

## Speciation of Organotin Compounds with Ion Pair-Reversed Phase Chromatography Technique

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### Abstract

The usage of ion-pair reversed phase Chromatography (IP-RP) technique for speciation of dibutyltin (DBT), tributyltin (TBT), and triphenyltin (TPhT) has been studied. These three species were able to separate on an ion pair-reversed phase chromatographic column. The eluates were detected on line by using hydride generation-quartz furnace atomic absorption spectrophotometry (HG-QFAAS) method. The eluent consisted of a mixture of methanol: water:acetic acid with a composition of 80:19:1, containing 1 mol L<sup>-1</sup> decane sulfonate acid as ion pairing reagent. The pH of the eluent was adjusted with 1 mol L<sup>-1</sup> H<sub>2</sub>SO<sub>4</sub>. The separation of all of the species at the above conditions was good, as shown by the values of fundamental chromatographic parameters. The capacity factor (*k'*) for DBT, TBT and TPhT species were 0.27, 2.54 and 5.92 respectively. The resolution (*R<sub>s</sub>*) 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. These data show the effectiveness of the developed chromatographic system.

### Keywords:

Organotin; Speciation; Chromatography; Ion pair; HG-QFAAS

### 1. Introduction

Tin atom can make covalent bond with one or more carbon atom to form an organometallic moiety which is commonly called organotin (Sn-C bond). The organotin compounds are generally anthropogenic compounds, except the methyltin which is probably produced by biomethylation in the environment. The tin atom in organotin compound is generally in the +4 oxidation state. The molecular formula of organotin is R<sub>n</sub>SnX<sub>4-n</sub>, where R represents alkyl or aryl group, e.g. methyl, butyl, phenyl, octyl, while X represents anionic species, e.g. chloride, hydroxide, mercaptoester, carboxylic, and sulfide [1].

One of the most useful organotins is tributyltin (TBT). This compound is widely used as an anti fouling agent because of its biocide property. TBT is used in a number of commercial applications including biocide additives in antifouling ship-paint formulation. These paints are then used to protect the underwater surface area of a ship's hull against marine organisms. TBT is also used as a wood preservative. Triphenyltin (TPhT) is commonly used as fungicide in the farm, especially in potato farmland. The degradation products of TBT, monobutyltin (MBT) and dibutyltin (DBT), are commercially used as PVC

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thermo stabilizer, catalyst, and glass coating. Although the toxicity of MBT and DBT are smaller than TBT, these two compounds are classified as dangerous compounds [2].

The toxicity of organotin is higher than its inorganic salt form. The empiric formula for the most toxic organotin is  $R_3SnX$  (triorganotin), e.g. TBT and TPhT [3]. TBT is reported as a compound which is destructive to the human immune system and hormonal performance [4]. TBT and TPhT could be released into the environment and accumulated in the sea sediment or marine organisms such as fish, shells, and squids, thus it will be toxic if it is consumed by human [5]. TBT with concentration less than 1 ng/L could make *imposex* impact to marine organisms [6].

Therefore, sensitive analytical methods suitable for speciation studies are desirable. Most successful approaches resulted from the interface between chromatographic techniques, providing the species differentiation ability, to a specific detector accounting for sensitivity. The determination of species will be more useful than the determination of the tin total amount because it gives accurate information about the tin's existence and toxicity [7]

Gas chromatography methods have high separation power and were connected to very sensitive detectors such as mass spectrometry, flame photometer detector, and atomic spectrometry [8-10]. High-performance liquid chromatography (HPLC) has been used as a separation technique, coupled with element-sensitive detection systems, because conventional HPLC detectors give a poor response to organotin compounds. A number of papers described the use of flame atomic absorption spectrometry (FAAS) [11], electrothermal atomization atomic absorption spectrometry (ETAAS) [12], inductively coupled plasma atomic emission spectrometry (ICP-AES), inductively coupled plasma mass spectrometry (ICP-MS) and mass spectrometry (MS) as detectors for HPLC [13]. FAAS gives poor sensitivity due to low analyte transport and atomization efficiency. ETAAS has excellent detection limits but it cannot provide a continuous recording of the HPLC eluate. ICP-AES equipped with Meinhard nebulizer, though able to record online the HPLC separation, presents, as a limiting factor, the instability of the torch when organic solvents are used. ICP-MS is a very sensitive technique and can detect online chromatographic effluent, but unfortunately is still not easy to handle for naturally occurring sample.

