Improving the Analysis of Organotin Compounds Using Retention Time Locked Methods and Retention Time Databases

Application

Environmental

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Abstract

The analysis of organotin compounds is becoming increasingly important in both environmental analysis and in food and consumer product analysis. This application note describes a retention time locked (RTL) gas chromatography/mass spectrometry (GC/MS) method for the analysis of derivatized organotin compounds. Three retention time locked libraries are made available, corresponding to three different derivatization methods. The retention time databases allow easy peak location and identification of the target solutes based on mass spectra and retention times.

Introduction

For many years, organometal speciation has been an important topic in environmental analysis, primarily due to increasing awareness of the toxicological effects of many organometal compounds. Within the class of organometalics, organotin compounds are probably the most widely spread in the environment due to their use as additives in polymers and in antifouling paints. Organotin compounds degrade in the environment into more polar metabolites [1]. Tributyltin, one of the most frequently used organotin additives (as tributyltinchloride or tributyltinoxide), for instance, degrades into dibutyltin and monobutyltin species. Consequently, a large diversity of organotin compounds can be detected in various environmental samples [2]. More recently, organotin contamination of diapers and printed T-shirts was reported and numerous analyses were performed on different consumer products, including all types of absorbent hygiene products.

Different methods were used for the extraction and analysis of organotin compounds in environmental, food, and consumer product matrices. Since the organotin compounds with less than four alkyl groups are very polar, they cannot be analyzed directly by GC and must be derivatized into tetraalkyltin compounds prior to analysis. Initially, most methods were based on extraction with tropolone (a complexing agent) and n-hexane, followed by Grignard derivatization and determination with GC-flame photometric detection (FPD) [3–9]. Recently, in-situ ethylation with sodium tetraethylborate ($NaBEt_4$) [10–13] has largely replaced Grignard derivatization. At the same time, mass selective detectors (MSD) and atomic emission detectors (AED) have replaced the FPD as the preferred GC detector for organotin compounds [11,13].

A few years ago, solid phase micro extraction (SPME) in combination with capillary gas chromatography-inductively coupled plasma mass spectrometry (CGC-ICP-MS) was used for the determination of volatile and semi-volatile organometal compounds, resulting in excellent sensitivity and selectivity [14,15]. SPME was performed in the headspace or directly in the aqueous sample using a 100 mm polydimethylsiloxane (PDMS) coated fiber. Using $NaBEt_4$, organotin compounds could be derivatized in-situ and simultaneously extracted into the PDMS phase.

More recently, stir bar sorptive extraction (SBSE) using a magnetic stir bar coated with a 0.5–1 mm PDMS layer was developed [16]. After extraction, the solutes were thermally desorbed online to GC/MS, GC-AED or GC-ICP-MS. SBSE in combination with CGC-ICP-MS was applied for the determination of organotins in environmental samples after in-situ derivatization with $NaBEt_4$, resulting in unsurpassed sensitivity with detection limits reaching the ppq (pg/L) level [17].

For standard applications such as the determination of organotin compounds in sediments, or soils, and in extracts or leachates of consumer products, these extremely high sensitivities are not required. For these applications, sufficient sensitivity is obtained using mass spectrometric detection. In comparison to AED or ICP-MS, where specific tin-chromatograms are obtained, the chromatograms obtained by mass spectroscopy are far more complex, even when using the selected ion monitoring (SIM) mode. Several ions per solute need to be monitored, and the derivatized sample extracts often contain many co-extracted solutes or by-products of the derivatization reaction. Therefore, data interpretation is more demanding requiring the use of extracted ion chromatograms, retention time matching, and calculation of the relative abundances of target and qualifier ions. In this respect, the use of retention time locked methods offers several advantages. If a selected ion method is used, the switching times between groups of monitored ions are fixed and do not need to be adjusted after column maintenance or column change, since the retention times of all solutes can be relocked. Moreover, quantification databases do not need to be updated for variations in retention times. Finally, a retention time locked database can be used, allowing easy peak allocation. Solute detection and confirmation are far more reliable using the results screener option [18,19], which combines the power of spectral matching with locked retention time matching.

