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Ion-pair reversed-phase chromatography for speciation of organotin compounds

Spéciation de quelques composés organiques de l'étain par chromatographie en phase inverse par appariement d'ions

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Abstract – Objective: Ion-pair reversed-phase chromatography (IP-RP) has been investigated for speciation of dibutyltin (DBT), tributyltin (TBT), and triphenyltin (TPhT). The influence of several parameters (pH, alkyl chain length, concentration of the ion pair reagent) has been studied to determine the best chromatographic conditions. **Methods:** Separation of the three organotin compounds was achieved on a C8-bonded silica column using a mixture of methanol, water, and acetic acid (80:19:1) as eluent, containing 1 mmol/L decane sulfonic acid as ion pairing reagent. The eluates were detected on-line by hydride generation-quartz furnace atomic absorption spectrophotometry (HG-QFAAS). **Results:** The resolving power of this developed method for separation of the organotins species at above conditions was shown by the values of fundamental chromatographic parameters. The capacity factors (k') for DBT, TBT and TPhT species were 0.27, 2.54, and 5.92 respectively. Resolution (R_s) values for DBT-TBT and TBT-TPhT separation were 2.92 and 2.42 respectively, while the selectivity for DBT-TBT and TBT-TPhT were 9.76 and 3.50 respectively. **Conclusion:** The developed IP-RP-HG-QFAAS chromatography technique can separate DBT, TBT, and TPhT with good performance which is shown by the chromatographic parameters produced.

Key words: Organotin compounds, speciation, ion-pair chromatography

Résumé – Objectif : La chromatographie d'appariement d'ions en phase inverse (IP-RP) a été étudiée pour la spéciation du dibutylétain (DBT), du tributylétain (TBT), et du triphénylétain (TPhT). L'influence de plusieurs paramètres (pH, longueur de la chaîne alkyle, concentration du réactif d'appariement d'ions) a été étudiée pour déterminer les meilleures conditions chromatographiques. **Méthodes :** La séparation des trois composés organo-étains a été réalisée sur une colonne de silice greffée C8 en utilisant un mélange de méthanol, d'eau et d'acide acétique (80:19:1) comme éluant, contenant 1 mmol / L d'acide décano sulfonique comme réactif d'appariement d'ions. Les éluats ont été détectés en ligne par spectrophotométrie d'absorption atomique à four en quartz après génération d'hydrures (HG-QFAAS). **Résultats :** Le pouvoir de résolution de cette méthode développée pour la séparation des organo-étains a été démontré par les valeurs des paramètres fondamentaux chromatographiques obtenus. Les facteurs de capacité (k') pour les espèces DBT, TBT et TPhT ont été 0.27, 2.54, et 5.92 respectivement. Les valeurs de résolution (R_s) pour la séparation des DBT-TBT et TBT-TPhT étaient 2,92 et 2,42 respectivement, tandis que la sélectivité des DBT-TBT et TBT-TPhT étaient de 9,76 et 3,50 respectivement. **Conclusion :** La technique de chromatographie IP-RP-HG-QFAAS élaborée permet de séparer les espèces DBT, TBT et TPhT avec une bonne résolution qui se traduit par les paramètres chromatographiques énoncés.

Mots clés : Organo-étains, spéciation, chromatographie en phase inverse par appariement d'ions

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1 Introduction

Environmental effects induced by organotin compounds have been recognised some time ago. These compounds are generally anthropogenic, except of methyltin which may be produced by biomethylation in the environment. The tin atom is generally in the +4 oxidation state. The molecular formula of organotin is R_nSnX_{4-n} , where R represents an alkyl or an aryl group, *e.g.* methyl, butyl, phenyl, octyl, while X represents an anionic species, *e.g.* chloride, hydroxide, carboxylate, and sulphide [1].

