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Determination of bis(tributyltin) oxide by GC–MS with on-line hydride derivatization: application to drug substance analysis

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Abstract

We report the determination of residual bis(tributyltin) oxide in a drug substance by GC–MS after extraction and on-line conversion to tributyltin hydride. Gas chromatography was performed using a 15 m × 0.25 mm i.d. DB-5 HT column with a temperature program from 100 to 160°C at 15°C min^{-1}. A mass range of 165–185 amu was monitored with the MS detector. Hydride generation is performed by placing a small amount of solid sodium borohydride in the injection port of a gas chromatograph and injecting samples and standards through this material. Conversion to tributyltin hydride is shown to be quantitative and linear for levels of bis(tributyltin) oxide between 1 and 100 ppm in the drug substance. The use of GC–MS provides sensitive and selective detection of tin containing species and the tin isotope pattern allows for confirmation of the presence of tin in chromatographic peaks. Recovery at 6 ppm was 89% with an injection precision of 6%. The limit of detection for bis(tributyltin) oxide in drug substance is 1 ppm.

Keywords: Determination of bis(tributyltin) oxide; Tributyltin hydride; Tributyltin chloride; On-line hydride derivatization; GC–MS determination of tributyltin; Selective-ion monitoring

1. Introduction

Alkyltin compounds are utilized in a wide variety of industrial applications. They have been used as pesticides, anti-foulants in boat paint, stabilizers for polyvinyl-chloride, wood preservatives [1] and reagents for organic synthesis [2–4]. Alkyltins are toxic and their use in these products has led to environmental concerns since these compounds have been shown to leach into sea and river water resulting in their accumulation in aquatic species. The presence of toxic alkyltins in water poses a threat to marine life and human health [5]. As a result, several methods have been developed for trace analysis of alkyltins in sea water and aquatic life. Bis(tributyltin) oxide, tributyltin chloride, as well as other mono-, di-, and tributyltin compounds are the predominant alkyltin compounds found.

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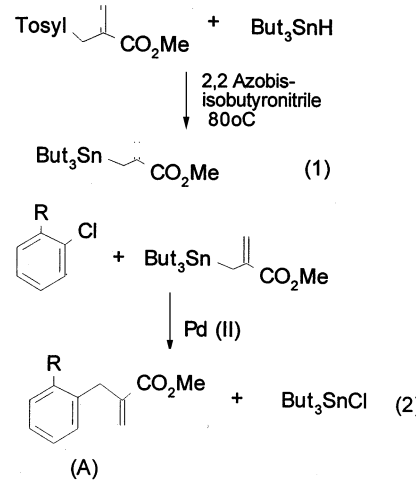
Gas chromatography (GC) and high performance liquid chromatography (HPLC) using specialized detection techniques have been most successful in the quantitation of alkyltin species. HPLC has been used with atomic absorption detection [6,7], ICP–MS detection [8] and fluorescence detection after derivatization with a fluorogenic reagent [9]. GC has been used with flame ionization quenching [10,11] to quantitate bis(tributyltin) oxide and triphenyltin hydroxide in boat paint and apple leaves. Sub-ppm levels of di- and tributyltins have been determined in fish by GC with flame photometric detection [12], electron capture detection [13], and atomic absorbance detection [14].

Unfortunately, alkyltin halides and oxides undergo undesirable side reactions with the silanol groups of the injector port liner and column of the GC [15]. Aue et al. doped the GC carrier gas with HCl to suppress this side reaction. Others have converted the alkyltin halides and oxides to their stable tetra-alkyltin analogs with Grignard reagents prior to separation by GC and detection by mass spectrometry [16,17] or flame ionization quenching [10].

The conversion of alkyltin halides and oxides to their volatile hydrides by reduction with sodium borohydride [18] and their quantitation using GC is the most common technique for alkyltin quantitation. The alkyltin hydride can be formed prior to injection by treating the sample with a solution of sodium borohydride. Variations of this procedure have been used for analysis of mono-, di- and tributyltins in urine [5], river sediment [19] and fish tissue [20]. Alkyltin pesticides were quantitated in agricultural products using GC–AA [21] with this same technique. More conveniently, it has been shown that the alkyltin hydrides can be formed on-line by placing solid sodium borohydride in the GC liner [1] or by doping the head of the GC column with sodium borohydride [22]. The alkyltin halide is injected directly and conversion to the hydride occurs on-line prior to separation and detection.

