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Analysis of Tributyltin Compounds in Shellfish by Using Gas Chromatography—Mass Spectrometry

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The slow leaching of "tributyltin" compounds from antifouling paints has resulted in pollution of waterways. This is particularly apparent where there are a number of pleasure crafts moored for long periods of time in sheltered estuarine locations (Cleary and Stebbing 1985). These locations may also be ideal places for the aquaculture of shellfish; in New Zealand, these areas include the Northland waterways and the Marlborough Sounds. There is a large and increasing export industry based on the farming of mussels and oysters in New Zealand.

Recent observations have been made that there is an increasing incidence of shell pertubation and malformation in some estuarine farmed-oysters, as well as a decrease in the spat production and in the flesh content of the shellfish. Concern that these effects may be the result of "tributyltin" contamination have been raised, and thus the analysis of a range of samples was required. There have been several methods that have been described using gas-liquid chromatography with electron-capture detection (Hattori et al. 1984, Tsuda et al. 1986). The electron-capture detector responds to a multitude of interfering compounds at the levels at which "tributyltin" appears to be active in the environment.

In order to allow for a more specific, and possibly more sensitive, method of analysis than those previously reported, a method based on high resolution gas chromatography- low resolution mass spectrometry with selected ion monitoring has been developed. This

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paper describes the method and the procedures required for the determination of "tributyltin" compounds in oyster samples.

MATERIALS AND METHODS

Acetone, hexane and dichloromethane were distilled before use. Methanol was purified by distillation from magnesium methoxide. Hydrochloric acid was 35%, BDH analytical reagent. Sodium chloride was Ajax Chemicals analytical reagent. Sodium sulphate was BDH analytical reagent. Sodium borohydride was BDH Spectrosol pellets. One pellet (about 0.25 g) was dissolved in 10 mL of methanol for hydrogenation of samples and standards. Silica gel was Merck Kieselgel 60 (70-230 mesh) rinsed on a Buchner funnel with dichloromethane and activated at 120°C for 48 h. Hydrochloric acid, 37.5 mL, was added to 150 g of activated silica gel. The mixture was shaken for 1 h.

Tributyltin hydride was prepared by the method of Van der Kerk et al. (1957) and stored under dry nitrogen. Standard solutions were prepared in hexane. Tributyltin chloride was Merck, distilled before use. Standard solutions were prepared in methanol. Bis(tributyltin)oxide was Fluka AG. Standard solutions were prepared in hexane.

A Hewlett-Packard 5890 gas chromatograph with a direct interface to a 5970B MSD with a 59970 workstation was used for detection of tributyltin hydride. GC conditions: Column 12 m HP1 (Hewlett-Packard) (0.2 mm id) splitless injector (240°C), transfer line 280°C, carrier gas helium (30 cm sec^{-1}), temperature programme 60°C (hold 0.5 min), 35°C min^{-1} to 120°C (hold 3.5 min), 55°C min^{-1} to 250°C (hold 2 min).

MSD conditions: 70 ev EI Selected Ion Monitoring of m/z 177, 179, 235. Dwell of 100 ms for each ion. (2.6 cycles s^{-1}).

All glassware was rinsed with acetone and then hexane before use.

10 g of homogenized oyster sample was shaken on a mechanical shaker for 30 min with 10 mL of hydrochloric acid, 30 mL of saturated sodium chloride

and 70 mL of dichloromethane. The mixture was centrifuged (2,500 r.p.m. for 5 min), and the dichloromethane extract was removed. The aqueous layer was shaken for 10 min with a further 50 mL of dichloromethane and then separated. The combined organic layers were filtered through sodium sulphate and evaporated under vacuum at 25°C. The extract was made up to 10 mL with dichloromethane.

A 10 mm i.d. column with a cotton wool plug and about 10 mL of dichloromethane was packed with 3 g of hydrochloric acid-silica gel and then 10 mm of sodium sulphate. A 2 mL aliquot of the sample extract was eluted with a further 15 mL of dichloromethane. This fraction was evaporated just to dryness under rotary vacuum.

1 and 2 µg of tributyltin chloride standard in methanol were evaporated just to dryness. The standards and the sample extracts were hydrogenated simultaneously in the following manner.

Sodium borohydride hydrogenation solution, 2mL, was added to the extract, and the mixture was left at room temperature for 15 min. Then, 2 mL of hexane and 5 mL of saturated sodium chloride were added. The mixture was shaken and the hexane layer separated.

A Pasteur pipet was packed with sodium sulphate, rinsed with hexane and dried at 100°C. The hexane extract was then filtered through the Pasteur pipet. A 2 µL sample of the extract was injected into the GC-MSD for analysis.

Tributyltin hydride was determined in the sample extracts by comparison of the areas of the peaks of ions m/z 177,179 and 235 with the areas of the peaks for the hydrogenated tributyltin chloride standards.

RESULTS AND DISCUSSION

The recovery of "tributyltin" from oysters was measured by fortifying with bis(tributyltin)oxide. 10 g samples of uncontaminated oysters were fortified with 0.94, 1.9 and 4.7 µg.

