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The determination of organotin compounds in fruit juices using gas chromatography–atomic absorption spectrometry

D S Forsyth, D Weber and L Barlow
Food Research Division, Bureau of Chemical Safety, Food Directorate, Health Protection Branch, Health and Welfare Canada, Ottawa, Ontario, Canada K1A OL2

A method for extracting butyl-, cyclohexyl-, octyl- and phenyl-tin compounds in fruit juices was developed using 0.05% tropolone in 25% pentane/diethyl ether. Methyl derivatives formed by Grignard reaction were quantified by gas chromatography–atomic absorption spectrometry. Several fruit juices contained low ng cm^{-3} levels of butyl- and octyl-tins. Gas chromatography–mass spectrometry, used for the confirmation of the butyl- and octyl-tins, also detected phenyl- and cyclohexyl-tin compounds at levels below the GC AA detection limits (0.03–0.05 ng Sn cm^{-3}).

Keywords: Butyltin, cyclohexyltin, octyltin, phenyltin, fruit juice, analysis, GC MS, GC AA

INTRODUCTION

Organotin compounds are used in a variety of applications, including poly(vinyl chloride) (PVC) stabilizers, antifoulants in marine paints, agricultural chemicals and wood preservation. Consequently, worldwide production of organotins has risen from 5000 tonnes in 1955 to at least 35 000 tonnes at present.[1]

Various dibutyl- or dioctyl-tin compounds are effective stabilizers in PVC formulations. Monoalkyltin compounds cause a synergistic effect when blended with the dialkyltins.[2] Octyltins have low mammalian toxicity and several are used in food-contact PVC products.[3]

Tributyltin compounds are used in some antifoulant marine paint and wood preservation products.[4] However, adverse effects on non-target organisms, particularly molluscs,[5–7] have resulted in restrictions on the use of alkyltin-based marine paints in recent years.

Organotin compounds for agricultural usage included tricyclohexyltin hydroxide (Plictran) for many years, as an acaricide on fruit crops, until 1987 when it was withdrawn from the world market by the manufacturer. Additional studies had found teratogenic effects in test animals at dosage levels that prevented attainment of adequate margins of safety, particularly for potential human occupational exposure.[8] Currently, triphenyltin compounds remain in use as fungicides on vegetable crops.

Our recent studies of Canadian wines for organotins[9,10] found elevated levels of butyltins in blended wines containing some foreign wines imported in non-food-grade PVC tanks. Since other foodstuffs, including fruit concentrates, are transported in these types of containers, this study reports on the levels of butyltins and other organotins present in fruit juices likely to be shipped in bulk-transport containers to Canadian distributors.

MATERIALS AND METHODS

Reagents and standards

All solvents used were distilled-in-glass grade (Caledon Laboratories Ltd, Georgetown, Ontario), and ACS reagent-grade inorganic chemicals were used. Methylmagnesium chloride was purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI, USA). Tropolone was obtained from Fluka Chemical Corp. (Ronkonkoma, NY, USA).

The source and purity of the organotin standards have been described elsewhere.[9,11]

Instrumentation

The details and operating conditions of the gas chromatograph (GC)–atomic absorption spectrometer (AA) system used in this study have been previously reported.[10–12]

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Table 1 Method detection limit (MDL)

Analyte	Mean[a] $N_{p\text{-}p}$(mV)[b]	N_{SD}(mV)[c]	Response factor[d]	LOD[e]	MDL[f] (1)[g]	MDL[f] (2)[h]
$BuMe_3Sn$	0.00613	0.00316	388.3	6.1	0.04	0.06
Bu_2Me_2Sn	0.00570	0.00258	427.2	5.7	0.04	0.07
Bu_3MeSn	0.00589	0.00223	410.2	5.2	0.03	0.08
Cy_2Me_2Sn	0.00657	0.00313	381.2	6.1	0.04	0.1
Cy_3MeSn	0.00537	0.00251	595.8	7.7	0.05	0.2
Ph_3MeSn	0.00485	0.00188	567.0	6.0	0.04	0.1

[a] Of 20 measurements. [b] Mean peak-to-peak baseline noise. [c] Standard deviation of $N_{p\text{-}p}$. [d] Inverse of slope from linear regression (pg Sn mV^{-1}). [e] Limit of detection; LOD = (mean $N_{p\text{-}p} + 3N_{SD}$) × response factor (pg Sn). [f] Method Detection Limit; MDL = {[(LOD × inj. vol.$^{-1}$) × extract vol.] × sample vol.$^{-1}$} × 10^{-3}. [g] As ng Sn cm^{-3}. [h] As ng $R_xSn^{(4-x)+}$ cm^{-3}; R = butyl, cyclohexyl or phenyl.