In this paper, a separation method is presented for organotin species, based on reverse phase-ion pair chromatography technique and followed by on line detection by use of a quartz furnace atomic absorption spectrophotometry (HG-QFAAS). The hydride generation combined with QFAAS has been used to increase the sensitivity of detection [14]. In order to get a good separation of DBT, TBT, and TPhT compounds, several parameters of conditions have been studied in this research. The optimum condition used for the HG-QFAAS is based on the previous research [15].

## 2. Experimental

### 2.1. Instrumentation and Reagents

#### 2.1.1. Instrumentation

HPLC (Waters<sup>®</sup>), Si-C<sub>8</sub> column (25 cm x 4.1 mm i.d.) (LiChrospher<sup>®</sup>100 RP-8 5 μm), a set of hydride generator equipment constructed by peristaltic pump and gas-liquid separator, atomic absorption spectrometer double beam GBC<sup>®</sup>-Avanta 6506 equipped with quartz furnace (EHG-3000), recorder system, and data analyzer Origin<sup>™</sup> 7.0., was used for all measurements.

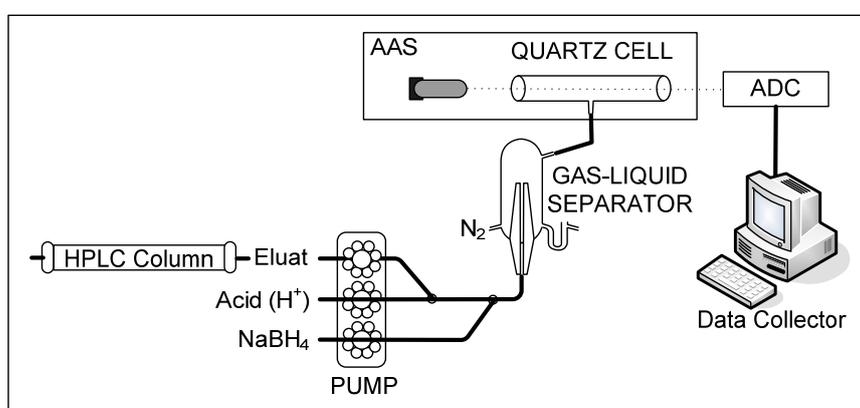
### 2.1.2. Reagents

All reagents were of analytical-reagent grade. A stock  $1000 \mu\text{g mL}^{-1}$  standard solution of DBT, TBT, and TPhT, methanol, pentane sulfonate acid, heptane sulfonate acid, decane sulfonate acid,  $\text{CH}_3\text{COOH}$ ,  $1 \text{ mol L}^{-1}$  of  $\text{H}_2\text{SO}_4$ ,  $1 \text{ mol L}^{-1}$  of  $\text{NH}_4\text{OH}$ , 0,4 % of  $\text{NaBH}_4$  (dissolved in 0,05 % of  $\text{NaOH}$ , b/v), HPLC grade water (18  $\text{M}\Omega$ ). Every reagent used in this research is pro analysis grade (Merck).

## 2.2. Experiment

### 2.2.1. Designing the IP-RP-HG-QFAAS system

The IP-RP-HG-QFAAS system is built by HPLC pump and Si- $\text{C}_8$  column, gas-liquid separator, peristaltic pump which is used to transfer the separated organotin species from HPLC column to hydride generator, hydride formed reagent, and a quartz cell tube which is put in the heating mantle. The whole system is integrated to an AAS detector. The whole equipment of IP-RP-HG-QFAAS system is shown by Fig.1.