Alkyltin compounds can also be used as reagents in the synthesis of drug substances and their intermediates. Tributyltin hydride can be used for free radical dehalogenation of alkyl, acyl, and aryl halides, the hydrostannation of olefins and alkynes as well as desulfuration, deamination, and decarboxylation reactions [2]. In addition, tributyltin hydride adds to olefins substituted with a suitable leaving group in the allyl position to give allyltins [3]. The allyltin compound can then be used in conjunction with a suitable palladium catalyst to transfer the vinyl group to an aryl halide thereby forming a substituted benzene with a new carbon–carbon bond [3,4]. A representative reaction scheme is presented below:



Utilization of this reaction scheme with (**A**) as a process intermediate requires effective rejection of the toxic tributyltin chloride before isolation of the final product. An appropriate analytical method is needed to test the final product for residual alkyltin.

We are currently in the process of analyzing drug substances synthesized using the above reaction scheme. The allylic double bond of intermediate (**A**) is hydrogenated and hydrolysis produces the corresponding carboxylic acid. Accounting for residual tributyltin species presents a challenging analytical problem.

The fate of the tributyltin chloride through the remainder of the process can be rationalized. Any residual tributyltin in intermediate (**A**) will be converted to the tributyltin hydride during the reduction step. During aqueous isolation, the tributyltin hydride will hydrolyze and dimerize to bis(tributyltin) oxide as presented below:

$$Bu_3SnCl \xrightarrow{\text{Reduction}} Bu_3SnH \xrightarrow{\text{Hydrolysis}} Bu_3SnOH \xrightarrow{\text{Dimerization}} Bu_3SnOSnBu_3 \quad (3)$$

Due to the toxic nature of bis(tributyltin) oxide, its presence at any level in the drug substance is a serious concern. It was necessary to devise an analytical method that could quantitate residual amounts of bis(tributyltin) oxide in the drug substance. Unfortunately, the use of atomic absorption (AA) spectroscopy is inadequate for the quantitation of alkyltin since AA cannot differentiate between alkyltin and atomic tin species. Thus, we have developed a method which uses liquid extraction, on-line hydride conversion and GC–MS with selected ion range monitoring to determine low levels of bis(trubutyltin) oxide in drug substance.

2. Experimental

2.1. Chemicals and reagents

Bis(tributyltin) oxide, tributyltin hydride, and tributyltin chloride were from Aldrich (Milwaukee, WI). HPLC grade hexane and methanol from Fisher (Fair Lawn, NJ) were used for solution preparations. Hydrochloric acid was A.C.S. Reagent grade from J.T. Baker (Phillisburg, NJ). Sodium borohydride was purchased from Morton Industries.

2.2. Instrumentation

A Hewlett-Packard (Avondale, PA) 5890 gas chromatograph equipped with a 5792 series mass selective detector (MSD) and a ChemStation B.02.04 was used for all analyses. A 10-μl injection was made through a 78×4 mm i.d. glass liner (Hewlett-Packard, Avondale, PA), the injector temperature was 270°C. The GC was fitted with a 15 m × 0.25 mm, 0.1 μm film thickness DB-5 HT column from J&W Scientific (Folsom, CA). The carrier gas was helium at a head pressure of 5 psi, the resulting column flow was 0.5 ml min^{-1}. The oven program was 100°C for 1 min followed by a 15°C min^{-1} temperature ramp to 160°C. The mass transfer-line temperature was 280°C. Peak areas were taken from the ion chromatograms generated with the 165–185 amu range.