Bis(tributyltin) oxide added (µg)	Recovery (%) as tributyltin hydride
4.7	88
1.9	87
0.94	91

The recovery of bis(tributyltin)oxide was initially determined by comparison of the fortified sample-extracts with tributyltin hydride standards prepared from bulk analytical standard material. However, these standards were found to be unstable. After a period of 48 h, the standards in hexane gave peak areas less than 80% of the areas of the same standard rehydrogenated with sodium borohydride solution.

Hydrogenation of 3 µg of tributyltin chloride with sodium borohydride solution gave conversions to tributyltin hydride of 100% and 104% when compared with a fresh standard of tributyltin hydride. The sample extracts were quantified with hydrogenated tributyltin chloride standards rather than standards prepared from synthetic tributyltin hydride because of the complete conversion of tributyltin chloride to tributyltin hydride and the instability of the standards prepared directly from analytical standards of tributyltin hydride. The sample and standard extracts were analysed by the GC-MS method immediately after hydrogenation.

The criteria for identification of sample peaks as tributyltin hydride were:

(a) The retention time of the sample peaks must agree with those of the standard to within 0.2%.

(b) The relative abundances of the three monitored ions should fall within ±10% of the values obtained for the standard.

(c) The signal-to-noise ratio of the least intense of the masses (ion m/z 235) being monitored should exceed three.

Tributyltin hydride could be identified in sample extracts without column cleanup. No interfering peaks were seen in the chromatographic time-window monitored of 4.5 to 6.5 min; the standard eluted in 5.0 min (Fig. 1.). However, sample extracts which were not subjected to column cleanup became viscous and frequently solidified; this caused difficulties with injections.

The extracts were subjected to column cleanup prior to hydrogenation. This allowed a concentration factor to be introduced into the analysis by rotary evaporation of the dichloromethane fraction from the column. There appeared to be no losses of tributyltin chloride from dichloromethane fractions concentrated in this manner. However tributyltin hydride could not be reliably concentrated in hexane extracts by rotary evaporation without losses.

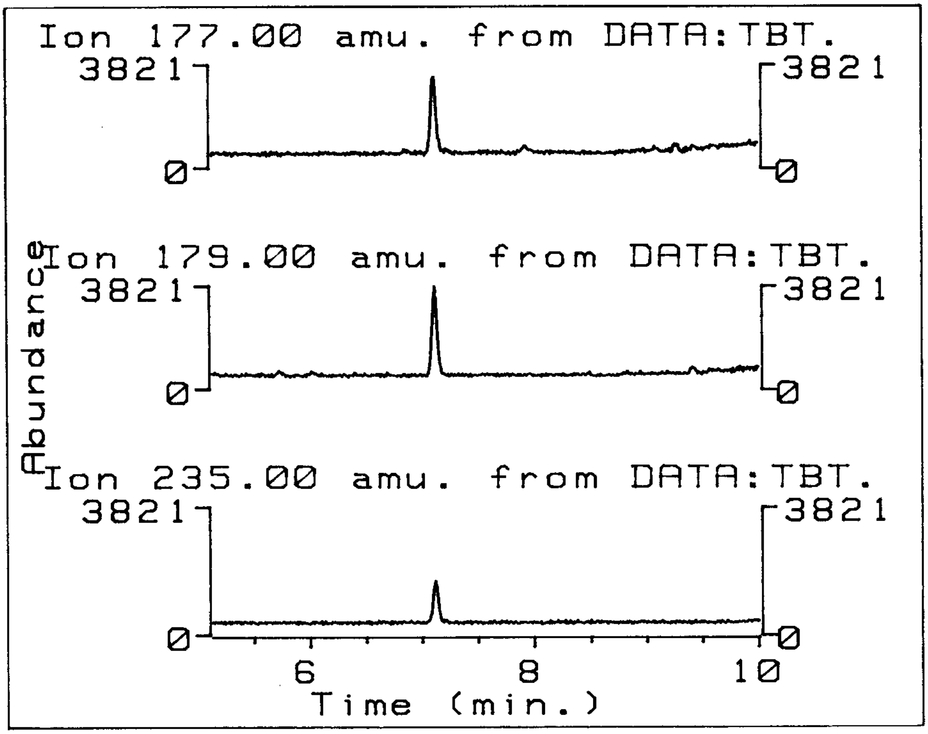

Figure 1. Tributyltin hydride from oyster extract.

The limit of detection for the method was 20 ng of "tributyltin" per gram of oyster. This was determined by a signal-to-noise ratio of three for ion m/z 235, which had a relative abundance of 33% of the most abundant ion, mass m/z 179. One oyster sample was analysed in triplicate.

Sample	"Tributyltin" as tributyltin hydride (ng g^{-1})
Kerikeri oysters	140, 140, 120
Oronga bay oysters	Less than 20
Ashby oysters	50
Ashby spat	Less than 20
Russel wharf oysters	60

The use of high resolution gas chromatography-low resolution mass spectrometry has allowed determination of "tributyltin" in oysters at the part per billion (ng g^{-1}) level. The method has demonstrated reproducibility and sensitivity adequate to indicate significant levels of contamination. The use of a mass-selective detector has improved specificity over the electron-capture detection. Oyster samples with shell perturbation and malformation had up to 140 ppb "tributyltin" in the flesh.

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