APPLIED ORGANOMETALLIC CHEMISTRY
Appl. Organometal. Chem. 2007; **21**: 412–424
Published online in Wiley InterScience
(www.interscience.wiley.com) DOI:10.1002/aoc.1235

Determination of migration of n-butyltins and n-octyltins to food simulants by gas chromatography–mass spectrometry

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Received 29 December 2006; Accepted 16 February 2007

A simple chromatographic method was developed for the simultaneous determination of butyl- and octyltin compounds in two aqueous-based food simulants, water and 3% (w/v) acetic acid. The procedure is based on one-step derivatization/extraction with sodium tetraethylborate directly in the aqueous phase in the presence of 1 ml of 0.05% (w/v) tropolone in hexane. Derivatization parameters such as extraction solvent, pH, reaction time and amount of the reagent were optimized. The corresponding ethylated compounds were analysed by GC-MS using tripropyltin (TPrT) and diheptyltin (DHT) as internal standards for butyl- and octyltin compounds, respectively. This new method was validated and evaluated for linearity, trueness and precision in both simulants. The detection limits were in the range 1.9 (MBT) to 8.8 (TBT) μg l^{-1} (as organic–metallic cation) in both simulants. The stability of the organotin compounds under migration conditions (10 days at 40 °C) was also tested. The tested organotins presented no significant losses under the migration conditions. The developed method was applied to some PVC food packaging materials from the market. Few organotins were detected at levels near the limit of quantitation. Copyright © 2007 John Wiley & Sons, Ltd.

KEYWORDS: organotins; dioctyltin; dibutyltin; diheptyltin; ethylation; migration; food simulants; stability; GC-MS

INTRODUCTION

Mono/diorganotin compounds, especially butyl- and octyltin species, are used in the PVC industry as heat and light stabilizers. In 2003, about 70% of the worldwide production of organotins was applied as PVC stabilizers.[1] These compounds seem to prevent the dehydroclorination reactions by binding hydrogen chloride.[2] Some of the most common stabilizers are organotin mercaptides (sulfur-contain) and organotin carboxylates. Mixtures of mono- and di-organotin compounds are frequently used to produce a synergistic effect.[3] Nowadays, PVC plastics have numerous applications, including food and beverages containers, potable water pipes and cling films.

Under certain conditions (e.g. high temperature), migration of tin stabilizers can take place from packaging material to food.[4,5] Foods are complex mixtures consisting of water, fat, proteins and carbohydrates. In order to avoid analysis of complicated food matrices, migration testing is usually carried out to the packaging item using food simulants. There are four types of food simulants used for testing migration, identified as simulant A (distilled water), simulant B (3% acetic acid), simulant C (10% ethanol) and simulant D (olive oil or sunflower oil), depending on the chemical nature of the food (for simulating neutral, acidic, alcoholic and fatty food, respectively). Testing conditions (time–temperature) correspond to the worst conditions of real use or storage of the food samples. There are many studies published in

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Contract/grant sponsor: General Chemistry State Laboratory of Athens.

the literature for the determination of organotins (OTs) in environmental samples, but only a few provide information on the migration of butyl- and octyltin compounds into foodstuffs.[6–12] Data on organotin migration from food packaging materials into food simulants are also scarce. There is one literature review,[4] stressing that there is a need to study the migration of intact organotins stabilizers or their degradation products into simulating solvents; however, to the best of our knowledge, there is no other report on the subject.

The toxicity of organotin compounds varies according to the number and nature of organic substitutes. In general, toxicity increases with increasing degree of alkylation and decreasing alkyl-chain length. Octyltin derivatives, therefore, are not considered highly toxic. Thus, octyltin compounds are permitted as PVC stabilizers in some European countries and the USA.[5] However, a recent study showed that octyltin stabilizers are embryo-fetotoxic and induce developmental effects.[13] Moreover, di-organotin stabilizers (dibutyltin, DBT, and dioctyltin, DOT) have been shown to interfere selectively with the immune system, causing suppression.[14–17] It is also possible that organotins can act as mutagens[18] or initiate the multistage process of carcinogenesis.[19] However, the health effects on humans are still not well understood.[1]

The EU Commission and the Scientific Committee for Food (SCF) established a specific migration limit (SML) for all di-*n*-octyltin (DOT) and mono-*n*-octyltin (MOT) stabilizers in PVC food and beverages packaging materials based on the proposed tolerable daily intake (TDI) value. Under the light of the toxicological findings for DOT, European Commission has decreased the SML of DOT to 0.006 mg kg^{-1} (as Sn) (which can be transformed to 0.012 mg/kg as cation) in food or food stimulant,[20] from 0.04 mg kg^{-1} (as Sn).[21] The established SML for MOT compounds is 1.2 mg kg^{-1} (as Sn).[20] DBT and MBT compounds are characterized as substances for which no or only scanty and inadequate data are available, and are included in the 'waiting list'.[22] This list includes substances not yet included in the Community lists, as they should be considered 'new' substances, i.e. substances never approved at national level.[22] These substances cannot be included in the Community lists, as they lack the data requested by the Committee. Consequently, a sensitive, accurate and, if possible, confirmatory method is required for the determination of OTs migration from PVC food packaging materials.

The most used analytical methods for the determination of OTs involve gas chromatography (GC) techniques. GC has the advantage of analysing wide range of OTs in one chromatographic run and couples easily to sensitive and selective detectors such as quartz-furnace atomic absorption spectrometry (QFAAS),[23] flame photometric detection (FPD),[24–26] inductively coupled plasma–mass spectrometry (ICP-MS),[27] microwave-induced plasma–atomic emission spectrometry (MIP-AES),[28] mass spectrometry (MS)[9,29–31] and tandem mass spectrometry (MS/MS).[32] GC-FPD is the most reported technique for the simultaneous determination of $NaBEt_4$-derivatized butyl- and octyltin compounds. These methods offer good analytical performance, but they do not provide structural confirmation of the target compound. GC-MS is capable of separating mixtures and identifying and quantifying their components. Frequently, GC-MS is used to confirm the presence of these compounds after quantitation with other detectors.[6,10,11] Tripropyltin (TPrT) is frequently applied as internal standard for the determination of butyltin compounds. Diheptyltin (DHT) has been applied once for the quantitation of octyltins in environmental samples.[33] In this work, two internal standards were used. TPrT was used to quantify butyltins and DHT was used to quantify octyltins.

For the GC determination of OTs, it is necessary to convert them into volatile derivatives before analysis. For their derivatization, three different approaches have been used: hydride generation ($NaBH_4$),[10,34] alkylation with Grignard reagents (RMgX)[6,7,32] and ethylation with sodium tetraethylborate ($NaBEt_4$).[26,29,30,35] A few years ago, $NaBPr_4$ was applied as derivatization reagent for the speciation of organotins.[36–38] Compared with alkylation by Grignard reagents, ethylation with $NaBEt_4$ and hydride generation are directly applicable to aqueous samples. In contrast to hydride generation, ethylation does not suffer from serious matrix interferences and usually provides more reproducible results.[38] Furthermore, simultaneous *in situ* derivatization/extraction is possible, making the whole procedure fast and simple.

Therefore, the aim of this work was to develop a confirmatory method for the simultaneous determination of migration of butyl- and octyltin compounds to food simulants, using $NaBEt_4$ derivatization and GC-MS analysis. TPrT and DHT were used for internal standardization. The mass spectra of the ethylated target organotin compounds and the two internal standards were reported and ions used for single ion monitoring (SIM) were assessed. The optimum conditions for simultaneous ethylation of these compounds were also investigated. The optimized method was validated. The stability of the analytes during the migration test (40 °C, 10 days) in water and 3% acetic acid was investigated for the first time. Finally, the method was applied to PVC samples from the Greek market.