In this application note, a GC/MS method is described for the analysis of organotin compounds in environmental, food, or consumer product extracts. Since derivatization by Grignard reaction and derivatization using $NaBEt_4$ are both easy and convenient, three types of derivatives are considered: methyl-derivatives using methylmagnesium bromide, pentyl- derivatives using pentylmagnesium bromide (both Grignard reagents), and ethyl-derivatives using $NaBEt_4$. The most important organotin compounds are listed in Table 1 together with typical ions for the mass spectra of all three derivatives. Tin has several isotopes and the mass spectra are characterized by typical isotope clusters. The relative abundances of the tin isotopes are Sn-116 (14.24%), Sn-117 (7.57%), Sn-118 (24.01%), Sn-119 (8.59%), Sn-120 (32.97%), Sn-122 (4.71%), and Sn-124 (5.98%). For the organotin compounds listed in Table 1, mass spectral libraries and retention-time-locked screener libraries were created for all three types of derivatives. After selecting the appropriate derivitization method, a library and screener database can be selected, allowing fast data interpretation. Sample extraction and clean-up are beyond the scope of this application note.

Experimental

Samples

The organotin compounds listed in Table 1 were purchased from Dr Ehrenstorfer, Augsburg, Germany (http://www.analytical-standards.com). For analysis, the standards were dissolved in methanol at a 1000 ppm (1mg/mL) concentration. These solutions were further diluted, depending on the derivatization method used. For creation of the databases, approximately 10 µg of compound was derivatized, resulting in a final concentration of 10 ppm.

Derivatization method 1: The sample extract is concentrated to 1 mL in an apolar solvent (typically hexane) in a reaction tube. To this solution, 0.5 mL methylmagnesiumbromide Grignard reagent (1.4 M in 75/25 toluene/THF, Sigma-Aldrich cat no 28,223-5) is added. The solution is vortexed for 10 s and allowed to stand at room temperature for 15 min. This procedure should be performed in a fume hood, since toxic vapors evolving from the reaction and the solvents are flammable. The reaction is stopped and the excess reagent is removed by adding 2 mL of a saturated ammoniumchloride solution in water or 2 mL 0.25 mol/L aqueous sulphuric acid. The mixture is vortexed for 10 s and the two phases are allowed to separate. The clear upper layer (apolar hexane phase) is transferred to an autosampler vial for analysis. The resulting organotin compounds are the methyl-derivatives.

Derivatization method 2: The sample extract is concentrated to 1 mL in an apolar solvent (typically hexane) in a reaction tube. To this solution, 0.5 mL pentylmagnesiumbromide Grignard reagent (2 M in diethylether, Sigma-Aldrich cat no 29,099-8) is added. The remaining steps in this procedure are identical to those used in derivitization method 1. The resulting organotin compounds are the pentyl-derivatives.

Derivatization method 3: The sample extract is concentrated to 1 mL in a polar solvent (typically ethanol) in a reaction tube. To this solution, 1 mL acetate buffer (82 g/L sodium acetate in water, adjusted to pH 4.5 with acetic acid) and 50 µL

Table 1: Organotin Compounds and Characteristic Ions for the Three Derivatization Products

Organotin solute Reagent Derivatives	Abbreviation	Derivatization 1 Methyl- magnesium bromide Methyl-	Derivatization 2 Pentyl- magnesium bromide Pentyl-	Derivatization 1 Sodium tetraethylborate Ethyl-
Triethyltin	TET	193, 191, 165, 163	179, 177, 249, 247	207, 205, 179, 177
Tetraethyltin	TeET	207, 205, 179, 177	207, 205, 179, 177	207, 205, 179, 177
Tripropyltin	TPT	179, 177, 221, 219	277, 275, 165, 163	235, 2331, 249, 247
Tetrapropyltin	TePT	249, 247, 207, 205	249, 247, 207, 205	249, 247, 207, 205
Monobutyltin	MBT	165, 163, 151, 149	319, 317, 193, 191	235, 233, 179, 177
Dibutyltin	DBT	151, 149, 207, 205	319, 317, 179, 177	263, 261, 207, 205
Tributyltin	TBT	193, 191, 249, 247	305, 303, 179, 177	291, 289, 207, 205
Tetrabutyltin	TeBT	291, 289, 179, 177	291, 289, 179, 177	291, 289, 179, 177
Monophenyltin	MPhT	227, 225, 223, 197	339, 337, 197, 195	255, 253, 197, 195
Diphenyltin	DPhT	289, 287, 285, 197	345, 343, 197, 195	303, 301, 197, 195
Triphenyltin	TPhT	351, 349, 347, 197	351, 349, 347, 197	351, 349, 347, 197
Tetraphenyltin	TePhT	351, 349, 347, 197	351, 349, 347, 197	351, 349, 347, 197
Tricyclohexyltin (Cyhexatin)	TCT	301, 299, 219, 217	357, 355, 205, 203	315, 313, 233, 231
Monooctyltin	MOT	165, 163, 263, 261	375, 373, 193, 191	291, 289, 179, 177
Dioctyltin	DOT	263, 261, 151, 149	417, 415, 375, 373	375, 373, 263, 261