Tributyltin (TBT) was widely used as active antifouling agent in paints for pleasure boats, large ships, nets, and cages in aquaculture and slimicides in cooling systems. Both mono (MBT) and dibutyltin (DBT) served as stabilizers in polyvinyl chloride (PVC) and as catalysts in the production of polyurethane foams and silicones. It is an aquatic pollutant with harmful effects at low levels on non-target marine organisms, such as shell malformation in oysters, larval mortality in mussel, and imposex in gastropods. MBT and DBT have lower toxicity than TBT [2], but nevertheless they are also classified as dangerous compounds [3,4]. Triphenyltin (TPhT) was commonly used as fungicide in agriculture, especially in potato farming.

TBT and TPhT are released from the aforementioned sources into the environment, are deposited in sea sediments and are accumulated by marine organisms such as fish, mussels, and squids. Numerous studies around the world have confirmed that organotin antifouling agents are highly toxic to a wide range of marine organisms. TBT at concentrations of less than 1 ng/L can induce imposex in marine organisms [5]. If consumed by humans, TBT may have adverse effects on the human immune system and hormonal performance [6, 7]. Many developed nations (*viz.* France, United Kingdom, USA, Switzerland, New Zealand, Japan, etc) have partially banned or restricted the use of TBT, but it is still widely used in developing countries in Asia, Africa and South America without control measures. This body of work has formed the basis of a resolution by the International Maritime Organisation (IMO) to progressively ban the use of TBT in antifouling products. The use of organotins is now restricted or prohibited in most countries, and the complete prohibition of TBT-based paints came into effect in September, 2008. Therefore, sensitive analytical methods for speciation studies are needed [8].

Gas chromatography has high separation power and may be connected to sensitive detectors such as a mass spectrometer, flame photometric detector, or atomic emission detector [9–11]. High-performance liquid chromatography (HPLC) has also been used, coupled with element-sensitive detection systems such as flame atomic absorption spectrometer (FAAS) [12], electrothermal atomization atomic absorption spectrometer (ETAAS) [13], inductively coupled plasma atomic emission spectrometer (ICP-AES), inductively coupled plasma mass spectrometer (ICP-MS), and mass spectrometer (MS) [14, 15]. FAAS gives poor sensitivity due to low analyte transport and atomization efficiency. ETAAS has excellent detection limits but cannot provide continuous recording of the HPLC eluate. ICP-AES equipped with a Meinhard nebulizer, though suitable for recording the HPLC separation on-line, presents as limiting factor an instability of the torch when

organic solvents are used. ICP-MS is a very sensitive technique and allows to monitor the chromatographic effluent on-line but is still not easy to handle for samples of natural origin.

In this paper, a separation method is presented for organotin species based upon reversed phase-ion pair chromatography technique and followed by on-line detection using a hydride generator combined with a quartz furnace atomic absorption spectrophotometer (HG-QFAAS) [16]. In order to achieve good separation of DBT, TBT, and TPhT, several parameters of separation have been studied here. The optimum conditions used for the HG-QFAAS were based on preliminary earlier research [17].

2 Experimental

2.1 Separation system

The system included a HPLC pump (Waters model 515, Milford USA), an external loop valve (Rheodyne, model 7125, volume 100 μ L) and separation was carried out on a C8-bonded silica column (LiChrospher 100 RP-8, 250 mm \times 4.6 mm i.d., particle size 5 μ m).

2.2 Hydride generation system

A continuous-flow hydride generator (GBC-HG3000, Dandenong, Australia) was used to convert DBT, TBT, and TPhT into volatile compounds (hydrides) after their chromatographic separation. Reductant ($NaBH_4$), acid concentrations and flow-rates were optimized to give the best signal for all the organotin species. Reagents were pumped by means of a peristaltic pump equipped with Tygon tubes and mixed with the chromatographic effluent in a mixing T without dead volume. The reaction mixture was carried to a gas-liquid separator through a nitrogen flow and the gas was introduced into the quartz cell of the atomic absorption spectrometer.

2.3 Quartz furnace atomic absorption spectrometer

A double beam atomic absorption spectrometer (GBC[®]-Avanta, Dandenong, Australia) was used as detector. The analogue signals from the detector were converted using an analogue-digital converter interface and treated by a data analyzer (Origin[™] Microcalc 7.0 system, Microcal Software Inc., USA). Operating conditions were as follows: detection wavelength = 224.6 nm; slit width = 0.7 nm; lamp = photron hollow cathode lamp, 10 mA; quartz cell temperature = 1100 $^{\circ}$ C (EHG 3000 electric cell heater).