EXPERIMENTAL

Reagents and chemicals

Monobutyltin trichloride (MBT, 95%), dibutyltin dichloride (DBT, 96%) and tributyltin chloride (TBT, 96%) as well as tripropyltin oxide (TPrT, 97%) were obtained from Sigma Aldrich (Wisconsin, WI, USA). Monooctyltin trichloride (MOT, 98%), dioctyltin dichloride (DOT, 98%) and diheptyltin dichloride (DHT, 99%) were purchased from LGC Promochem (Wesel, Germany).

Sodium tetraethylborate ($NaBEt_4$, 97%) was obtained from Sigma Aldrich. A 2% (w/v) $NaBEt_4$ solution was prepared

DOI: 10.1002/aoc

daily by dissolving 0.04 g in 2 ml of water and stored at 4 °C in the dark.

Buffer solutions of CH_3COOH/CH_3COONa with pH values between 4 and 6 were prepared by dissolving 8.2 g of sodium acetate (Merck, Darmstadt, Germany) in 1 l of water followed by pH adjustment with glacial acetic acid (Carlo Erba, Rodano, Italy). A 3% (w/v) acetic acid solution was prepared in water as an aqueous acidic food simulant.

A solution of 0.05% (w/v) tropolone (98%, Acros Organics, Geel, Belgium) was prepared in hexane and an ammonium hydroxide solution (2 M NH_4OH, from analytical reagent grade 25%, Fluca, Buchs, Switzerland) was prepared in water. The water used throughout the experiments was obtained from a Milli-Q water system (Millipore, Milford, MA, USA). All solvents used (methanol, hexane, isooctane) were of analytical grade.

Standard solutions

Standard stock solutions of individual organotin compounds of 1000 mg l^{-1} (as cation) were prepared by dissolving appropriate amounts of the respective compounds in methanol and stored at 4 °C for at least one year. Intermediate standard solutions of individual compounds of 10 mg l^{-1} (as cation) were prepared in Milli-Q water by dilution of the individual stock solutions and stored at 4 °C in the dark for at least one week.

Working solutions of individual organotin compounds (0.10–5.0 mg l^{-1}) in simulants were prepared daily from the intermediate solutions. For internal standardization a solution of TPrT and DHT containing 0.50 mg l^{-1} (as cation) of each compound, in the simulating solvent, was also prepared daily from the intermediate solutions.

Moreover, intermediate standard mixtures (50 mg l^{-1} of each cation) were prepared weekly from the individual stock solutions. More dilute mixed standard solutions were prepared daily in water and 3% acetic acid.

Instrumentation

All chromatographic analyses were performed by an Agilent 6890 gas chromatograph coupled to an Agilent 5973N mass-selective detector. The separation was carried out on a HP-5MS capillary column (30 × 250 μm i.d. × 0.25 μm film thickness). The instrumental parameters of the GC-MS system are shown in Table 1.

Table 1. Instrumental parameters

GC parameters	
Column	HP-5MS 30 m × 0.25 mm × 0.25 μm
Carrier gas	He, 1.2 ml min^{-1}
Injection mode	Splitless
Injection volume	1 μl
Injection *T*	250 °C
Temperature program	
Initial *T*	80 °C for 1 min
$Rate_1$	10 °C min^{-1}
Intermediate *T*	210 °C for 15 min
$Rate_2$	20 °C min^{-1}
Final *T*	300 °C for 5 min
QMS parameters	
EI mode	70 eV
Solvent delay	3 min
Transfer line temperature	280 °C
Source temperature	230 °C
MS quad temperature	150 °C

Analytical procedure

Derivatization

After the migration test, 10 ml of the water food simulant was placed in a narrow-necked flask. Then, 1 ml of mixed internal standard solution (TPrT and DHT, 0.50 mg l^{-1} of each cation) was added. The pH was adjusted at 4.8 with 20 ml of 0.1 mol l^{-1} acetate buffer solution and derivatization was performed with 200 μl of 2% (w/v) $NaBEt_4$ solution. Immediately, 1 ml of 0.05% (w/v) tropolone in hexane was added and the mixture was shaken on a vortex for 1 min and on a mechanical table (290 rpm) for 15 min. After phase separation, the organic layer was collected with a Pasteur pipette and analysed by GC-MS.

Accordingly, 10 ml of the acetic acid food simulant was mixed with 1 ml of mixed internal standard solution (TPrT and DHT, 0.50 mg l^{-1} of each cation). Afterwards, 2 ml of an ammonium hydroxide solution 2 M and 20 ml of 0.1 mol l^{-1} acetate buffer were added to adjust pH at 4.8 and ethylation extraction was performed with 200 μl of a 2% (w/v) $NaBEt_4$ solution into 1 ml of 0.05% (w/v) tropolone in hexane, as described for water.

Quantitation

The determination of organotin concentrations in samples were performed using calibration curves (as described in the following section) using TPrT and DHT as internal standard for butyl- and octyltin compounds, respectively. Blank analyses of each simulant were performed with each batch of samples to check the laboratory and reagent contamination. Concentrations and detection limits of organotins given in this work are expressed as amount of the respective organometallic cation per litre.

Validation of the method

The method was validated using organotin standard solutions prepared in water and 3% acetic acid. TPrT and DHT (0.5 mg l^{-1} of each cation) were used as internal standards for butyl- and octyltin compounds, respectively. The linear range of detection was established by measuring simulant standard solutions in the range 0.10–5.0 mg l^{-1} (0.10, 0.20, 0.30, 0.50, 0.80, 1.0, 2.0, 3.0 and 5.0 mg l^{-1}) with 0.50 mg l^{-1} of each internal standard. Usually two calibrations curves were constructed, one in the low concentration

DOI: 10.1002/aoc

range (0.10–0.80 mg l^{-1}) and one in the high concentration range (1.0–5.0 mg l^{-1}). Each standard was measured twice. Calibration curves were constructed by plotting the values of the relative peak areas (analyte peak area : internal standard peak area) vs analyte standard solution concentration.

In order to examine if the calibration can be performed with mixtures of organotin compounds, standard mixed solutions of DBT and DOT in water were prepared at five concentrations (0.10, 0.20, 0.30, 0.50 and 0.80 mg l^{-1}). Then, it was investigated whether the calibration curves obtained by the mixed standard solutions were statistically equivalent to calibration curves obtained by individual standard solutions of each compound. For this purpose a correlation curve was plotted of the analytical parameters obtained from the aqueous mixed solutions against the analytical parameters obtained from the individual standard solutions.

The instrumental limit of detection (LOD) for each of the five organotin compounds were defined as $(3.3 \times S_{y/x})/b$ and the LOQ as $(10 \times S_{y/x})/b$. $S_{y/x}$ and b are the residual standard deviation and the slope of a calibration curve for concentrations ranging from 0.10 to 0.80 mg l^{-1}, respectively. The method LOD was 10 times lower than the instrumental LOD, taking into account the preconcentration ratio (1 : 10).

Precision was determined by performing six replicates analyses of each compound in both simulants, at a concentration of 0.10 and 1.0 mg l^{-1} over one day (repeatability) and one analysis at six different days at the level of 1.0 mg l^{-1} (reproducibility).