**Fig.1.** Schematic diagram of IP-RP-HG-QFAAS

The condition parameters during measurement are shown by Table 1.

**Table 1.** The parameters and analytical condition of IP-RP-HG-QFAAS method

|                                    |                      |  |
|------------------------------------|----------------------|--|
| IP-RP<br>HPLC Waters®              | Column               | Si- $\text{C}_8$ (25 cm x 4.1 mm i.d.) |
|                                    | Eluent               | methanol:water:acetic acid             |
|                                    | Ion pair reagent     | alkyl sulfonate acid                   |
|                                    | pH of eluent         | 3 - 8                                  |
|                                    | Flow rate of eluent  | 1.5 mL/minute                          |
| HG-QFAAS<br>AAS GBC® - Avanta 6506 | Hollow cathode       | Sn (6 mA)                              |
|                                    | Wavelength           | 224.6 nm                               |
|                                    | Band pass            | 0.7 nm                                 |
|                                    | Temperature EHG-3000 | 1100 °C                                |
|                                    | Gas-liquid separator | 9 x 3 cm                               |

### 2.2.2. Optimization of the Quality of DBT, TBT, and TPhT Separation

In order to optimize the separation of DBT, TBT, and TPhT, measurement of some parameters has been done. The parameters are eluent composition, pH of the eluent, the chain length of alkyl sulfonate acid, the concentration decane sulfonate acid. The concentration of

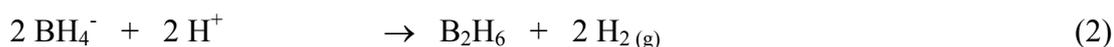
standard solutions of DBT, TBT, and TPhT during these measurements is 10 mg L<sup>-1</sup>. The measurements have been done against each individual compound and also mixture of some compounds.

### 3. Results and Discussion

The speciation of DBT, TBT, and TPhT in this research was held by using IP-RP Chromatography with Si-C<sub>8</sub> column. To obtain the high resolution of separation the best composition of eluent has been figured out. The quality of speciation of organotin species in a non polar column can also be enhanced by adding alkyl sulfonate acid as ion-pair reagent, through increment of the species hydrophobicity. The ion Sn(IV) of species DBT, TBT, and TPhT will combine with ion-pair reagent to form a metal-ion pair complex. The complex formed has good affinity against 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, then atomized in the quartz cell tube which is put in the heating mantle [16] The mechanism of hydride generation is shown by reactions below.



The hydride generation process takes place in the gas-liquid separator HG. Several factors which influence the metal ion determination using HG methods are acid and reductant concentrations and the type of acids used. The optimum analytical performance can be achieved under conditions stated in the previous research [15], which can be seen in Table 2.

The condition parameters stated in Table 2 are applied in the speciation process of DBT, TBT, and TPhT species using IP-RP-HG-QFAAS system.

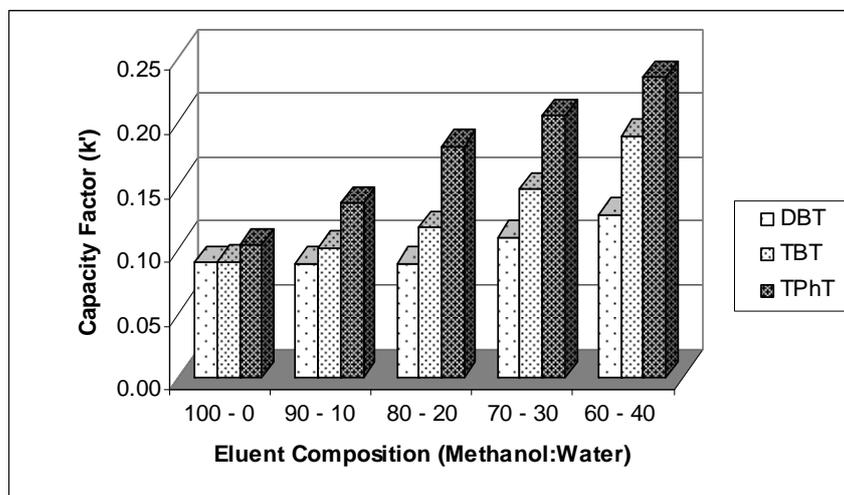
**Table 2.** Analytical performance of HG-QFAAS for determination of Sn(IV)