2.4 Reagents

All reagents were of analytical-reagent grade. Stock solution of 1000 μ g/mL DBT, TBT, and TPhT, were prepared from commercially available chloride salt (E. Merck) without further purification. Eluents were prepared using various concentrations of sodium salts of either pentanesulfonic acid (PS), decanesulfonic acid (DS), or heptanesulfonic acid (HS) as ion-pairing reagent, and following mixing with methanol

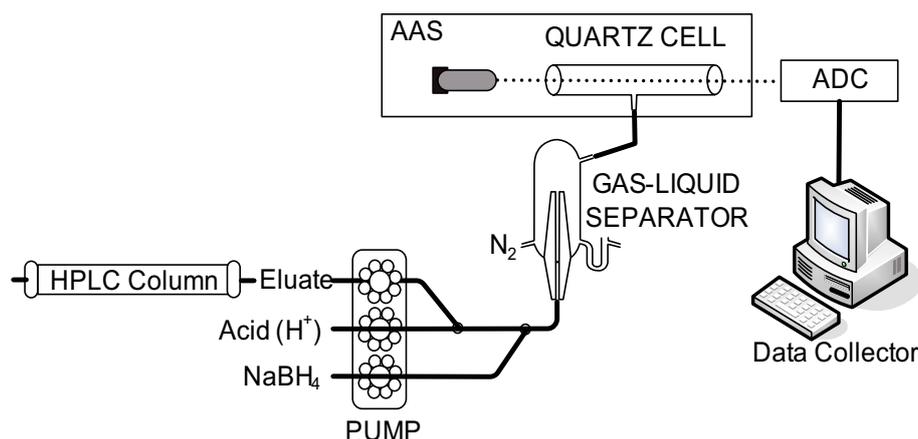


Fig. 1. Schematic representation of the IP-RP-HG-QFAAS instrument.

Table I. The operating parameters and the instrument settings used for the IP-RP-HG-QFAAS.

IP-RP separation system	Column: C8-bonded silica column (LiChrospher 100 RP-8, 250 mm × 4.6 mm i.d., particle size 5 µm). Eluent: methanol:water:acetic acid Ion pair reagent: alkyl sulfonate pH of eluent: 3–8 Eluent flow rate: 1.5 mL/minute
HG-QFAAS detection system	Hollow Cathode Lamp: Sn (10 mA) Wavelength: 224.6 nm slit width: 0.7 nm Quartz cell temperature: 1100 °C Gas-liquid separator : 9 × 3 cm

and acetic acid were filtered on a 0.2 µm cellulose acetate membrane (Millipore). pH was adjusted with 1 M sulfuric acid solution. All the solutions were prepared using doubly deionised water (Barnstead EASYpure 18 MΩ, Germany).

The sodium tetrahydroborate stock solution, 10% (m/V) for hydride generation was prepared by dissolving 10 g NaBH₄ in 100 ml 0.05% (m/v) NaOH. The solution was kept in a refrigerator and was stable for up to four weeks. The working solution of sodium tetrahydroborate was prepared daily by dilution of the stock solution with 0.05% (m/v) NaOH.

2.5 Experiment

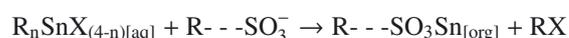
The schematic diagram of the IP-RP-HG-QFAAS operation system is shown in figure 1. The chromatographic separation was achieved using a C8-bonded silica column analytical column. A Rheodyne Model 7125 injection valve with a 100 µL sample loop was used for the introduction of the sample. The effluent from column was delivered to a T-cross and mixed with acid firstly and then reacted with NaBH₄. A peristaltic pump was used to introduce acid and NaBH₄. The produced hydrides were separated in a gas-liquid separator and carried by nitrogen to the atomizer. The operating parameters and the instrument settings were adjusted according to the manufacturer's recommendations. Table I lists the analytical condition and instrument settings.