A VG Analytical 7070EQ mass spectrometer coupled to a Varian VISTA 6000 GC was used for mass-spectral (MS) confirmation. The system was operated in the electron impact mode (70 eV) using the conventional magnetic sector only. Mass resolution was 1000. GC operating conditions were as previously reported.[10] Ions were selected for monitoring at *m/z* 205 and 207 for butyltrimethyltin ($BuMe_3Sn$), 225 and 227 for phenyltrimethyltin ($PhMe_3Sn$), 205 and 207 for dibutyldimethyltin (Bu_2Me_2Sn), 261 and 263 for octyltrimethyltin ($OcMe_3Sn$), 247 and 249 for tributylmethyltin (Bu_3MeSn), 231 and 233 for dicyclohexyldimethyltin (Cy_2Me_2Sn), 261 and 263 for dioctyldimethyltin (Oc_2Me_2Sn), 299 and 301 for tricyclohexylmethyltin (Cy_3MeSn), and 349 and 351 for triphenylmethyltin (Ph_3MeSn).

Table 2 Mean recoveries of organotin compounds from juice

Analyte	Matrix	Spiking level (ng cm^{-3})	Mean[a] recovery ± SD (%)
$BuSnCl_3$	Apple juice	8.9	98 ± 2
		1.8	94 ± 3
	Passion blend	8.9	100 ± 5
		1.8	104 ± 5
Bu_2SnBr_2	Apple juice	8.9	95 ± 3
		1.8	92 ± 3
	Passion blend	8.9	103 ± 8
		1.8	99 ± 4
Bu_3SnBr	Apple juice	14.5	91 ± 1
		2.9	87 ± 2
	Passion blend	14.5	98 ± 6
		2.9	98 ± 2
Cy_2SnBr_2	Apple juice	13.6	95 ± 3
		2.7	98 ± 3
	Passion blend	13.6	101 ± 7
		2.7	98 ± 2
Cy_3SnBr	Apple juice	13.8	82 ± 1
		2.8	82 ± 4
	Passion blend	13.8	90 ± 5
		2.8	79 ± 3
Ph_3SnCl	Apple juice	13.7	95 ± 1
		2.7	97 ± 2
	Passion blend	13.7	101 ± 6
		2.7	99 ± 2

[a] $N = 5$.

Sample collection

A selection of fruit juices was purchased from local grocery stores and stored at room temperature. Opened samples were refrigerated for the duration of use. Most of the products sampled contained juice from non-indigenous fruit.

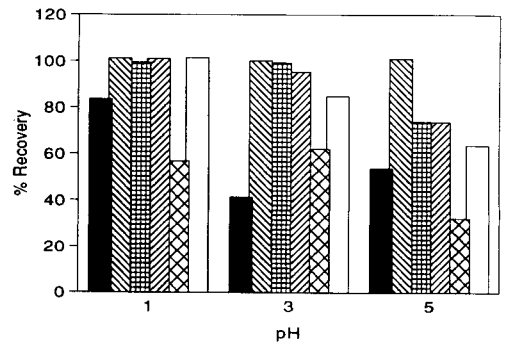

Figure 1 The effect of sample pH on recovery of monobutyltin (■), dibutyltin (▨), tributyltin (▤), dicyclohexyltin (▨), tricyclohexyltin (▩), and triphenyltin (□), using 0.05% tropolone in pentane.