| Parameter                               | Results  |
|---|--|
| Temperature of EHG-3000                 | 1100 °C  |
| CH <sub>3</sub> COOH concentrations     | 0.3 M  |
| NaBH <sub>4</sub> concentrations        | 0.4 % (v/v, dissolved in NaOH 0.05 %, b/v)                       |
| Mixing technique of the reactant system | mixing in the coil before introduced to the gas-liquid separator |
| Limit of detection                      | 3.74 µg/L  |
| Reproducibility                         | 1.12 % for 50 µg/L standard concentrations                       |

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 acid, and ion-pair reagent concentration.

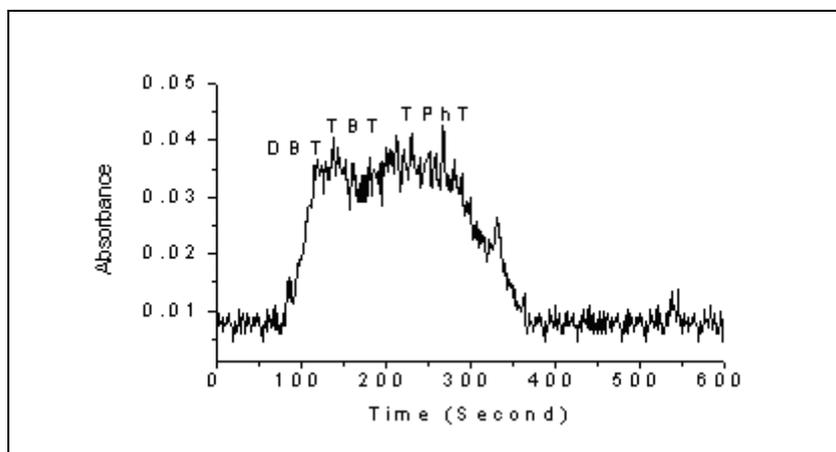
### 3.1. The Influence of Eluent Composition

In this step, variation of the eluent composition of methanol: water acetic acid has been conducted. Selection of this composition was based on the nature of insoluble organotin species in water, so that the required strength of eluent composition is utilised, as can be seen by Fig.2.



**Fig.2.** Effect of eluent composition methanol:water on capacity factor ( $k'$ ) of organotin species.

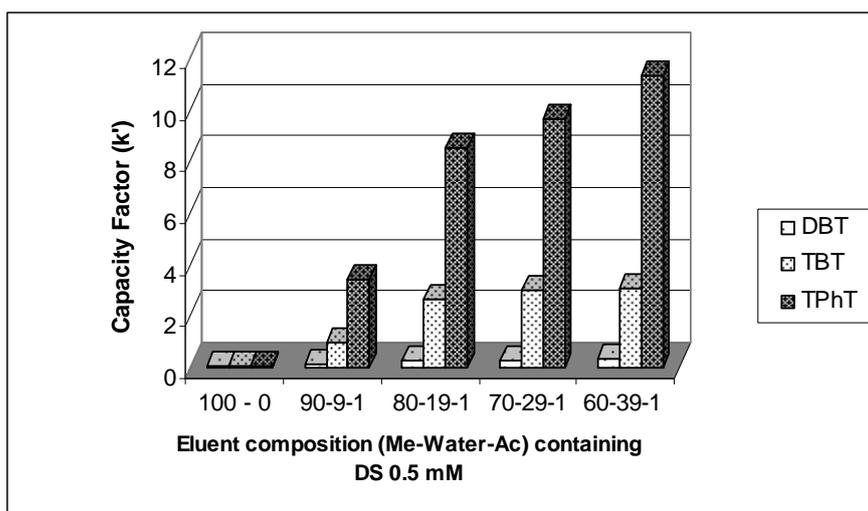
Fig.2. shows that there is no significant difference between capacity factor of DBT, TBT, and TPhT, which means that these three compounds have similar retention property. Thus, with this composition of eluents the separation will not happen. Fig.3 shows the uneffective separation using this eluent composition.