derivatization reagent are added. The derivatization reagent is prepared by dissolving 2 g $NaBEt_4$ (Sigma-Aldrich cat no 48,148-3) in 10 mL ethanol. This solution should be freshly prepared. The sample is shaken and allowed to react for 30 min. After addition of 5 mL water, the derivatized compounds are extracted in 1 mL hexane. The mixture is vortexed for 10 s and the two phases are allowed to separate. The clear upper layer (apolar hexane phase) is transferred to an autosampler vial for analysis. The resulting organotin compounds are the ethyl-derivatives.

These derivatization methods can be adapted to the type of sample analyzed. For example, derivatization method 3 is often applied to aqueous samples directly, combining *in-situ* derivatization and simultaneous extraction. This method is also used for sediment samples. Typically 1 g sample (dry weight) is extracted with 10 mL acetate buffer, 7 mL methanol and 10 mL hexane. Four mL of a 5% $NaBEt_4$ solution is added while stirring. The derivatized organotin compounds are simultaneously extracted into the hexane layer.

Analytical Conditions

All analyses were performed on an Agilent 6890-5973N GC-MSD system. Automated splitless injection was performed using an Agilent 7683 automatic liquid sampler. The instrumental configuration and analytical conditions are summarized in Table 2. The retention time of tetrabutyltin (used as the locking standard) was locked at 16.000 min. To duplicate this method, the initial column head pressure can be set to the pressures indicated in Table 2 (nominal pressure). Then the retention time locking (RTL) calibration runs can be performed automatically (at –20%, –10%, +10% and +20% of the nominal pressure) [18]. The retention time versus head pressure curve is then calculated and stored in the method. Agilent's RTL software uses this curve to set the column head pressure so that retention time of the locking standard (tetrabutyltin) is 16.000 min.

Table 2. Instrumentation and Conditions of Analysis

Instrumentation	
Chromatographic system	Agilent 6890 GC
Inlet	Split/Splitless
Detector	Agilent 5973 N MSD
Automatic sampler	Agilent 7683
Liner	Splitless liner (part number 5062-3587)
Column	30 m × 0.25 mm id × 0.25 µm HP-5MS (Agilent part number 19091S-433)
Experimental conditions	
Inlet temperature	280 °C
Injection volume	1 µL
Injection mode	Splitless, purge time: 1 min, purge flow: 50 mL/min.
Carrier gas	Helium
Head pressure	Tetrabutyltin is retention time locked at 16.000 min (pressure around 45 kPa at 50 °C, 34 cm/s at 50 °C)
Oven temperature	50 °C, 1 min, 10 °C/min to 300 °C, 4 min.
Transfer line temperature	300 °C
Detector	Scan (40–550 amu), threshold 100, MS quad 150 °C, MS source 230 °C. Solvent delay: 4 min
	SIM mode: 50 ms dwell time per ion, ions listed in Table 3

Results and Discussion

A typical chromatogram, for an organotin standard mixture, derivatized using method 3 (ethyl-derivatives with $NaBEt_4$), is shown in Figure 1. The compounds elute according to their boiling point, and the elution sequence can be predicted by calculating the total number of carbon atoms after derivatization. With this derivatization, the elution sequence of the butyltin compounds is MBT (10 C atoms) < DBT (12 C atoms) < TBT (14 C atoms) < TeBT (16 C atoms). The spectrum obtained for tributyltin (as tributylethyltin) is shown in Figure 2. The typical ion clusters, resulting from the different tin isotopes, are clearly detected.