2.3. Sample preparation and extraction

A 50 mg sample of drug substance was dissolved in 0.50 ml of methanol in a stoppered test tube. A 50-μl aliquot of concentrated hydrochloric acid was added and the test tube was shaken for several minutes. The solution was then extracted with five (1.5 ml each) portions of hexane. The combined extracts were evaporated to dryness and re-diluted to 0.50 ml with hexane. The samples were then injected. The sample preparation converts all bis(tributyltin) oxide to tributyltin chloride. The tributyltin chloride is then separated from the drug substance using hexane as presented below:

$$But_3SnOSnBut_3 + HCl \rightleftharpoons But_3SnCl_{(MeOH)} \rightleftharpoons But_3SnCl_{(Hexane)} \quad (4)$$

The tributyltin chloride can then be converted to the tin hydride on-line without interference from the drug substance which remains in the methanol layer.

2.4. Standard addition

Quantitation was performed by standard addition. A sample of drug substance was prepared using methanol containing 6.0×10^{-4} mg ml^{-1} bis(tributyltin) oxide (this amounts to 6 ppm of bis(tributyltin) oxide relative to the drug substance). The sample was extracted and analyzed as described above. The amount of bis(tributyltin) oxide in the sample was calculated as follows:

$$\text{ppm} = \frac{C_s \times W_{std} \times 10^6}{(C_{std} - C_s) \times W_s}$$

where C_s is the peak area of the tributyltin peak of the sample, C_{std} is the peak area of the trib-

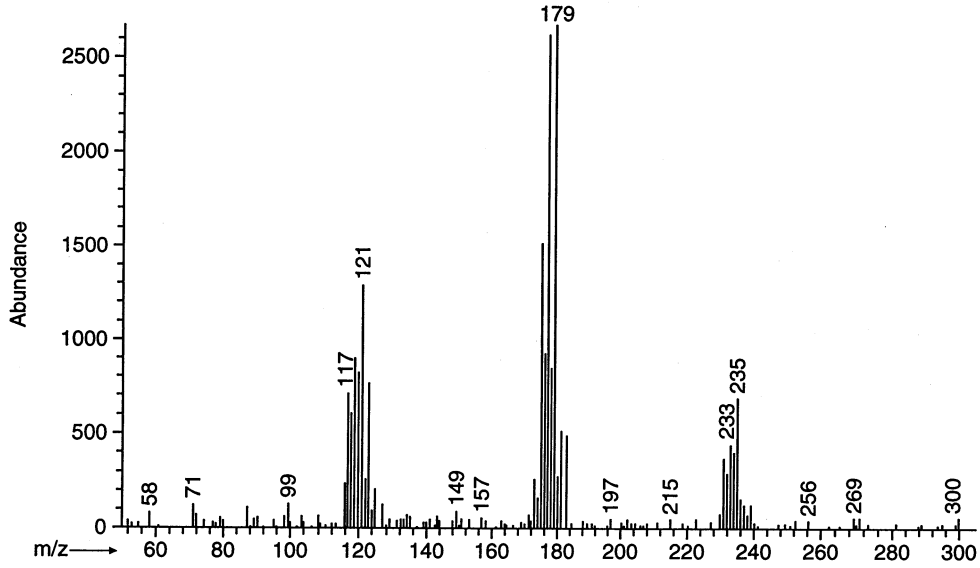


Fig. 1. Mass spectrum of an authentic standard of tributyltin hydride.

utyltin peak of the sample with a known amount of bis(tributyltin) oxide added, W_s is the weight of the sample in milligrams that was dissolved in 0.50 ml of methanol and W_{std} is the concentration of the bis(tributyltin) oxide in methanol multiplied by the dilution volume (0.50 ml).

2.5. On-line hydride conversion

Approximately 100 mg of sodium borohydride was placed between two 5 mm plugs of glass wool inside the injection port liner. The hexane extract containing the tributyltin chloride was injected through the solid borohydride. Borohydride deactivation was not observed: there was no decrease in peak areas even after more than 50 injections of tributyltin chloride samples ranging from 0.001 μg to 0.02 μg.