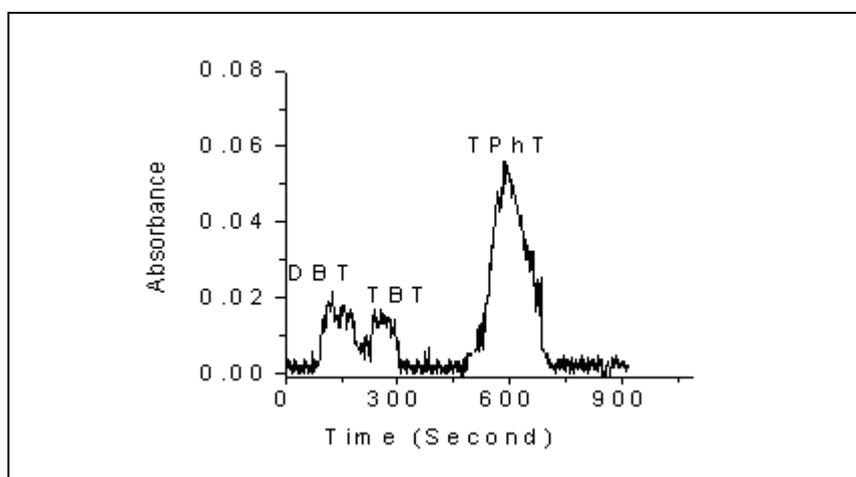
**Fig.3.** Chromatogram profile of organotin species using eluent methanol:water (80:20).

In order to enhance the separation effectively, 0.5 mM decane sulfonate acid was added into the eluent as an ion pair reagent. The influence of ion pair reagent against the capacity factor is shown by Fig.4.

From Fig.4, it can be assumed that the best separation will happen using the eluent ratio of methanol:water:acetic acid (80:19:1) with addition of 0.5 mM decane sulfonate acid. In fact, the three compounds separated well using this composition of eluent, which is shown by Fig.5.



**Fig.4.** Effect of eluent composition on capacity factor ( $k'$ ) of organotin species.



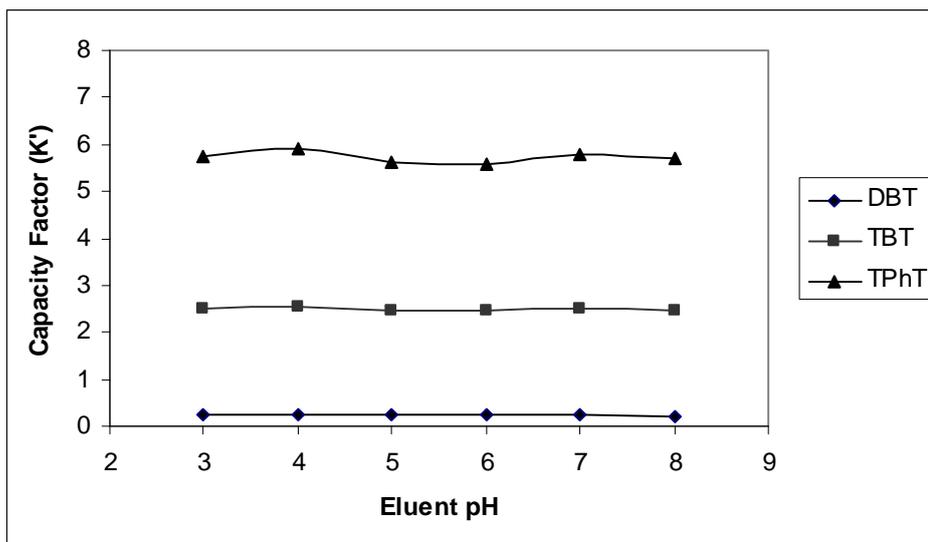
**Fig.5.** Chromatogram profile of organotin species using eluent methanol:water:acetic acid (80:9:1) containing 0.5 mM decane sulfonate acid.