Trueness was evaluated at two levels (0.10 and 1.0 mg l^{-1}) by analysing independently these two standard solutions in both simulants. The relative error (%E) was calculated by subtracting the concentration found from the calibration curve from the nominal concentration and divided by the nominal concentration. Blanks of both simulants were analysed regularly, along with calibration standards and the analysed samples.

Stability experiments

A stability test was carried out in water and 3% acetic acid under the migration conditions for both butyl- and octyltin compounds, during a period of 10 days. Individual standard solutions of MBT, DBT, MOT and DOT (1.0 mg l^{-1} of each cation) were kept for 10 days at 40 °C. Aliquots of each solution (1 ml) were analysed at regular time intervals during that period. Each day, a freshly prepared mixed standard solution (at the same concentration) containing the organotins of interest was analysed for comparison and for quantitation of the analytes in the stimulant solution.

Samples

Six food packaging materials from PVC, such as cling films ($n = 3$), containers ($n = 2$) and one water pipe, were tested with respect to migration of organotins to water and 3% acetic acid as food simulants. Migration tests were performed as follows:[39–41]

For the migration of OTs from the PVC films, special calibrated migration cells were used, where only the surface intended to be in contact with foodstuffs was in intimate contact with the simulating solvent: 1 dm^2 of the inner surface area was in contact with 100 ml of food simulant.

The PVC containers were filled with 100 ml of the appropriate food stimulant and covered to prevent evaporation. The specific area under migration for container A and container B was 2.320 and 0.6302 dm^2, respectively.

Migration tests for the PVC pipe were performed by immersing 0.825 dm^2 of the surface area into 100 ml of simulating solvent. All samples were placed in an oven at 40 °C for 10 days. Blank tests for both simulants were also included. After migration tests, food simulants were analysed according to the protocol described above.

RESULTS AND DISCUSSION

Development of the GC-MS method

The first step in GC analysis of organotin compounds is derivatization. In this work organotins were ethylated with $NaBEt_4$ and separated on a HP-5MS capillary column. The mass spectrometer was operated in the electron ionization (EI) mode. Figure 1 presents the full scan mass spectra obtained for each of the five ethylated organotin compounds and the two internal standards. The identity of each ethylated butyl- and octyltin compound was determined by comparison of the extracted mass spectra with spectra library databases and fragmentation patterns given in previous works. All mass spectra found were in good agreement with those found in literature for the ethylated butyltins[29,35] and octyltins.[42] The m/z numbers of Fig. 1 refer to isotopes occurring with maximum intensity in the group (i.e. with ^{120}Sn). The mass spectra of these compounds consist of fragment peaks at m/z 121 [SnH^+], 149 [$SnEt^+$] and 179 [$SnEt_2H^+$], which are characteristic ion fragment masses for organotins. The mass spectra and the fragmentation pattern of ethylated octyltins are identical with those reported previously.[42] The only additional fragment at the mass spectrum of DOT is at m/z 208 [Fig. 1(e)], corresponding to the cluster [$SnEt_3H^+$]. TPrT and DHT have been used as internal standards before, but their mass spectra have never been reported in the literature. A detailed MS interpretation for the mass spectra of these compounds [Fig. 1(f, g)] is given in Table 2.

Based on the individual mass spectrum, three specific ions per analyte were chosen according to the highest abundances and lowest background levels and monitored by the selected ion monitoring (SIM) mode. MOT was quantified using two ions in order to avoid background contamination occurred at m/z 149 during the SIM window of MOT. In the case of DBT and TBT, high contamination was noticed at m/z 207 [$BuSnEtH^+$]; thus this fragment was not chosen. Table 3 gives the corresponding data for the SIM program. A SIM chromatogram of a standard mixture of the organotins of

DOI: 10.1002/aoc

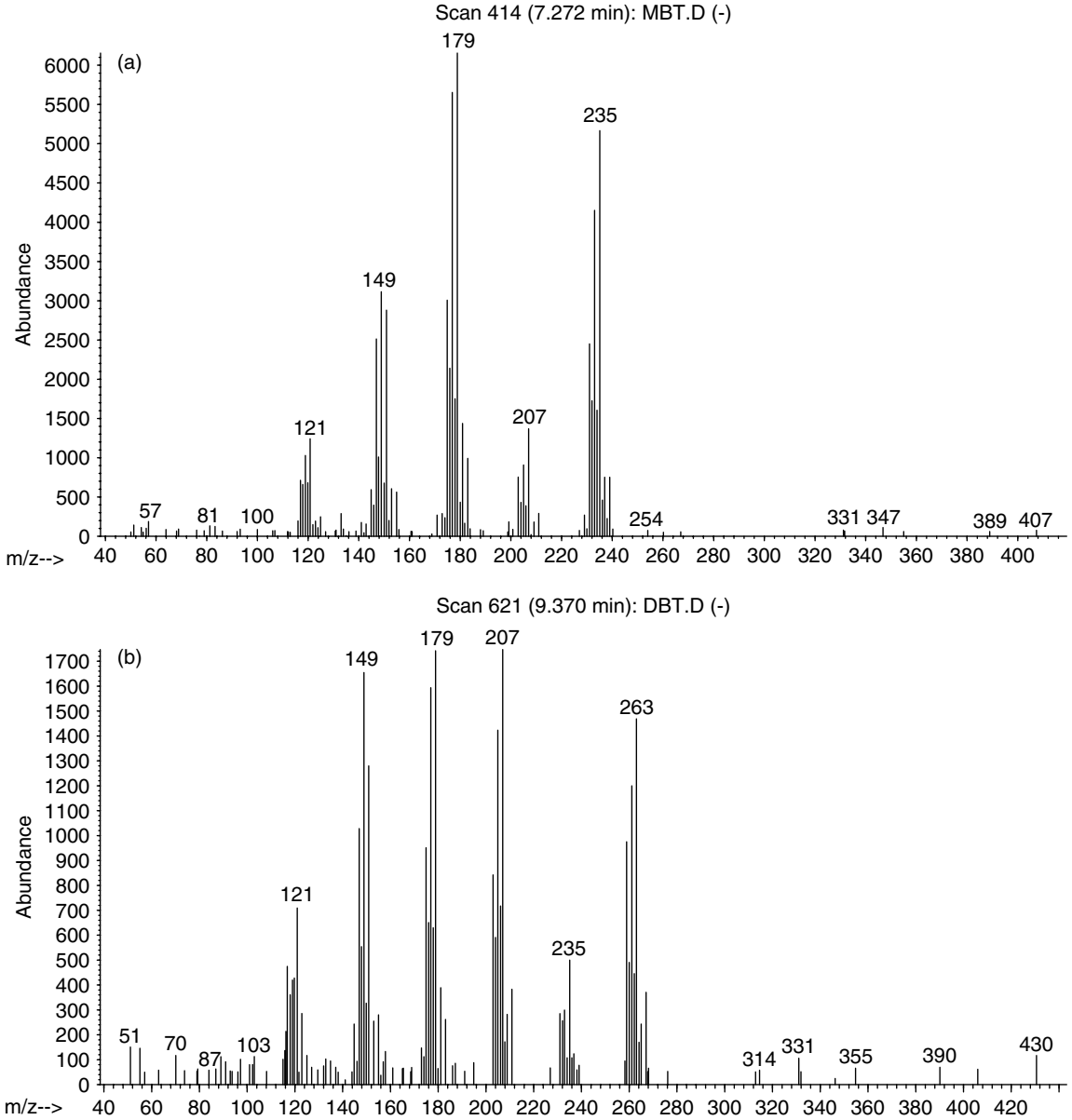

Figure 1. Mass spectra of the seven ethylated organotin compounds: (a) MBT; (b) DBT; (c) TBT; (d) MOT; (e) DOT; (f) TPrT; and (g) DHT.