In order to optimize the separation of DBT, TBT, and TPhT, the influence of several parameters (pH, alkyl chain length and concentration of the ion pair reagent) has been

studied to determine the best chromatographic conditions. The concentration of DBT, TBT, and TPhT standard solutions during these measurements was 10 mg/L. The evaluation of fundamental chromatographic values has been conducted for each individual species and also the mixture of organotin species.

3 Result and discussion

The speciation of DBT, TBT, and TPhT in this research was held by using IP-RP chromatography with C8-bonded silica column. Eluent composition was adjusted to achieve optimum separation. The quality of speciation of organotin species in a non polar column can also be enhanced by adding alkyl sulfonate as ion-pair reagent, through augmentation of the species hydrophobicity. The Sn (IV) ion of DBT, TBT, and TPhT species will combine with ion-pair reagent to form a metal-ion pair complex. The complex formed has good affinity for the reverse-phase column, thus the separation will be more effective. The formation of metal-ion pair complex is shown by reaction below.



The separated species will then be detected by AAS detector. In order to increase the detection limit of tin measurement using AAS, the hydride generation (HG) technique is used. Through the hydride generation method, the Sn ion is derivatized into gaseous covalent hydride, and then atomized in the quartz cell tube which is put in the electric cell heater [18]. The hydride generation and separation of resulting hydride take

Table II. The optimum condition and analytical performance of the HG-QFAAS for determination of Sn(IV).

Parameter	Results
Quartz cell temperature (using electrical cell heterer EHG-3000)	1100 °C
CH ₃ COOH concentrations	0.3 M
NaBH ₄ concentrations	0.4% (v/v), dissolved in NaOH 0.05%, (m/v)
Mixing technique	mixing occurred in the reaction coil before introduced to the gas-liquid separator
Reproducibility	1.12 % for 50 µg/L standard concentrations
Linearity	10–500 µg/L (R ² = 0.9904)
Limit of detection	3.74 µg/L
Accuracy, expressed as recovery (%) of spiked water samples.	≥ 95.00%

place in the gas-liquid separator HG. Several factors which influence the metal ion determination using HG method are acid and reductant concentrations and the type of acid used. The optimum analytical performance can be achieved under condition stated in the previous research [17], which can be seen in table II. The condition parameters stated in table II are applied in speciation process of DBT, TBT, and TPhT species using IP-RP-HG-QFAAS system.

This research is focused on studying the optimum condition parameters in organotin speciation using reverse-phase chromatography. The parameters which are optimized are the eluent composition, pH of the eluent, the chain length of alkyl sulfonate, and ion-pair reagent concentration.

3.1 Influence of eluent composition

In order to find out the influence of eluent composition on separation, eluent composition was adjusted to achieve optimum separation. Eluents were prepared using various compositions of methanol, water and acetic acid. The choice of this composition based on the nature of insoluble organotin species in water, so that require being arranged strength of eluent composition. Experimental results shows that there is no significant difference between capacity factor of DBT, TBT, and TPhT, which means that these three compounds have similar retention property. Thus, with this composition of eluent the separation will not be effective.

In order to enhance the separation effectivity, 0.5 mM decane sulfonate was added into the eluent as ion pair reagent. The influence of ion pair reagent adding against the capacity factor is shown by figure 2. From figure 2 can be assumed that the best separation will happen using the eluent methanol:water:acetic acid (80:19:1) with addition of 0.5 mM decane sulfonate. In fact, the three compounds are separated well using this composition of eluent. This result shows that TPhT has the strongest retention in the stationary phase. This phenomenon happens because TPhT is the less polar compound compared with DBT and TBT. With addition of ion pair reagent, the non polarity of TPhT is increasing. Thus, the TPhT compound will be stronger retained in the non polar stationary phase.