Fig.5 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 increased. Thus, the TPhT compound will have stronger retention in the non polar stationary phase.

### 3.2. Influence of the Eluent pH

The optimum eluent composition as described in 3.1. is applied to the experiment of determination of eluent pH influence. The pH of eluent is adjusted by adding 1M of  $H_2SO_4$  or 1M of  $NH_4OH$ . The chart of eluent pH against the capacity factor is shown by Fig.6.

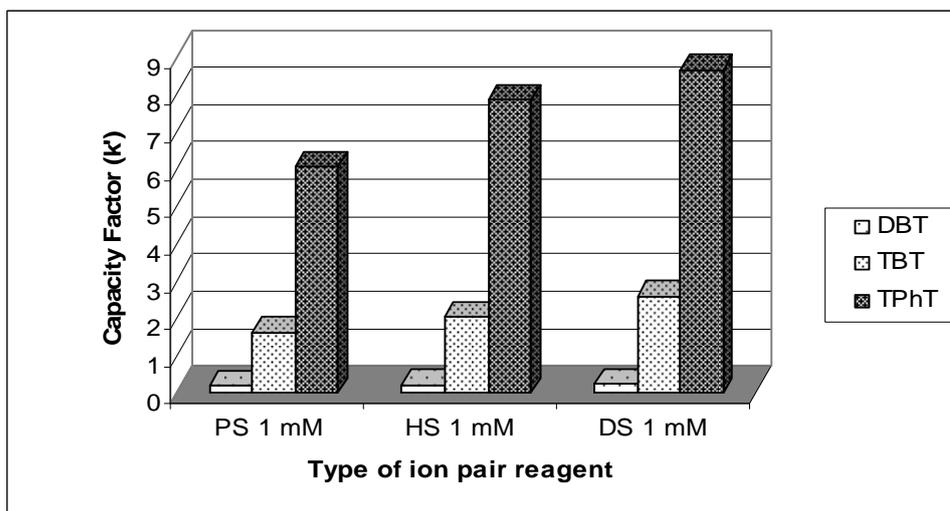
Fig.6 showed that the eluent pH doesn't give significant influence to the capacity factor. Thus, any pH of eluent can be applied in the separation process. Even though the eluent pH doesn't give significant influence to the separation process, it will be better if the pH is not extremely acid or extremely basic in order to avoid the hydrolysis of organosilane bonds in the  $Si-C_8$  stationary phase.