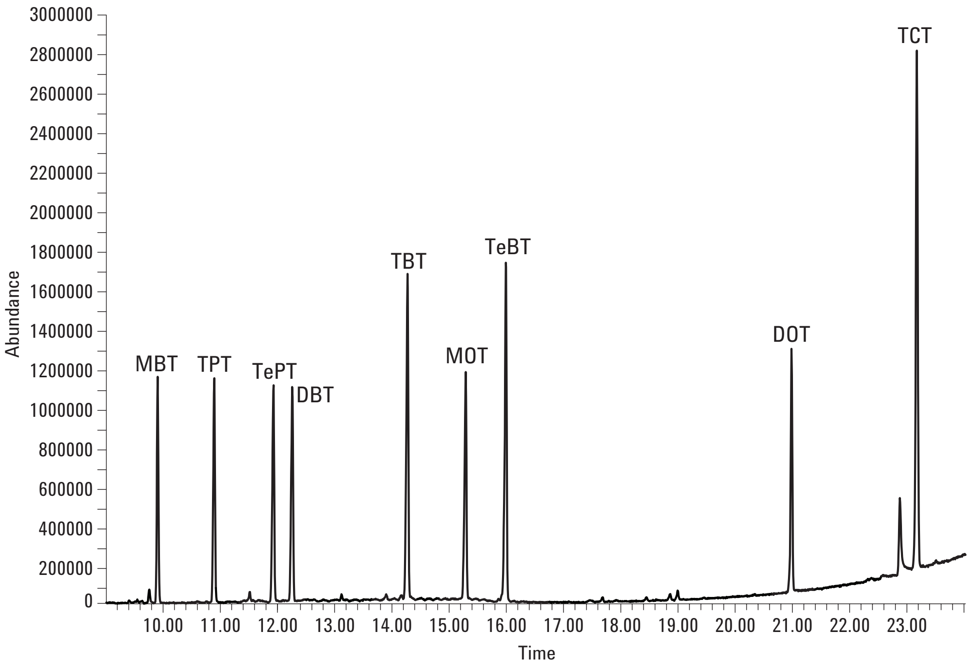


Figure 1. GC/MS chromatogram for the analysis of an organotin standard mixture after derivatization with $NaBEt_4$ (ethyl-derivatives).

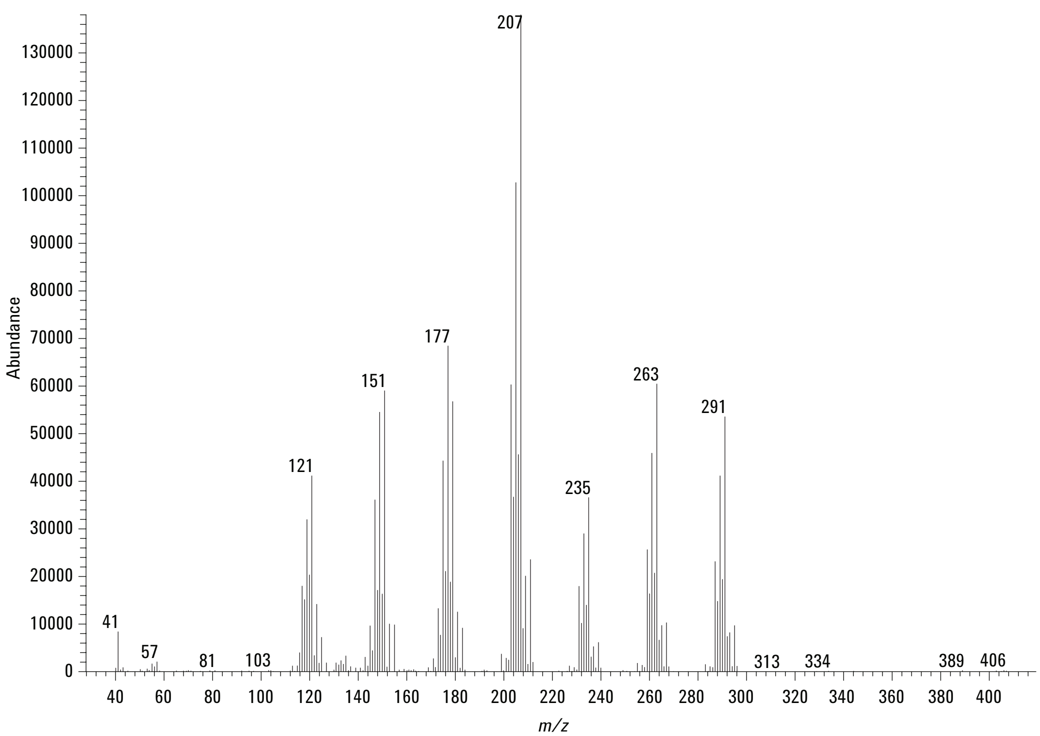


Figure 2. Mass spectrum of tributyltin after derivatization with $NaBEt_4$ (ethyl-derivative).