Table 2. Main EI fragments for ethylated tripropyltin and diheptyltin derivatives (based on ^{120}Sn)

Pr_3SnEt		Hep_2SnEt_2	
m/z	Fragments	*m/z*	Fragments
249	Pr_3Sn^+	347	Hep_2SnEt^+
235	Pr_2SnEt^+	277	$HepSnEt_2^+$
206	Pr_2Sn^+	249	$HepSnEtH^+$
193	$PrSnEtH^+$	219	$HepSn^+$
163	$PrSn^+$	179	$SnEt_2H^+$
151	$SnEtH_2^+$	149	$SnEt^+$
121	SnH^+	121	SnH^+

interest in water is shown in Fig. 2. All peaks were sharp and well separated.

Optimization of the derivatization conditions

In order to analyse organotin compounds by chromatographic techniques, a derivatization step is necessary to convert them into thermally stable, volatile derivatives. Sodium tetraethylborate was first used in the derivatization of butyltins by Craig and colleagues[43,44] for their environmental analysis. Some years later, Craig and Mennie[42] introduced its use for octyltin derivatives.

Advantages of derivatization by $NaBEt_4$ are that ethylation can be carried out in the aqueous phase and extraction can

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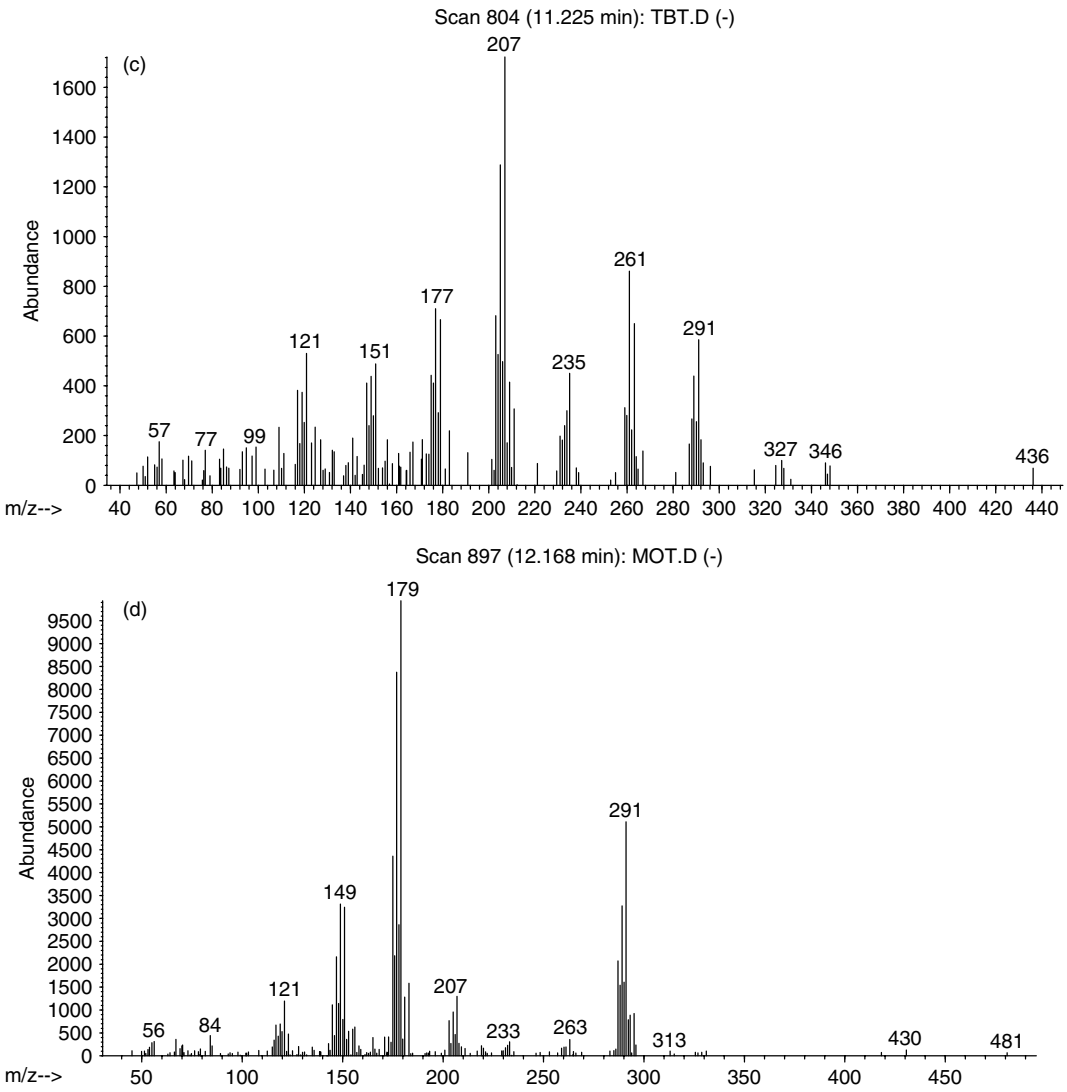

Figure 1. (*Continued*).

Table 3. GC-MS parameters

Group	Retention time (min)	Start time[a] (min)	Selected ions (m/z)
MBT	6.35	3	149, 179, 235
TPrT	7.22	7	121, 193, 235
DBT	8.45	8	149, 179, 263
TBT	10.31	10	179, 263, 291
MOT	11.25	10.7	179, 291
DHT	14.70	12	149, 249, 347
DOT	16.12	15	179, 263, 375

[a] Time at which the detector begins to measure.

be performed simultaneously. However, this procedure is strongly affected by pH, reaction time and amount of the reagent.[30,31,45–47] Therefore, these parameters were optimized. The optimization experiments were carried out with aqueous standard mixed solutions of DBT and DOT. Owing to the extensive use of DBT and DOT as PVC heat stabilizers, only these compounds were considered. The influence of the organic solvent on the efficiency of the extraction was also examined using a mixture of MBT, DBT, MOT and DOT in deionized water. The concentrations were 1.0 mg l^{-1} (as cation) for each compound. It must be noted that the optimization of the *in situ* ethylation of octyltin species has, to the best of our knowledge, never been examined before.