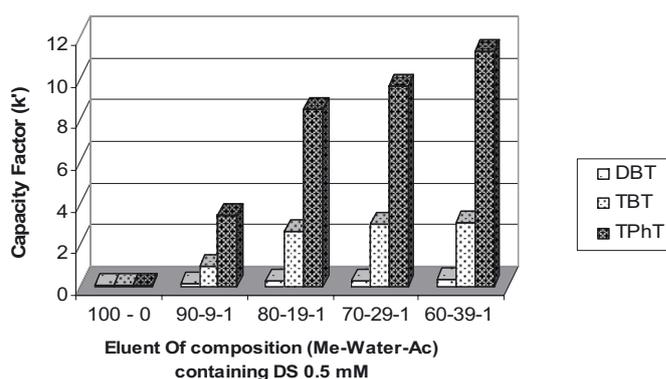


Fig. 2. Effect of eluent composition (containing decane sulfonate) on capacity factor (k').

3.2 Influence of the eluent pH

The optimum composition of eluent which has been figured out is applied to the experiment for eluent pH influence studies. The eluent pH is adjusted by adding 1M H₂SO₄ or 1M NH₄OH. The influence of eluent pH against the capacity factor of organotin species. That plot shows that the eluent pH doesn't give significant influence to the capacity factor. Thus, any pH of eluent can be applied in the separation process. Even though the eluent pH doesn't give significant influence to the separation process, it will be better if the pH is not extremely acid or extremely alkaline in order to avoid the hydrolysis of organosilane bonds in the Si-C₈ stationary phase.

3.3 Influence of the alkyl chain length of the ion-pair reagent

Various alkyl chain lengths of the ion-pair reagents are added into the eluent in order to discover the influence of the alkyl chain length to the speciation performance. The variants of ion-pair reagents are pentane sulfonate, heptane sulfonate and decane sulfonate sodium salt. From the result shown by figure 3, it can be estimated that the best separation takes place when the ion-pair reagent with the longest alkyl chain length added into the eluent. The longer the alkyl chain length of the ion pair reagent, the lower its polarity, so that when DBT, TBT

Table III. Influence of ion-pairing reagent concentrations on resolution and selectivity values of DBT, TBT and TPhT.

Decane sulfonate Concentrations (mM)	α		Rs	
	DBT-TBT	TBT-TPhT	DBT-TBT	TBT-TPhT
0.1	3.07	11.68	2.07	2.64
0.5	3.18	9.89	2.32	2.87
1	3.50	9.76	2.42	2.92
5	6.05	5.52	2.11	2.81
10	5.94	5.89	1.60	2.74

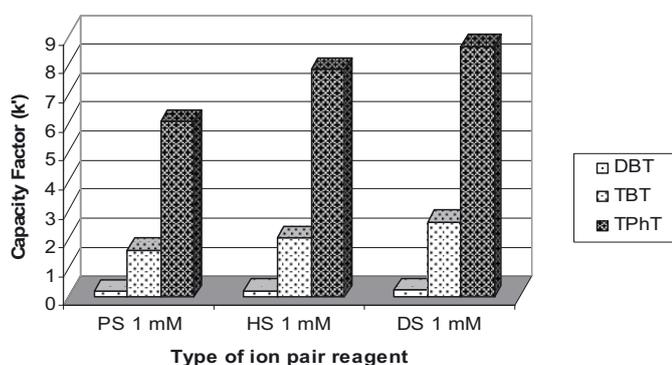


Fig. 3. Effect of the alkyl chain length of ion pair reagent on capacity factor (k'). (PS: Pentane Sulfonate, HS: Heptane Sulfonate, DS: Decane Sulfonate)

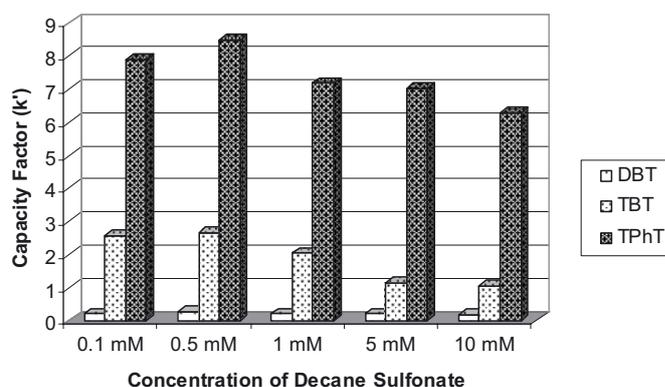


Fig. 4. Effect of decane sulfonate concentrations on capacity factor (k').

and TPhT species form an ion-pair complex with decane sulfonate, organotin species became more hydrophobic. Through augmentation of species hydrophobicity, the species will be more retained by the non polar stationary phase, and the separation will be better.