3. Results and discussion

The current risk based concentration (RBC) level of bis(tributyltin) oxide in water is 1.1 μg l^{-1} tap water [23]. Assuming that an adult consumes 2 l of water per day, a maximum consumption of 2.2 μg bis(tributyltin) oxide per day is tolerable. Assuming the RBC levels of bis(tributyltin) oxide are applicable to acceptable daily intake (ADI) of pharmaceuticals and a daily dose is 50 mg of drug per day, the allowable amount of bis(tributyltin) oxide in a drug substance would be ~44 ppm. A method should be developed that can afford at least that limit of detection.

A mass selective detector set to scan a narrow mass range can increase signal/noise ratio and eliminate interfering peaks thereby providing a lower limit of detection. A mass spectrum of a standard of tributyltin hydride is given in Fig. 1. To maximize sensitivity, the range of 165–185 amu was monitored for all further experiments. This narrow range corresponds to the most intense fragment for tributyltin hydride and includes the characteristic range of isotopes for tin.

Prior to sample analysis, the effectiveness of the on-line hydride conversion was assessed. Known standards (2 μg ml^{-1}) of tributyltin hydride and tributyltin chloride were prepared separately and injected. The area counts were recorded and the molar response (counts mol^{-1}) of each compound was calculated. The molar response ratio of tributyltin chloride to tributyltin hydride was 1.08:1. This ratio indicates that the conversion of tributyltin chloride to tributyltin hydride in the GC liner is quantitative.

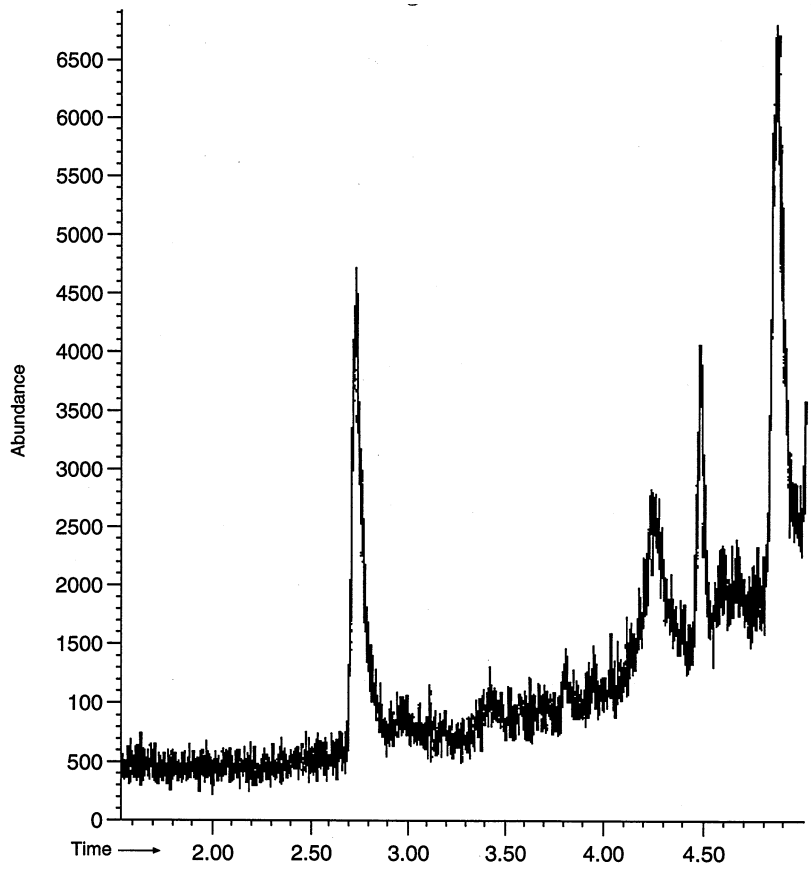


Fig. 2. Chromatogram of drug substance sample.