Evaluation of the extraction

These experiments aimed at evaluating the extraction efficiencies of two organic solvents (hexane and isooctane) and the addition or not of a complexing agent (tropolone). Table 4 summarizes the influence of the extraction solvent (isooctane, hexane or tropolone/hexane) on the peak area

DOI: 10.1002/aoc

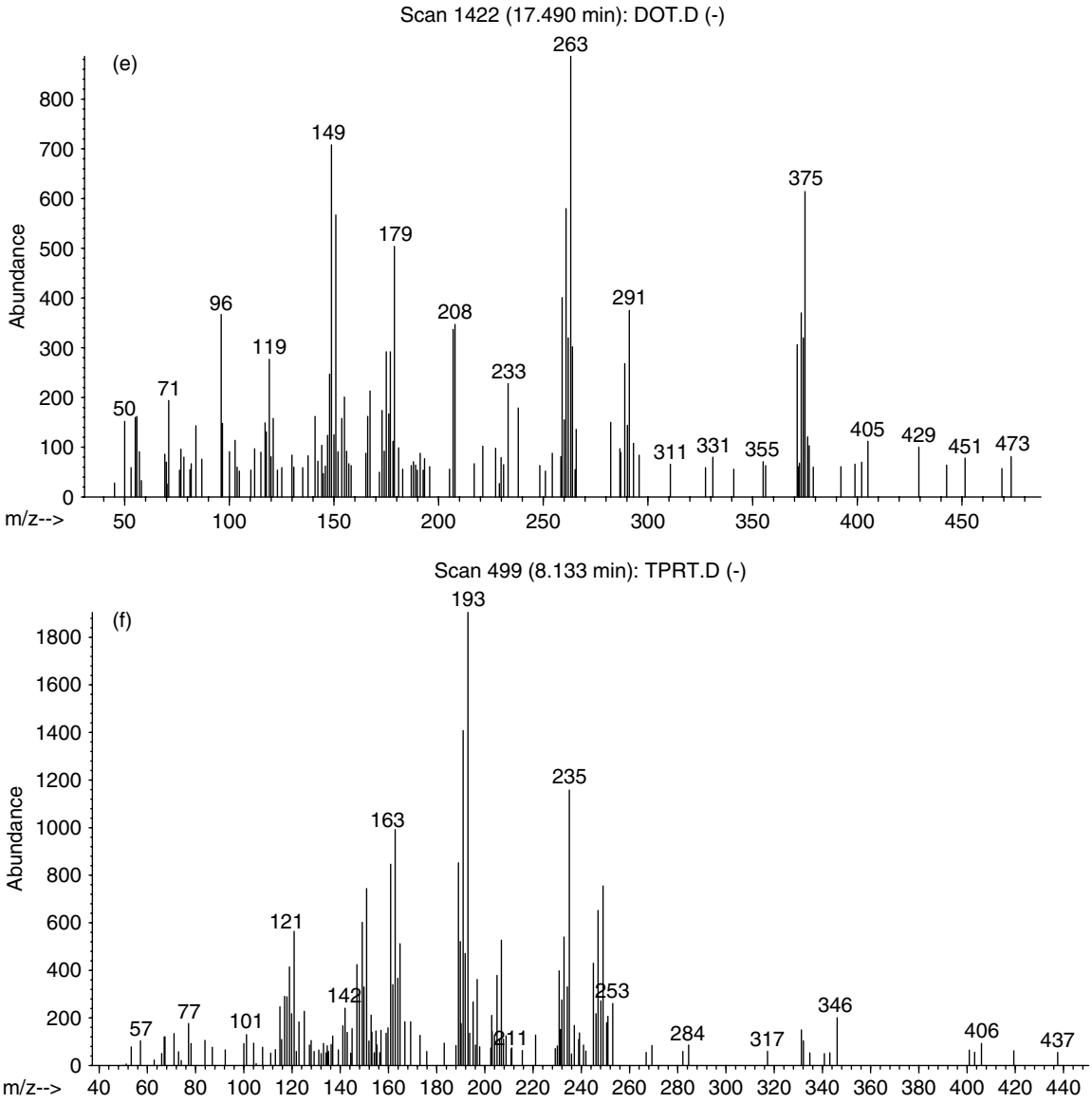

Figure 1. (*Continued*).

of the analytes and internal standards and on the analyte peak area : internal standard peak area ratio. From the results obtained, organic solvent does not seem to be very critical. Better sensitivity was obtained with isooctane extraction for mono-substituted analytes (MBT and MOT), but the sensitivity for DOT was worse in isooctane than in hexane. Since DOT analysis is of primary importance in migration studies due to the low SML value and this work was particularly focused on this analyte, hexane was chosen as the extraction solvent. In addition, the use of tropolone improved the extraction efficiency of the high polar mono- and di-organotin compounds. It is worth noticing that the MBT and MOT peak areas were increased up to 2-fold after addition of 0.05% (w/v) of tropolone in hexane. On the contrary, the peak area for TPrT proved to be almost independent of the presence of tropolone due to its low polarity and ease of extraction. Therefore, a tropolone–hexane 0.05% (w/v) solution was chosen for further experiments.

pH optimization

The influence of the pH on the derivatization yield of DBT, DOT, TPrT and DHT is shown in Fig. 3. Ethylation reactions are generally performed with a pH above 4, which is the optimum range for reagent stability and for better efficiency for the transfer of ethyl groups from sodium tetraethylborate.[45] According to the results, the peak area of DBT is the highest in the range 4–4.8, which is in agreement with results reported in previous investigations.[24,25,45,48] The optimum pH range was found to be similar for DOT and DHT (4.5–5.5). The ethylation reaction of organotins is basically

DOI: 10.1002/aoc

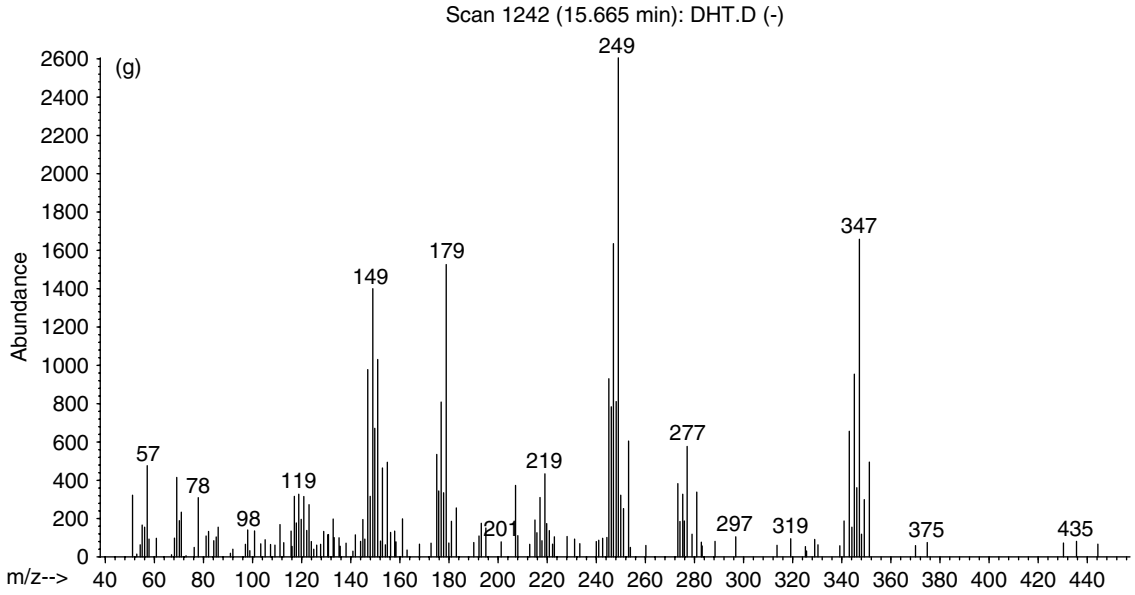

Figure 1. (*Continued*).