The analysis of a coastal sediment sample is shown in Figure 3. In this case, derivatization method 2 (Grignard reaction with pentylmagnesium bromide) was applied and a complex chromatogram was obtained. Using the extracted ion chromatogram at *m/e* 179 the butyltin compounds were easily detected (Figure 4). Tetrabutyltin, eluting at 16.000 min, was added as internal standard. In this case, pentyl- derivatives are analyzed. Therefore the elution order is reversed since the derivatization adds a C5-group for every free valency. The elution sequence is now TeBT (16 C atoms = unchanged) < TBT (17 C atoms) <DBT (18 C atoms) < MBT (19 C atoms). The mass spectrum obtained for the pentyl derivative of tributyltin is shown in Figure 5.

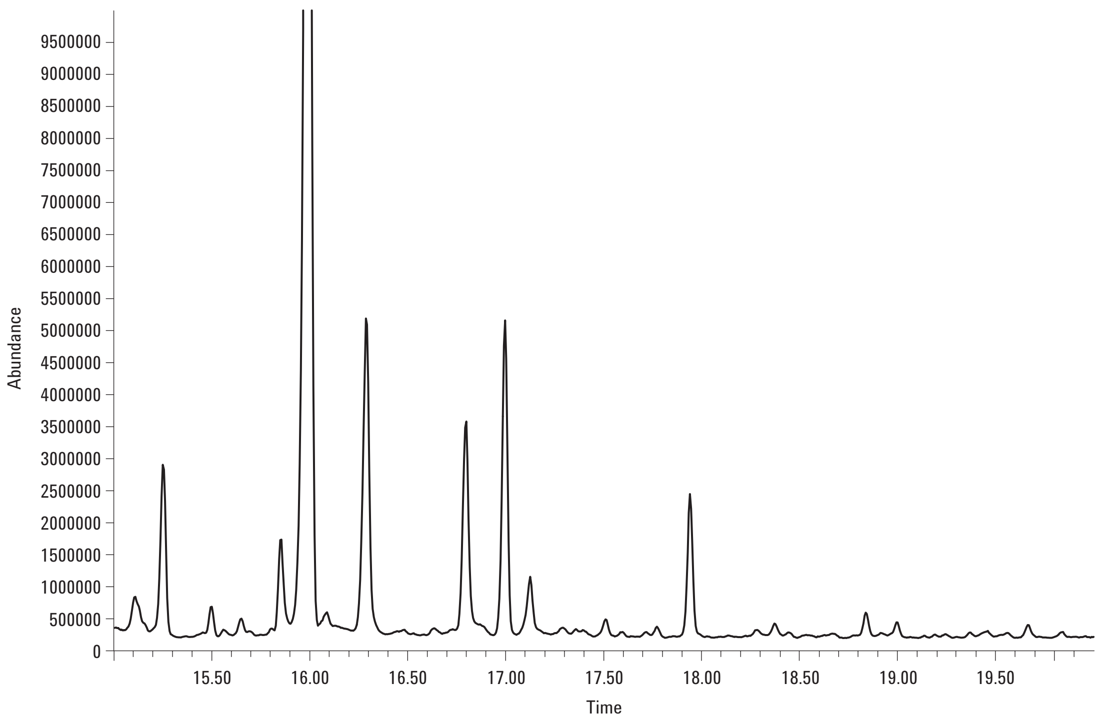


Figure 3. GC/MS chromatogram for the analysis of a coastal sediment sample after derivatization with pentylmagnesium bromide (pentyl-derivatives).