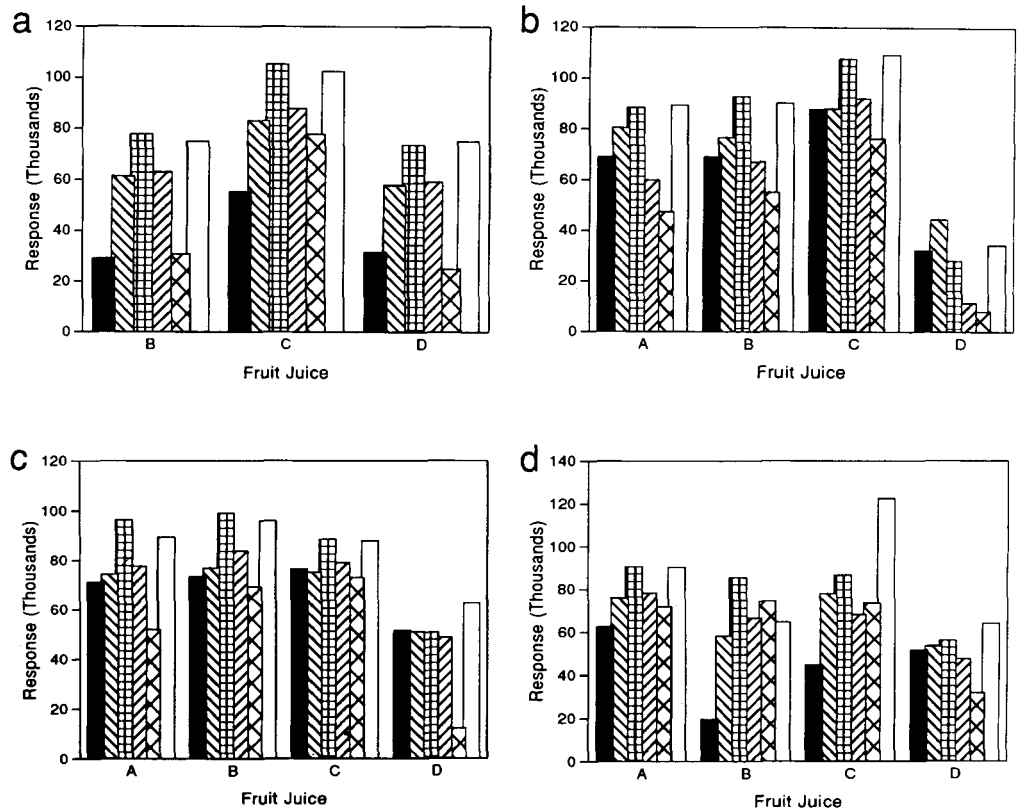

Figure 2 Recovery of monobutyltin (■), dibuytyltin (▨), tributyltin (▦), dicyclohexyltin (▨), tricyclohexyltin (▨), and triphenyltin (□), using (a) pentane, (b) methylene chloride, (c) diethyl ether or (d) ethyl acetate from Juice A—citrus blend, B—citrus punch, C—grape punch, and D—fruit punch.

Extraction

A sample (30 cm^3) of fruit juice was measured into a 50 cm^3 centrifuge tube and ascorbic acid (0.5 g) was added. The pH was adjusted to 1 using concentrated hydrochloric acid (HCl) and the sample extracted (rotary-tumbled, 65 rpm) for 20 min with 10 cm^3 of 0.05% tropolone in a 25% pentane/diethyl ether solution. Following centrifugation (2100 rpm, 10 min) the organic extract was first transferred into a 15 cm^3 centrifuge tube, residual aqueous carry-over was allowed to settle, and then the organic layer transferred into a 10 cm^3 tube with care to prevent transfer of any aqueous phase. The 10 cm^3 tube was placed in a water bath at 30 °C under a gentle stream of nitrogen and the extract volume reduced to approx. 2 cm^3. After the aqueous carry-over had been returned to the fruit-juice sample, the above extraction and transfer step was repeated once. Following transfer of the second extract to the 10 cm^3 tube, the 15 cm^3 tube was rinsed once with diethyl ether (2 mL) with anhydrous sodium sulfate (approx. 300 mg) present. The diethyl ether was then added to the pooled extract in the water bath and the volume was reduced to 1 cm^3. To remove most of the remaining diethyl ether, additional pentane (5 cm^3) was added to the sample extract and the volume reduced to 1 cm^3 by evaporation as before.