3.4 Influence of the ion pair reagent concentration

The optimum concentration of ion-pair reagent has to be figured out in order to enhance the speciation performance and to avoid the destruction of the chromatography column. In this research the concentration of decane sulfonate added into the eluent methanol:water:acetic acid (80:19:1) is varied. The influence of the ion pair reagent concentration to the capacity factor is shown in figure 4.

The data in figure 4 shows that the best speciation of DBT, TBT, TPhT takes place when the concentration of the ion-pair reagent is 1 mM. This assumption is supported by the calculation of selectivity value (α) and resolution (Rs). The calculated α and Rs are summarized in table III.

The high concentration of decane sulfonate is always avoided in order to prevent the destruction of chromatography column. The chromatogram profile of DBT, TBT, and TPhT speciation is shown by figure 5.

From the data of influence of concentrations of ion pair reagent to resolution value, we can be proposed the retention mechanism of DBT, TBT and TPhT species in the non polar stationary phase. The retention of organotin compounds takes place because there is an interaction between the alkyl sulfonate reagent and the Si-C₈ stationary phase. First, the

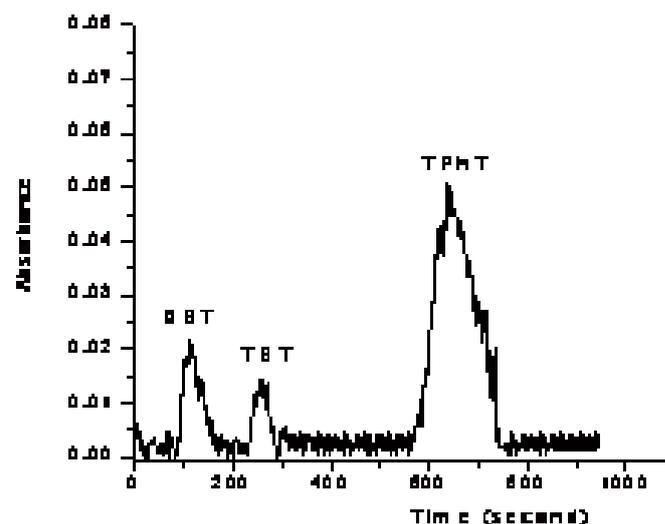


Fig. 5. Chromatogram profile of DBT, TBT and TPhT separation using methanol:water:acetic acid (80:19:1) eluent containing 1 mM decane sulfonate.

ion-pair reagent will flow into the pores of stationary phase and then interact with the Sn(IV) of the organotin compounds. The principle of this retention mechanism is similar to the ion exchange which is commonly known. The difference is that the ion exchange mechanism in this research takes place dynamically. Thus, the more concentrated the ion-pair reagent, the faster the organotin compounds will be eluted from the stationary phase. It happens because the capacity factor will be decreasing (see figure 4).

4 Conclusion

The developed IP-RP-HG-QFAAS chromatography technique can separate DBT, TBT, and TPhT with good performance which is shown by the chromatographic parameters produced. The optimum composition of eluent is methanol:water:acetic acid (80:19:1) containing 1mM decane sulfonate as ion-pair reagent. The capacity factor for DBT, TBT, and TPhT is 0.27, 2.54 and 5.92 respectively. The resolution value (R_s) of DBT-TBT and TBT-TPhT speciation is 2.42 and 2.92 respectively. The selectivity value (α) of DBT-TBT and TBT-TPhT is 3.50 and 9.76. These data show the effectiveness of developed chromatographic system.

Conflict of interest. The authors declare that there are no conflicts of interest.

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