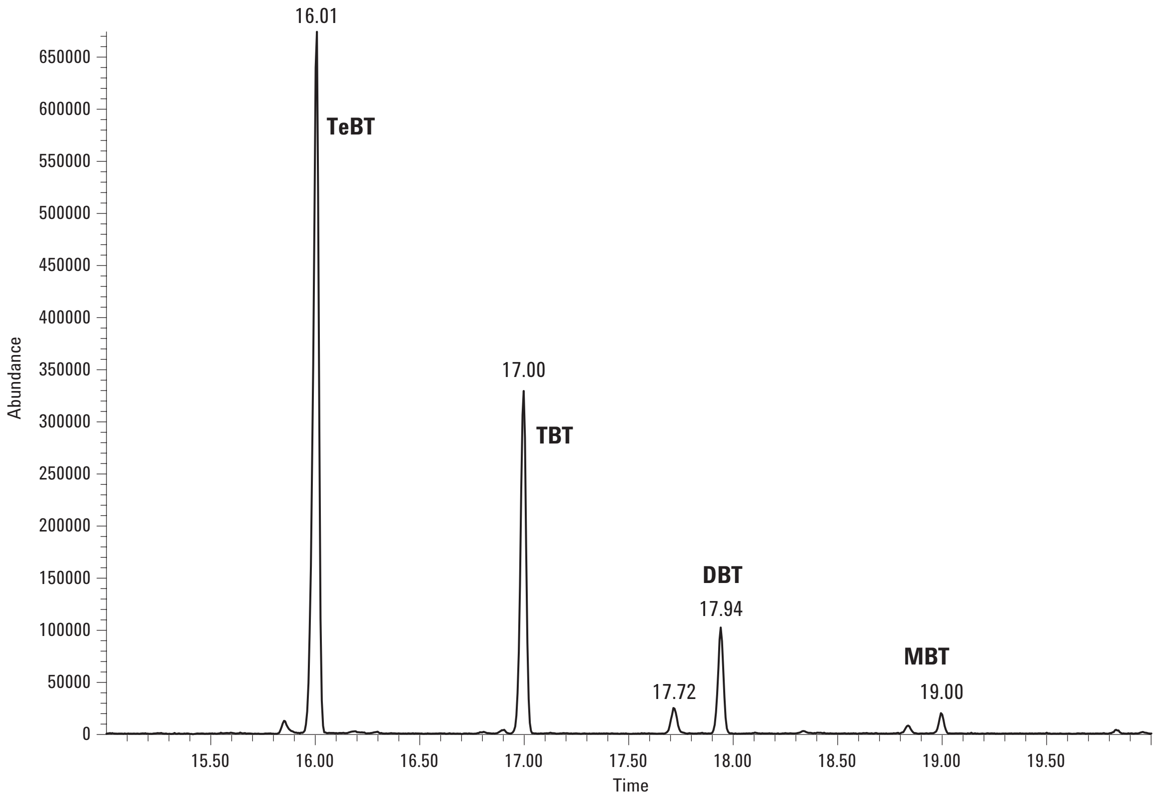


Figure 4. **Extracted ion chromatogram showing the presence of butyltin compounds in the coastal sediment sample extract(shown in Figure 3) after derivatization with pentylmagnesium bromide (pentyl-derivatives).**