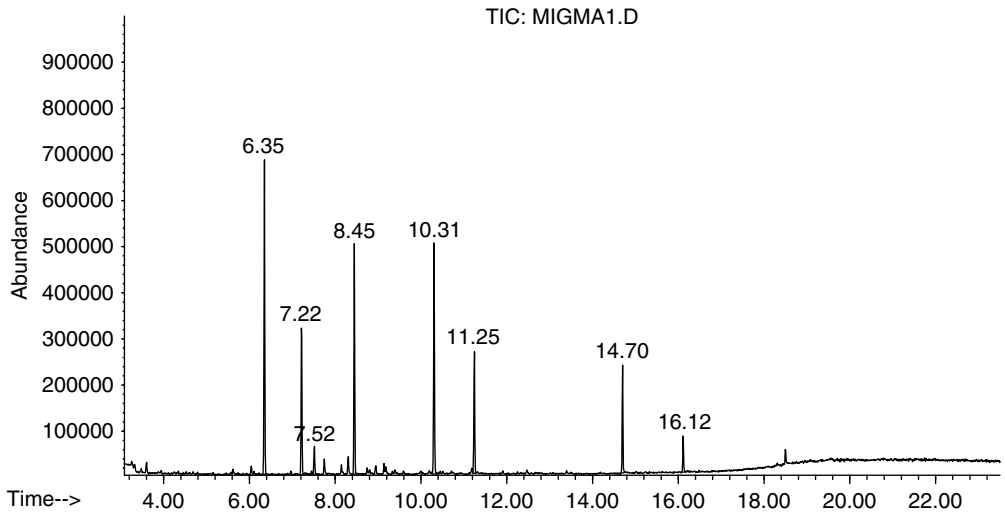

Figure 2. SIM chromatogram of a standard mixture of organotins (1.0 mg l^{-1} of each cation) after ethylation with $NaBEt_4$. Peaks t_R (min): 6.35 (MBT), 7.22 (TPrT, internal standard 0.50 mg l^{-1} as cation), 8.45 (DBT), 10.31 (TBT), 11.25 (MOT), 14.70 (DHT, internal standard 0.50 mg l^{-1} as cation) and 16.12 (DOT).

a nucleophilic type of reaction. The attractive power of organotins for nucleophilic groups may depend on their level of substitution. DHT and DBT, both disubstituted, should then have a similar capacity to attract nucleophilic chemical groups. Moreover, organotins compounds act as weak acids, thus the amount of organotin cation involved in ethylation depends on the pH value. It is also interesting to observe that TPrT gave quite stable responses (peak areas) in the tested pH range. As the pH ranged from 4 to 6, the best responses (relative peak area: *analyte peak area to IS peak area* ratio) were obtained at pH 4.8 for both DBT and DOT, which is in agreement with pH values for ethylation used by other researchers.[24,45]

Amount of $NaBEt_4$

The effect of $NaBEt_4$ amount on the derivatization of DBT, DOT, TPrT and DHT at pH 4.8 is demonstrated in Fig. 4. Various aliquots of 2% $NaBEt_4$ solution were investigated. The peak areas of both DBT and TPrT are higher for low amounts of $NaBEt_4$, whereas the derivatization of DHT and DOT required a higher amount of $NaBEt_4$. The nature of the alkyl groups could explain the need for different volumes

DOI: 10.1002/aoc

Table 4. Influence of extraction solvent and tropolone on the extraction efficiencies of ethylated organotin species

Extraction solvent	Compound	Analyte peak area	Internal standard peak area[a]	Analyte peak area : internal standard peak area
Tropolone/ hexane	MBT	303 516	65 413	4.640
	DBT	147 616	65 413	2.256
	MOT	265 767	82 316	3.228
	DOT	102 146	82 346	1.241
Hexane	MBT	139 746	59 830	2.336
	DBT	118 351	59 830	1.978
	MOT	114 403	40 705	2.810
	DOT	61 669	40 705	1.515
Isooctane	MBT	273 845	89 756	3.051
	DBT	148 187	89 756	1.651
	MOT	199 417	63 027	3.164
	DOT	40 526	63 027	0.643

[a] TPrT for butyl- and DHT for octyltin compounds.

of $NaBEt_4$ solution. As already mentioned, ethylation is a nucleophilic reaction and the degree of alkylation and/or the nature of the alkylation may influence its yield.[45,49] The highest responses (relative peak area) for DBT and DOT were obtained by adding 300 and 400 µl of 2% $NaBEt_4$, respectively. However, 200 µl was chosen, as a compromise for low background level[29,50] and protection of the column.[23]

Reaction/extraction time

The effect of the derivatization time was investigated at the optimized conditions described above. Two different systems were investigated: viz. vortex and mechanical table. Vortex was tested from 0.5 to 2 min and mechanical table for 15–45 min. Vortex was chosen in order to achieve vigorous and homogeneous mixing of organic and aqueous phase. As time in vortex varied from 0.5 to 2, no significant changes were observed in the relative signal of the analytes. With regard to different polarity and extraction efficiency of the ethylated organotins, a long reaction time may be needed. Therefore a mechanical table was used. No significant changes in the peak areas were observed for butyltins and TPrT between the shaking times tested. However for octyltins and DHT, a decrease in the signal was observed for times over 30 min. Therefore, a derivatization/extraction time of 1 min in vortex and 15 min in mechanical shaker was chosen.

Method performance

All calibration graphs were linear with a correlation coefficient (r) higher than 0.993. Table 5 presents the linear ranges, the slopes (b) and the correlation coefficients (r) for all analytes in the two different simulants. TBT was also included in the validation experiments.

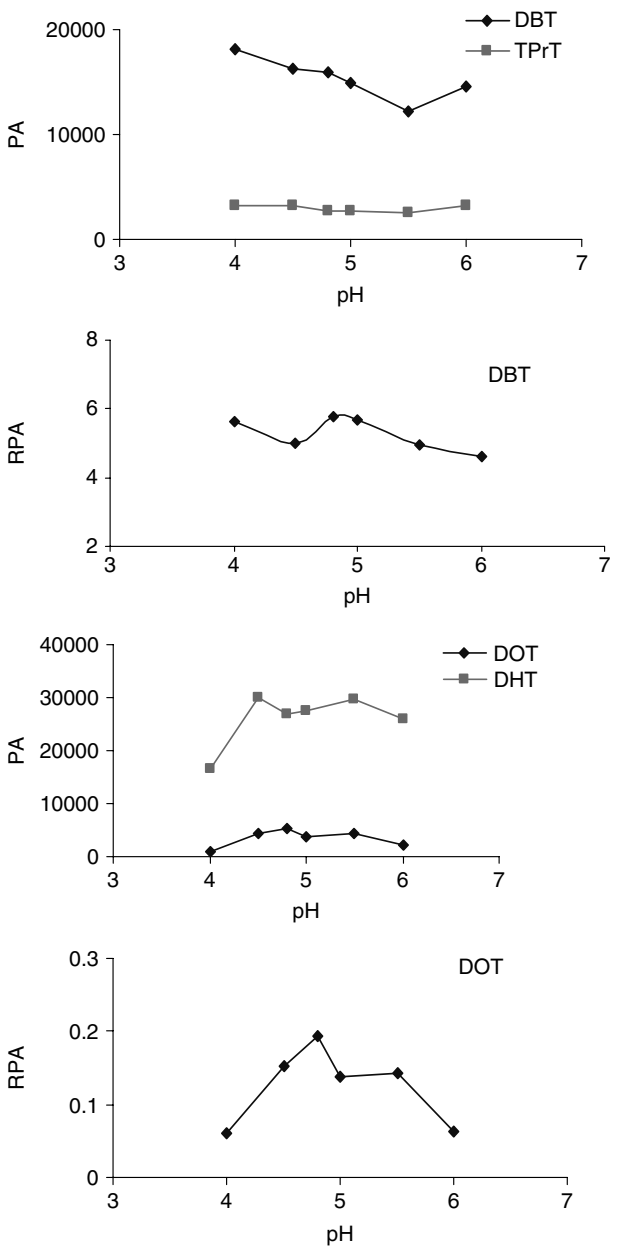

Figure 3. Influence of pH on the peak area (PA) and the relative peak area (RPA) of DBT and DOT. TPrT and DHT were used as internal standards, respectively.