Derivatization

Tetrahydrofuran (1 cm^3) and methylmagnesium chloride (0.5 cm^3) were added to the sample extract. The sample was then capped under nitrogen, vortexed briefly, and rotary-tumbled (10 min, 25 rpm). After the sample had been cooled in ice, prechilled nitric acid (0.5 mol dm^{-3}) was slowly added until the total volume was 10 cm^3. Iso-octane (0.9 cm^3) was added, and the sample was tumbled (2 min, 25 rpm), and centrifuged briefly (up to 2500 rpm). The aqueous layer was removed and deionized water (18 MΩ cm^{-1})

Table 3 Alkyltin levels[a] in fruit drinks (GC AA method)

Sample	Brand	Container[b]	Analyte concentration (ng $R_xSn^{(4-x)+}$ cm^{-3})			
			$BuSn^{3+}$	Bu_3Sn^+	$OcSn^{3+}$	Oc_2Sn^{2+}
Pineapple	1	Can	<0.06	<0.08	nd[c]	nd
Grapefruit	1	Box	<0.06	<0.08	nd	nd
Pineapple/mandarin	1	Box	<0.06	<0.08	nd	nd
Pineapple/orange/passion	1	Box	<0.06	<0.08	nd	nd
Cranberry	2	Box	<0.06	<0.08	nd	nd
Citrus blend	3	Box	<0.06	<0.08	nd	nd
Citrus blend	3	Box	<0.06	<0.08	nd	nd
Passion blend	4	Glass	<0.06	<0.08	nd	nd
Apple	4	Box	<0.06	<0.08	nd	nd
Citrus blend	4	Box	<0.06	<0.08	nd	nd
Grape	5	Box	<0.06	<0.08	nd	nd
Fruit blend	5	Box	<0.06	<0.08	nd	nd
Apple	6	PVC	<0.06	<0.08	4.8	4.3
Apple/pineapple	6	Box	0.1	<0.08	nd	nd
Fruit blend	6	Box	0.1	<0.08	nd	nd
Orange	6	Box	0.2	0.3	nd	nd
Papaya	6	Box	<0.06	<0.08	nd	nd
Banana	6	Box	<0.06	<0.08	nd	nd
Passion blend	7	Box	<0.06	<0.08	nd	nd
Fruit blend	7	Box	<0.06	<0.08	nd	nd
Pineapple	7	Box	<0.06	<0.08	nd	nd
Banana/Orange	7	Box	<0.06	<0.08	nd	nd
Citrus punch	8	Box	<0.06	<0.08	nd[c]	nd
Citrus blend	8	Box	<0.06	<0.08	nd	nd
Orange	8	Box	<0.06	<0.08	nd	nd
Pineapple	8	Box	<0.06	<0.08	nd	nd
Grapefruit	8	Box	<0.06	<0.08	nd	nd
Papaya	9	Box	<0.06	<0.08	nd	nd
Fruit blend	9	Box	<0.06	<0.08	nd	nd
Citrus blend	9	Box	<0.06	<0.08	nd	nd
Apple/orange/pineapple	10	Glass	0.1	<0.08	nd	nd
Passion blend	11	Glass	<0.06	<0.08	nd	nd
Kiwi	12	PVC	<0.06	<0.08	4.9	1.0
Papaya	12	PVC	<0.06	<0.08	11.7	nd
Peach	12	PETE	<0.06	<0.08	nd	nd
Grape	12	PETE	<0.06	<0.08	nd	nd
Banana	12	PETE	<0.06	<0.08	nd	nd
Citrus	12	PETE	<0.06	<0.08	nd	nd
Watermelon	12	PETE	<0.06	<0.08	nd	nd
Lemonade	12	PETE	<0.06	<0.08	nd	nd
Banana	12	PVC	<0.06	<0.08	4.5	0.9
Lemonade	12	PVC	<0.06	<0.08	16.3	nd

[a] Uncorrected for recovery. [b] Container type: Box = Tetra-Pak® or similar box; PVC = poly(vinyl chloride); PETE = poly(ethylene terephthalate). [c] nd, Not detected.

was added until the total volume was 10 cm^{-3}. Tumbling and centrifugation were repeated as before. Following removal of the aqueous layer, the sample extract was adjusted to 2.0 cm^3 with hexane, dried over anhydrous sodium sulfate and stored in an autoinjector vial.