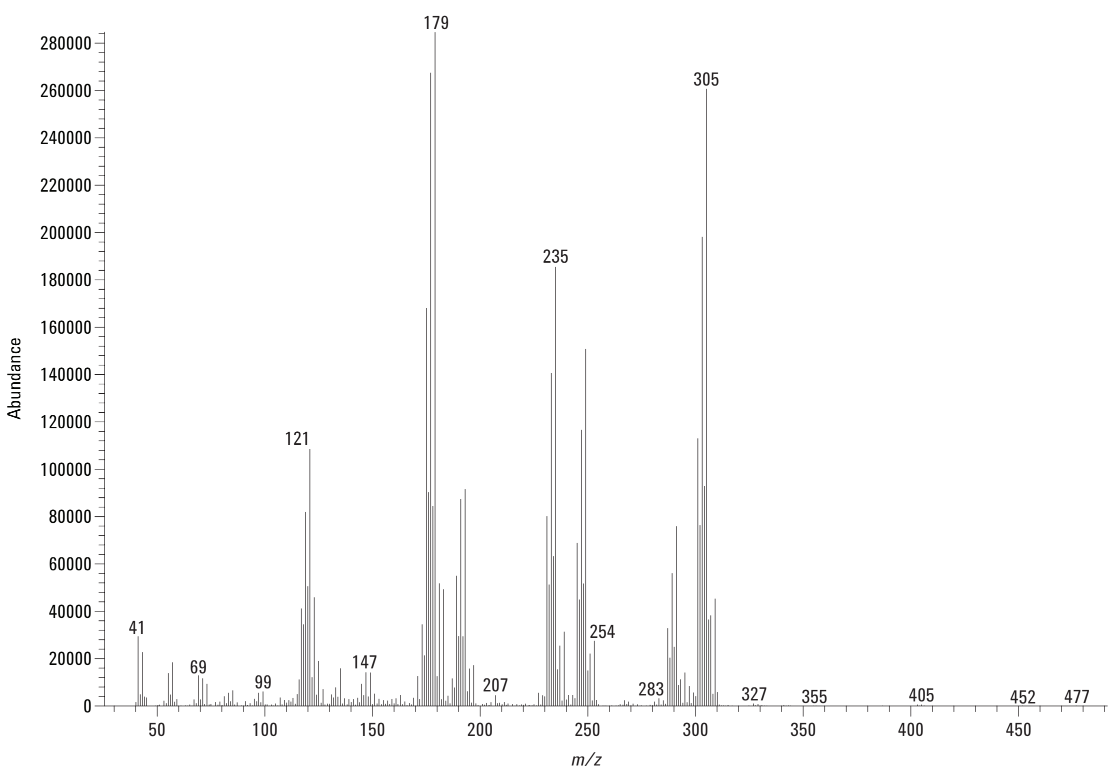
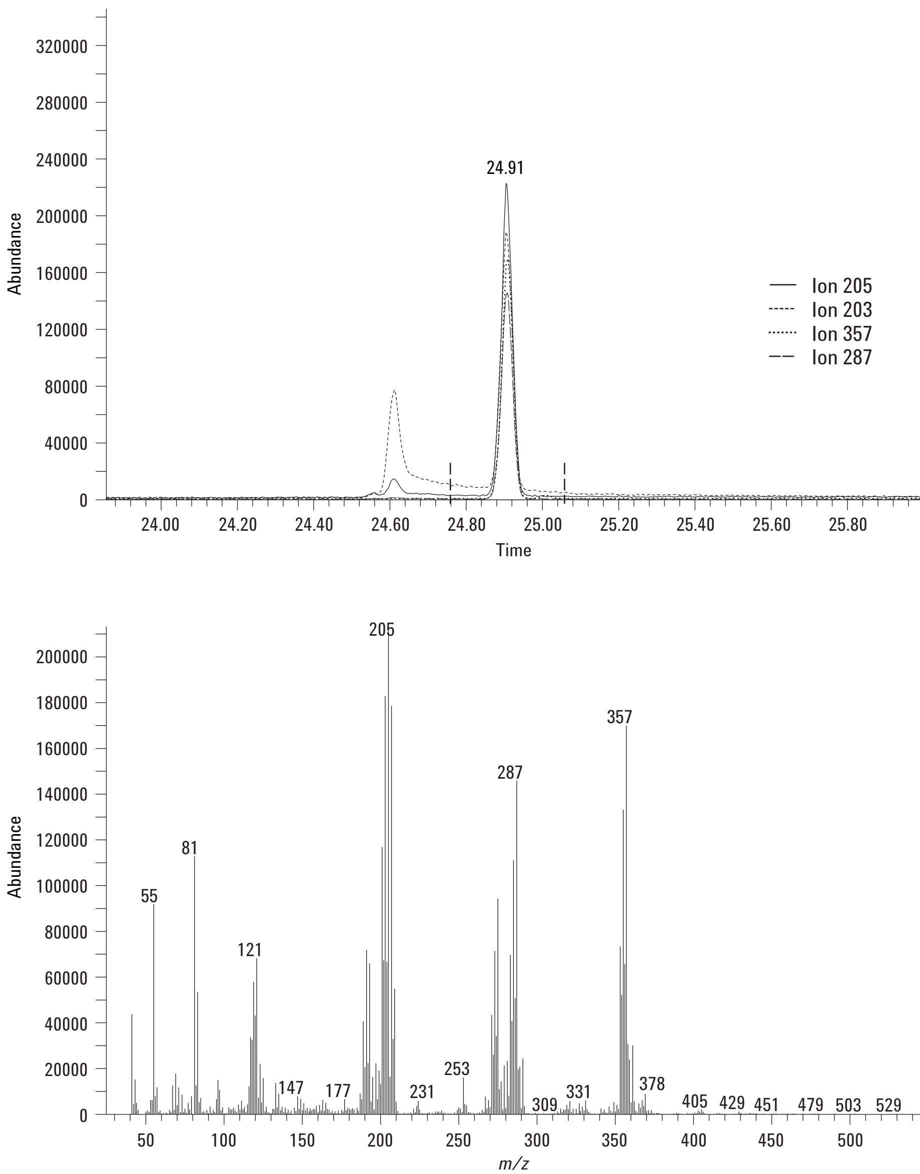


Figure 5. **Mass spectrum of tributyltin after derivatization with pentylmagnesium bromide (pentyl-derivative).**

Using the Agilent results screener and the appropriate screener library, the files can be screened for the presence of all compounds listed in the screener database. Figure 6 shows a typical result, with the identification of pentyltricyclohexyltin at 24.908 min. The target ions for this compound are extracted and overlaid in the top window. For easy comparison, the apex mass spectrum is displayed. Though not shown in Figure 6, the Agilent RTL Screener Software can display the library and apex spectra together for easy spectral comparison. In addition, the relative abundances of the target ion and qualifiers are measured and compared to the library data. What distinguishes the Agilent screener methods from conventional GC/MS techniques is the comparison of a peak's locked retention time to values stored with the RTL database. In this case, the locked retention time of pentyltributyltin is within 0.002 min (0.12 s) of the database value. The Agilent results screener compares locked retention times and spectral information for fast peak allocation and more reliable identification.