As can be observed, higher sensitivity (higher slope) was obtained with the less substituted organotin compounds in both simulants. This may be related to the degree of substitution and the masses selected for the ion monitoring. The latter plays an important role on the response of the analyte. The analytical signal is obtained by measuring the peak area of these ions. In general, similar sensitivity was obtained in both simulants for the same compound.

The method LOD and LOQ for each of the five organotins in both simulants are listed in Table 6. Blank values were taken into account. LOD for DOT was 3.5 and 2.4 µg l^{-1} in water and

DOI: 10.1002/aoc

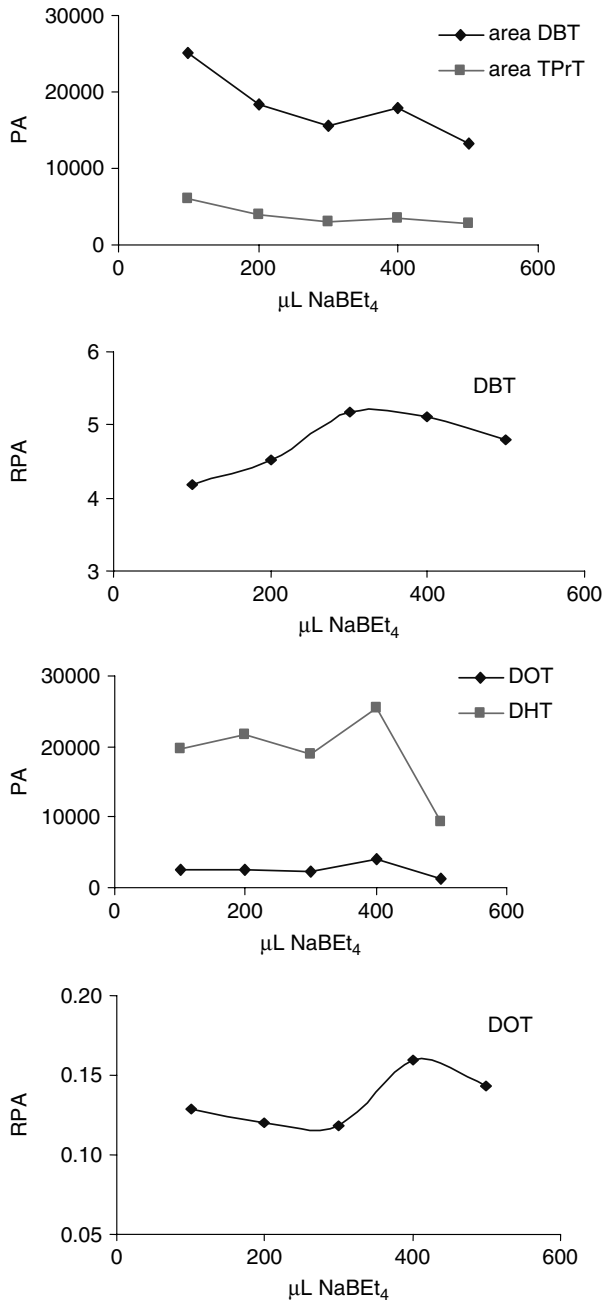

Figure 4. Influence of the volume of derivatizing reagent (2% $NaBEt_4$) on the peak area (PA) and the relative peak area (RPA) of DBT and DOT.

3% acetic acid, respectively. These values are lower than the established migration limit (12 μg l^{-1} as cation), indicating that the developed method is fit for the determination of migration of DOT. Low LODs were also obtained for DBT (1.9 and 5.8 μg l^{-1}, in water and acetic acid media, respectively).

The precision is given by the relative standard deviation, RSD (%), of the found concentrations. Satisfactory RSD (%) values were obtained under both repeatability (RSDs <13%) and reproducibility (RSDs <16%) conditions. Migration methods are empirical methods with a 100% recovery of the analytes from the tested packaging material. Trueness was estimated as the relative error (%E) of the analyte concentration obtained from the calibration curves. Although no reference material was available for such analysis, the results obtained are good evidence that the method presents acceptable accuracy.[51] The results are summarized in Table 7.

To check the stability of the GC-MS system, measurements ($n = 20$) of organotin standard solutions with different concentration levels were performed in both simulants within a long time of period (3 months). The RSD (%) values of the relative retention times (analyte t_R/IS t_R) ranged from 0.06% (DBT) to 0.38% (TBT). The good reproducibility allows the use of relative retention time as an additional confirmation tool of the presence of organotins in samples, apart from the selected ions and their relative abundances in the spectra.

Table 5. Slopes and correlation coefficients of calibration curves in aqueous food simulants

		Slope (b)		Correlation coefficient (r)	
Range (mg l^{-1})	Compound	Water	3% acetic acid	Water	3% acetic acid
0.10–0.80	MBT	6.004	4.774	0.9972	0.9992
	DBT	2.628	2.648	0.9998	0.9981
	TBT	1.236	1.363	0.9985	0.9957
	MOT	3.317	3.752	0.9982	0.9974
	DOT	1.724	2.099	0.9995	0.9988
1.0–5.0	MBT	6.256	5.799	0.9999	0.9999
	DBT	2.367	2.822	0.9985	0.9998
	TBT	1.479	2.499	0.9985	0.9995
	MOT	3.147	4.129	0.9979	0.9984
	DOT	1.627	1.778	0.9976	0.9930

Table 6. Limits of detection (LOD) and quantitation (LOQ) for the determination of organotin compounds in water and 3% acetic acid simulants

	LOD (μg l^{-1})		LOQ (μg l^{-1})	
Compound	Water	3% acetic acid	Water	3% acetic acid
MBT	6.6	4.0	20	11
DBT	1.9	5.8	5.8	18
TBT	5.1	8.8	16	27
MOT	6.4	6.2	19	19
DOT	3.5	2.4	11	7.3

Calibration with the mixed standard solutions of organotins

The calibration curves obtained for both DBT and DOT in their mixed standard solutions were linear with similar

DOI: 10.1002/aoc

Table 7. Precision and trueness for the in situ ethylation of organotins in aqueous food simulants

Concentration level (mg l^{-1})	Compound	Repeatability, RSD (%), $n = 6$		Reproducibility, RSD (%), $n = 6$		Trueness, E (%)	
		Water	3% acetic acid	Water	3% acetic acid	Water	3% acetic acid
0.10	MBT	13	6.8	—	—	−33	2.0
	DBT	6.1	4.5	—	—	−20	−8.7
	TBT	13	9.2	—	—	−18	−15
	MOT	6.8	3.7	—	—	−15	21
	DOT	2.9	6.0	—	—	6	15
1.0	MBT	2.8	10	7.6	6.8	−19	−8.3
	DBT	5.3	4.4	5.1	6.2	−19	−1.1
	TBT	3.9	2.4	11	7.0	20	4.9
	MOT	8.7	5.5	11	12	−21	−9.0
	DOT	10	2.9	16	5.6	−6.4	12

slopes and correlation coefficients (r) greater than 0.99. These curves were compared with correlation curves obtained from standard solutions of individual compounds. The comparison was accomplished by constructing a correlation curve in which the analytical parameters (relative peak areas: analyte peak area : internal standard peak area ratio) obtained from the mixed standard solutions at the five concentration levels (0.10–0.80 mg l^{-1}) were plotted on the y-axis and the analytical parameters obtained from the standard solutions of individual compounds at the five corresponding concentrations were plotted on the x-axis.