**Fig.6.** Effect of eluent pH on capacity factor ( $k'$ ) of organotin species

### 3.3. Influence of the Alkyl Chain Length of the Ion Pair Reagent

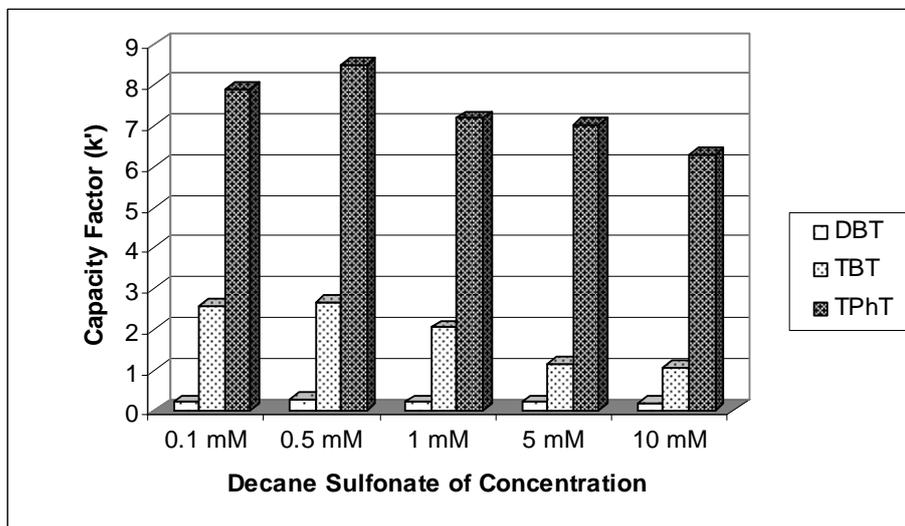
Various alkyl chain lengths of the ion-pair reagents were added into the eluent in order to determine the influence of the alkyl chain length to the speciation performance. The variants of ion-pair reagents are pentane sulfonate acid, heptane sulfonate acid and decane sulfonate acid. From the results shown by Fig.7, we can estimate that the best separation takes place when the ion-pair reagent with the longest alkyl chain length is added into the eluent. The longer the alkyl chain length of the ion pair reagent, the lower its polarity, so that when DBT, TBT and TPhT species form complex reaction with decane sulfonate acid, hence hydrophobic interactions will be reduced.. Thus, the retention of the species by the non polar stationary phase will be enhanced, and the separation will be better.



**Fig.7.** Effect of the alkyl chain length of ion pair reagent on organotin species capacity factor ( $k'$ ). (*PS: pentane sulfonate, HS: heptane sulfonate, DS: decane sulfonate*)

### 3.4. Influence of the Ion Pair Reagent Concentration

The optimum concentration of ion-pair reagent has to be determined in order to enhance the speciation performance and to avoid the destruction of the chromatographic column. In this research the concentration of decane sulfonate acid 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 by Fig.8.



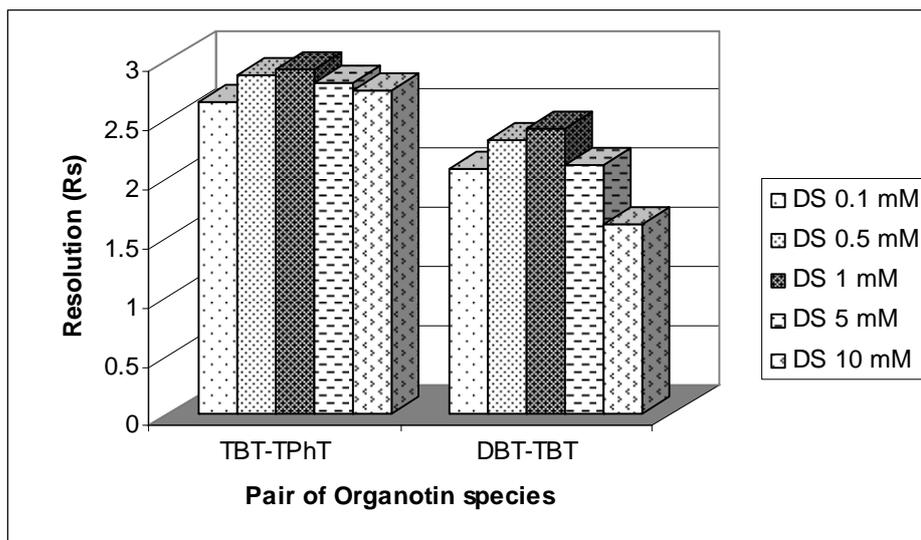
**Fig.8.** Effect of decane sulfonate acid concentrations on capacity factor ( $k'$ ) of organotin species

The data in Fig.8 shows that the best speciation of DBT, TBT, TPhT takes place when the concentration of the ion-pair reagent is 1mM. This assumption is supported by the calculation of selectivity value ( $\alpha$ ) and resolution ( $R_s$ ). The calculated  $\alpha$  and  $R_s$  are summarized in Table 3.