After confirming the quantitative conversion of the tributyltin chloride to the tributyltin hydride in the GC liner, the linearity of the conversion was evaluated. Eight standards of tributyltin chloride in hexane were prepared ranging from 0.12 to 12 μg ml^{-1} and injected. A plot of detector response vs. tributyltin chloride concentration was linear with a correlation coefficient of 0.999. When taking into account sample size, molecular weights, and the stoichiometry of conversion of bis(tributyltin) oxide to tributyltin hydride, this linearity range corresponds to 1 to 100 ppm bis(tributyltin) oxide in the drug substance. The limit of detection was determined (S/N = 3:1) to be 0.12 μg ml^{-1} tributyltin chloride. This level of tributyltin chloride equates to 1 ppm of bis(tributyltin) oxide in drug substance.

Samples of drug substance were prepared with known amounts of bis(tributyltin) oxide added as described above. Initially, analysis of the samples was attempted without extraction. However, precipitation of the excess drug substance in the liner inhibited the on-line hydride conversion and recoveries of bis(tributyltin) oxide were low. Therefore, the procedure was modified such that the tributyltin species were extracted from the polar organic solution into hexane thereby removing the excess drug substance prior to analysis. Hexane was a suitable extraction solvent since the polar nature of the drug substance had little affinity for the hexane. It has been shown that tributyltin chloride is effectively extracted from a polar medium using hexane [24].

A typical chromatogram of extracts from an authentic sample of drug substance is shown in Fig. 2. The selected ion mass spectrum of the tributyltin hydride peak is given in Fig. 3. The later eluting

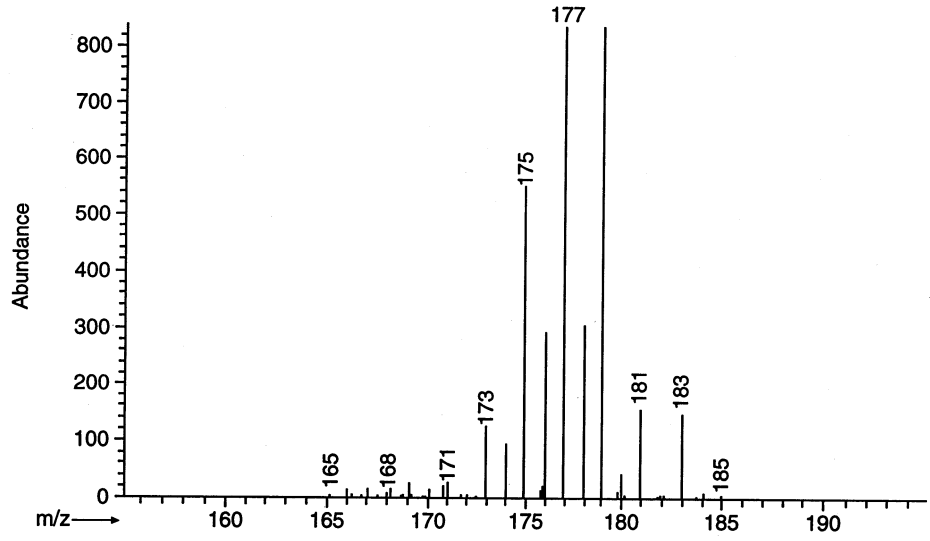


Fig. 3. Mass spectrum of tributyltin hydride peak from drug substance chromatogram. Ions scanned from 165–185 amu.

peaks are unknown; the mass spectra of these peaks do not have the characteristic isotope pattern for tin.

The level of bis(tributyltin) oxide in the sample was found to be 2.9 ± 0.5 ppm ($n = 2$). Recovery of bis(tributyltin) oxide was determined by comparing the response due to a sample with 6 ppm of bis(tributyltin) oxide added to a 6 ppm external standard of bis(tributyltin) oxide. The recovery was determined to be 89%. The injection precision at this level was 6% R.S.D.

4. Conclusion

Development of drug substance syntheses utilizing toxic alkyltin reagents requires that an appropriate analytical method be in place to monitor the final product for residual alkyltin. We have developed a method using on-line hydride derivatization combined with GC–MS to determine ppm levels of bis(tributyltin) oxide in a drug substance. This method offers an advantage over traditional atomic absorption methods because it can differentiate between alkyltin species and inorganic tin. This method may also be adapted for the analysis of other types of samples such as water, soil or tissue.

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