(13) Pentyltricyclohexyltin
24.908 min (-0.002)
response 4581312

Ion	Exp %	Act %
205.00	100	100
203.00	82.90	83.87
357.00	77.20	80.36
287.00	66.70	68.98

Figure 6. Screener result for the detection of tricyclohexyl tin in a sample extract after derivatization with pentylmagnesium bromide (pentyl-derivative).

For added specificity and sensitivity, SIM methods were developed for all three alkyl derivatives of the target tin compounds. Table 3 lists the SIM ions and target compounds in each group. Note that the start time for each SIM group is also listed. Normally, this timing could not be published with confidence, because of retention time differences between instruments. However, RTL allows analysts to duplicate locked methods directly and reproduce all analyte retention times within a few thousandths of a minute. Thus, it is possible to apply this method directly, including the SIM group timing, after locking tetrabutyltin to the method-specified retention time of 16.000 minutes.

Table 3. SIM Groups and Timing for Methyl, Pentyl, and Ethyl Derivatives of the Target Tin Compounds Listed in Table 1. The GC/MS Method Shown in Table 2 was used with the Retention Time of Tetrabutyltin Locked to 16.000 Minutes.

	Start time (min)	Solutes	Ions
Derivatization 1			
1	5.00	TET, MBT	193, 191, 165, 163, 151, 149
2	6.50	TeET	207, 205, 179, 177
3	8.00	MPhT, DBT, TPT	227, 225, 223, 151, 149, 207, 205, 179, 177, 221, 219
4	10.50	MOT, TePT	165, 163, 263, 261, 249, 247, 207, 205
5	12.50	TBT, TeBT	193, 191, 249, 247, 291, 289, 179, 177
6	16.40	DPhT, DOT	289, 287, 285, 197, 263, 261, 151, 149
7	21.00	TCT, TPhT	301, 299, 219, 217, 351, 349, 347, 197
8	25.00	TePhT	351, 349, 347, 197
Derivatization 2			
1	5.00	TeET	207, 205, 179, 177
2	9.00	TET, TePT	179, 177, 249, 247, 207, 205
3	13.50	TPT, TeBT	277, 275, 165, 163, 291, 289, 179, 177
4	16.50	TBT, DBT, MBT	305, 303, 179, 177, 319, 317, 193, 191
5	20.00	MPhT, MOT, DPhT	339, 337, 197, 195, 375, 373, 193, 191
6	22.80	DPhT, TCT	345, 343, 275, 273, 357, 355, 205, 203
7	24.00	DOT, TPhT	417, 415, 375, 373, 351, 349, 347, 197
8	26.00	TePhT	351, 349, 347, 197
Derivatization 3			
1	5.00	TeET (=TET)	207, 205, 179, 177
2	8.50	MBT, TPT	235, 233, 179, 177, 249, 247
3	11.40	TePT, DBT	249, 247, 207, 205, 263, 261, 207, 205
4	13.00	MPhT, TBT	255, 253, 197, 195, 291, 289
5	14.80	MOT, TeBT	291, 289, 179, 177, 291, 289
6	17.00	DPhT, DOT	303, 301, 197, 195, 375, 373, 263, 261
7	22.00	TPhT, TCT	351, 349, 347, 197, 315, 313, 233, 231
8	25.00	TePhT	351, 349, 347, 197

Conclusion

A GC/MS method is presented for the analysis of organotin compounds in extracts of environmental, food, or consumer product samples. Three different derivatization methods are described. For each derivatization method, mass spectral and retention time-locked screener databases were created. By itself, RTL is a valuable tool for maintaining GC and GC/MS methods and for comparing results among different laboratories. It also allows analysts to duplicate methods exactly, including SIM group timing and peak timing in quantitative methods.

When combining RTL with locked mass spectral database searching, peak identifications become far more convenient and reliable. While many compounds can have similar spectra, they usually do not have similar spectra and identical retention times. Agilent's ability to reproduce retention times for a given method on any 6890 GC makes it possible to differentiate closely-related compounds and to screen for large numbers of analytes in a matter of seconds. This rapid GC/MS screening technique is now available for a wide variety of important tin compounds.

The three organotin databases are available for free from the Life Sciences and Chemical Analysis portion of the Agilent web site (www.agilent.com).

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