Table 8 contains all statistical data obtained for the correlation curves of DBT and DOT after linear regression analysis. The two calibration curves (the one obtained from the standard mixed organotin solution and the other from the individual organotin solutions) proved to be statistically equal at a confidence level of 95%, as the confidence interval of the slope and the intercept contains the values 1 and 0, respectively. Therefore calibration could be performed with mixed standard solutions.

Stability

Figure 5 and 6 show the influence of the migration conditions on the stability of organotins compounds in 3% acetic acid and water, respectively. All organotin compounds were shown to be stable for both simulants, except MOT in acetic acid media and DOT in water stimulant after the 8th day. The observed variation (as %RSD) for all the OTs was within the reproducibility of the method (Table 7), ranging between 5% (DBT in water) and 16% (MBT in water), except for MOT in acetic acid (17%) and DOT in water (25%). These observations support the stability of organotins under the conditions of the migration test. Moreover, the acidic conditions do not seem to have any significant influence on the stability of these compounds. These results are in agreement with reports in literature concerning the stability of the Sn–C bond.[1] Therefore, the migration test can be performed without any significant loss of the analytes.

Table 8. Statistical data of the correlation curves of the analytical parameters for DBT and DOT obtained from mixed standard solutions and individual standard solutions.

	DBT	DOT
Slope (b)	0.9162	1.0272
Standard deviation of slope (S_b)	0.0311	0.0244
Intercept (a)	−0.0571	0.0083
Standard deviation of intercept (S_a)	0.0386	0.0149
Correlation coefficient (r)	0.9977	0.9966
Number of experimental pairs	4	8
t_{theor} (confidence level 95%)	4.303	2.447
Confidence interval of b	From 0.7825 to 1.0499	From 0.9674 to 1.0871
Confidence interval of a	From −0.2231 to 0.1089	From −0.0282 to 0.0448

Application of the method to real samples

The optimised method described here was applied to six PVC samples: three cling films, two containers and a water pipe. The results are summarized in Table 9. The identity of the target compounds was confirmed by the relative retention time and the relative abundances of the selected ions. It is not surprising that, for the same sample, higher concentrations were obtained in 3% acetic acid than in water, indicating better extraction of organotins in acidic medium than in water. MBT, DBT and MOT were detected in five out of the six samples. It is interesting to note that DOT was not detected in any sample. MOT was detected in three out of the six samples, although at levels well below the established SML value (which is quite high in comparison with the DOT

DOI: 10.1002/aoc

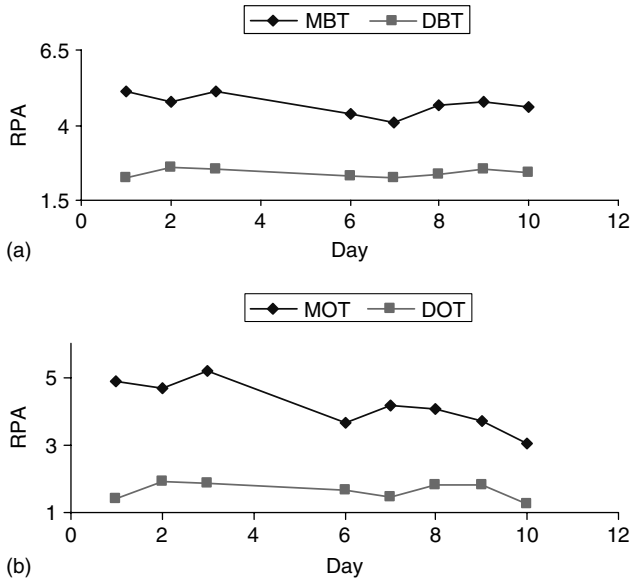

Figure 5. Stability of the organotins in the 3% acetic acid simulant as a function of the migration testing time at 40 °C (RPA: relative peak area).

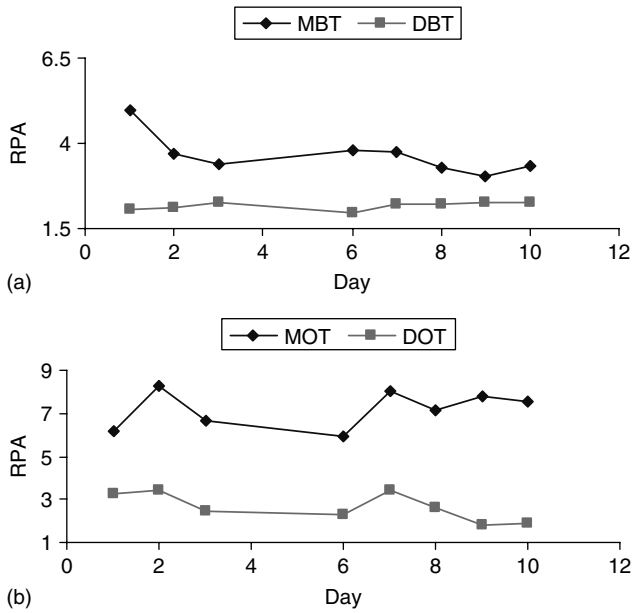

Figure 6. Stability of the organotins in the water simulant as a function of the migration testing time at 40 °C (RPA: relative peak area).

SML). Butyltins were also detected in three out of six samples. In particular, in two samples, relatively high concentrations of DBT and MBT were found. However, SMLs for these compounds have not been established yet. DBT and MBT compounds were characterized as substances for which no or only scanty data was available and are included in the 'waiting list'.[22] This list includes substances not yet included in the Community lists, as they should be considered 'new' substances, i.e. substances never approved at national level.[22] These substances are included in the Community lists, lacking the data requested by the Committee. The results of this study, even if few, indicate that further studies should be carried out.

Table 9. Organotin migration levels (in μg l^{-1}) from PVC food packaging items

	Concentration of the organotin compound in food simulant (μg l^{-1})	
	Water simulant	3% acetic acid simulant
Cling film A	<LOD[a]	<LOD
Cling film B	<LOD	11.5 (MOT)
Cling film C	<LOD	6.83 (DBT)
Container A	<LOD	110 (DBT)
		14.3 (MOT)
Container B	10.6 (MOT)	73.0 (MBT)
		18.6 (MOT)
Water pipe	<LOD	<LOD

[a] <LOD, concentrations were below the LOD values reported in Table 6 for all the compounds.

CONCLUSIONS

A GC-MS method for the migration of organotins, in two aqueous food simulants was developed. The results obtained indicate that the ethylation conditions have to be carefully optimized. It was demonstrated that the presence of tropolone improves the efficiency of organotin extraction. Better derivatization efficiencies were obtained at pH 4.8 by adding 200 μl of a 2% (w/v) sodium tetraethylborate solution. The method was validated and satisfactory figures of merit were obtained in both water and 3% acetic acid simulating solvents (official food simulants). The mass spectra of all the analytes and the two internal standards were obtained and compared with literature. These compounds presented no significant losses under the migration conditions according to stability experiments. Finally, the method was applied to PVC food packaging materials for butyl- and octyltin migration. Organotins were found at low but detectable levels in most of the samples. The proposed, confirmatory method is a rapid and simple one, suitable for the simultaneous determination of butyl- and octyltin compounds in both aqueous food simulants.

Acknowledgements

The authors thank the General Chemistry State Laboratory of Athens for financial support of this work.

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DOI: 10.1002/aoc