**Table 3.** Resolution and selectivity values organotin species at the various decane sulfonate acid concentrations

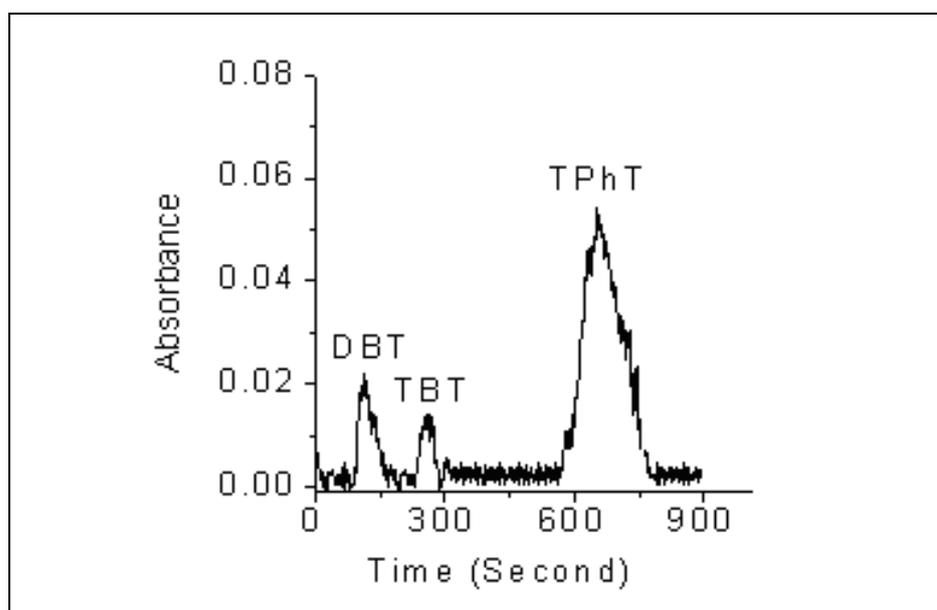
| Decane sulfonate acid concentration (mM) | $\alpha$ |          | $R_s$   |          |
|--|----------|----------|---------|----------|
|  | DBT-TBT  | TBT-TPhT | DBT-TBT | TBT-TPhT |
| 0.1                                      | 3.07     | 11.68    | 2.07    | 2.64     |
| 0.5                                      | 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     |

The high concentration of decane sulfonate acid is avoided in order to prevent the destruction of chromatography column. The chart of resolution value against the concentration of decane sulfonate acid is shown by Fig.9.



**Fig.9.** Effect of decane sulfonate acid concentrations on resolution values (Rs) of organotin species

The chromatogram profile of DBT, TBT, and TPhT speciation is shown by Fig.10.



**Fig.10.** Chromatogram profile of organotin species using eluent methanol:water:acetic acid (80:19:1) containing 1 mM decane sulfonate acid.

The data obtained from the influence of concentrations of ion pair reagent to the resolution value (Fig. 9), may be used to illuminate 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 acid reagent and the Si-C<sub>8</sub> stationary phase. First, the ion-pair reagent will flow into the pores of the stationary phase and then interact with the Sn(IV) of the organotin compounds. The basis of this retention mechanism is similar to the ion exchange mechanism which is commonly known. The difference is that the ion exchange in this research takes place dynamically. Thus, the more concentrated the ion-pair reagent, the faster the organotin compounds will be retained in the stationary phase. Thus, the capacity factor will decrease (see Fig.8).

#### 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 acid 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 ( $\alpha$ ) of DBT-TBT and TBT-TPhT is 3.50 dan 9.76. These data show the effectiveness of the developed chromatographic system.

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