the strongly adsorptive nature of the OTs. The pH value was adjusted to 3.0 by adding $NH_3 \cdot H_2O$ to enhance the stability of the OTs.

3.2.2. Standard work curves and detection limit of method

The stock solutions with a concentration of 1 mg Sn/ml were obtained by dissolving individually accurately weighed TMT, DBT, TBT, DPhT and TPhT into methanol, respectively. Working standard solutions were freshly made before analysis. All the solutions were stored at 4 °C in the dark before use. A series of standard solutions from 0.5 μg/L to 500 μg/L concentrations containing TMT, DBT, TBT, TPhT and DPhT mixed samples were prepared in the HPLC mobile phase and measured by HPLC-ICP-MS. The calibration line was linear with correlation coefficient of 0.998. The HPLC-ICP-MS chromatogram of the 5 mixed OTs standards is shown in Fig. 1d.

When injection volume was 20 μL and flow rate was 0.4 mL/min, the detection limit (3 times S/N) of TMT, DBT, TBT, DPhT and TBT were 0.24 μg/L, 0.31 μg/L, 0.25 μg/L, 0.25 μg/L and 0.37 μg/L, respectively.

Table 4
Recovery of OTs in R.P.

Sample	Extraction	OTs	RT (min)	OTs in R.P. (μg/L)	Spiked in R.P. (μg/L)	Recovery (%)
Fresh sample	3 g Fresh sample extracted by 5 ml mobile phase	Unknown 1	4.35	18.00	–	–
		Unknown 2	6.36	26.82	–	–
		Unknown 3	6.89	ND[a]	–	–
		TMT	7.89	26.8	19.70	0
		DPhT	10.67	153.9	15.16	0
		DBT	14.92	7.22	24.23	24.0
		TPhT	16.86	71.79	29.16	55.4
		TBT	31.20	12.63	19.31	52.7
Freeze-dried sample	0.2 g Freeze-dried sample extracted by 3 ml mobile phase	Unknown 1	4.35	ND	–	–
		Unknown 2	6.36	9.35	–	–
		Unknown 3	6.89	14.63	–	–
		TMT	7.89	ND	253.6	86.9
		DPhT	10.67	ND	184.6	43.9
		DBT	14.92	6.00	215.3	52.4
		TPhT	16.86	266.0	514.0	104.3
		TBT	31.20	46.18	303.4	97.8
Freeze-dried sample	0.2 g Freeze-dried sample extracted by 1.5 ml 1 M HAC-NaAC and 1.5 ml methanol	Unknown 1	4.35	ND	–	–
		Unknown 2	6.36	ND	–	–
		Unknown 3	6.89	ND	–	–
		TMT	7.89	ND	253.6	81.2
		DPhT	10.67	ND	184.6	43.2
		DBT	14.92	ND	215.3	45.6
		TPhT	16.86	17.20	514.0	80.7
		TBT	31.20	12.59	303.4	82.3

[a] ND shows that the value is lower than the detection limit.

3.2.3. OTs speciation in R.P.

Fresh and freeze-dried R.P. samples were extracted by the mobile phase and HAC-NaAC solution, the same procedure repeated two times, and the HPLC-ICP-MS chromatogram is shown in Fig. 1. The figure shows (a) the results from the analysis of 3 g of fresh sample extracted by 5 ml mobile phase, (b) 0.2 g freeze-dried sample extracted by 1.5 ml, 1 M HAC-NaAC and 1.5 ml methanol, (c) 0.2 g freeze-dried sample extracted by 3 ml mobile phase and (d) was spectrum of 5 mixed OTs standards 10 μg/L. It can be seen that there are seven different kinds of OTs (including three kinds of unknown OTs, according to Compound Independent Calibration principle, CIC, it means that the calibration is based on elemental concentration, the different chemical structure will interfere little to the response of the elements), unknown OTs were quantitative analysed by TMT standards, and also shown in Table 4. In R.P. sample, TPhT and TBT were major form. In Fig. 1b and c, the samples were extracted by the mobile phase and HAC-NaAC solution, the extraction efficiency of OTs in freeze-dried samples extracted by the mobile phase was better than those of the HAC-NaAC solution. When the mobile phase was used to extract fresh and freeze-dried samples, the types of OTs in fresh sample was more than that of the freeze-dried samples, due probably to lose of OTs during the process of freeze-drying.

A known amount of standard were added to fresh and freeze-dried samples and extracted by ultrasound. Then, concentrations of OTs in samples were measured to calculate the recovery. The results are shown in Table 4. The recoveries of TMT, TBT and TPhT were more than 80%, but the recoveries of DBT and DPhT were relatively low, about 40% and 50%, respectively. The reason might be attributed to the decomposition and absorption of those compounds during the extraction procedure. Further study on this subject is in progress.

4. Conclusions

A HPLC-ICP-MS method was established to simultaneously determine five OTs in the shellfish samples. In order to reduce the solvent loading on the plasma, the double-pass spray-chamber was peltier cooled to −5 °C. O_2/Ar mixed gas (30%) was mixed into the make-up gas and added post-nebulisation in order to convert organic carbon to CO_2 in the plasma, thus avoiding carbon build-up on the plasma cones. The shellfish samples were treated by ultrasonic extraction with HPLC mobile phase as extraction solution for 30 min. Seven OTs including DBT, TBT, DPhT and TPhT in shellfish samples were detected with the developed method. The dominate Sn species in the samples were TBT and TPhT. Standard recoveries for TMT, TBT and TPhT were over all 80%. However, the recoveries for DPhT and DBT were relatively low, about 40% and 50%. The reason might be attributable to the decomposition of these compounds during the extraction procedure, and further study on this subject is in progress.

Acknowledgement

The Speed wave MW-3$^+$ microwave digestion system was supplied by Leeman Company in China.

References

Chiron, S., Roy, S., Cottier, R., & Jeannot, R. (2000). Speciation of butyl and phenyltin compounds in sediments using pressurized liquid extraction and liquid chromatography–inductively coupled plasma mass spectrometry. *Journal of Chromatography A, 879*(2), 137–145.

Minganti, V., Capelli, R., & Pellegrini, R. (1995). Evaluation of different derivatization methods for the multi-element detection of Hg, Pb and Sn compounds by gas chromatography-microwave induced plasma-atomic emission spectrometry in environmental samples. *Fresenius' Journal of Analytical Chemistry, 351*(1), 471–477.

Munoz, J., Gallego, M., & Valcarcel, M. (2005a). Speciation of organometallic compounds in environmental samples by gas chromatography after flow preconcentration on fullerenes and nanotubes. *Analytical Chemistry, 77*(16), 5389–5395.

Munoz, J., Gallego, M., & Valcarcel, M. (2005b). Speciation analysis of mercury and tin compounds in water and sediments by gas chromatography–mass spectrometry following preconcentration on C_{60} fullerene. *Analytica Chimica Acta, 548*(1–2), 66–72.

Vinas, P., Lopez-Garcia, I., Merino-Merono, B., Campillo, N., & Hernandez-Cordoba, M. (2004). Liquid chromatography-hydride generation-atomic absorption spectrometry for the speciation of tin in seafoods. *Journal of Environmental Monitoring, 6*, 262–266.