Analysis

The sample was quantified by comparison with external standards using GC AA. A 10 μl sample was injected by autosampler. Since octyltin standards were unavailable at the time of the study, response factors (expressed as Sn) from

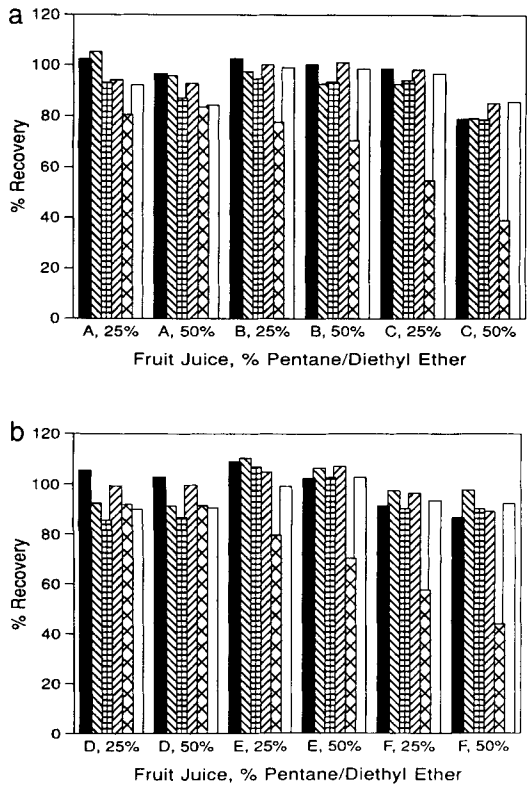

Figure 3 Recovery of monobutyltin (■), dibutyltin (▨), tributyltin (▦), dicyclohexyltin (▨), tricyclohexyltin (▩) and triphenyltin (□), using 25% pentane/diethyl ether or 50% pentane/diethyl ether from (a) Juice A—apple, B—passion blend, C—citrus blend and (b) D—grape punch, E—fruit blend, and F—grapefruit.

existing alkylmethyltin standards were used for quantification. The octyltin compounds were identified by GC MS. Reagent blanks were run with each sample series. Method detection limits ranged from 0.03 to 0.05 ng Sn cm^{-3} (Table 1).

Method development

Solvent study

Four solvents (pentane, diethyl ether, methylene chloride and ethyl acetate) were evaluated as 0.05% tropolone solutions for extracting alkyltins from citrus and non-citrus fruit juices: 15 cm^3 of spiked juice was extracted once (same conditions as given in the 'Extraction' section) with 5 cm^3 of 0.05% tropolone solution. Derivatization was as described in the 'Derivatization' section. Solvent mixtures (25% pentane/diethyl ether and 50% pentane/diethyl ether) were also evaluated as 0.05% tropolone solutions with a variety of different juices: 30 cm^3 of spiked juice was extracted twice with tropolone solution using the same conditions reported in the 'Extraction' and 'Derivatization' sections.

pH study

The pH of a juice blend containing both citrus and non-citrus fruit was adjusted to 1, 3 and 5 with concentrated HCl or ammonium hydroxide. Spiked samples were extracted twice with 0.05% tropolone in pentane. Extraction and derivatization conditions were as reported in the 'Extraction' and 'Derivatization' sections.

Recovery experiments

Sample of apple or passion fruit juice blend were spiked at two levels (1.8–2.9 or 8.9–14.5 ng cm^{-3}) with a mixture consisting of Bu_3SnBr, Bu_2SnBr_2, $BuSnBr_3$, Cy_3SnBr, Cy_2SnBr_2, and Ph_3SnCl just prior to extraction. The percentage recovery of each analyte was calculated by dividing the mean peak area of the compound recovered from the spiked samples by the mean peak area of the compound in a blank apple or passion fruit juice blend extract spiked just prior to derivatization.

RESULTS AND DISCUSSION

Extraction method

Organotin recoveries from apple and from passion fruit juice blend (containing citrus and non-citrus fruit) averaged 92 and 98% respectively using the reported method (Table 2). Octyltin recoveries could not be conducted, as standards were not available. Coextractives from the juice matrix did not affect derivatization yields.

Initial work using a method developed for wines[10] indicated that there was wide variation in recoveries from juice samples. A variety of modifications were examined, including sample pH, NaCl addition, extraction solvent and halide present from acid (Cl or Br). Sodium chloride addition did not improve recoveries and there was little difference in recoveries between HBr- and HCl-acidified samples. However, sample pH and extraction solvent were found to influence the extraction efficiency. Most organotin recoveries, except that for dibutyltin, generally decreased as the sample pH was increased from 1 to 5 (Fig. 1). Recoveries were not improved by lowering the

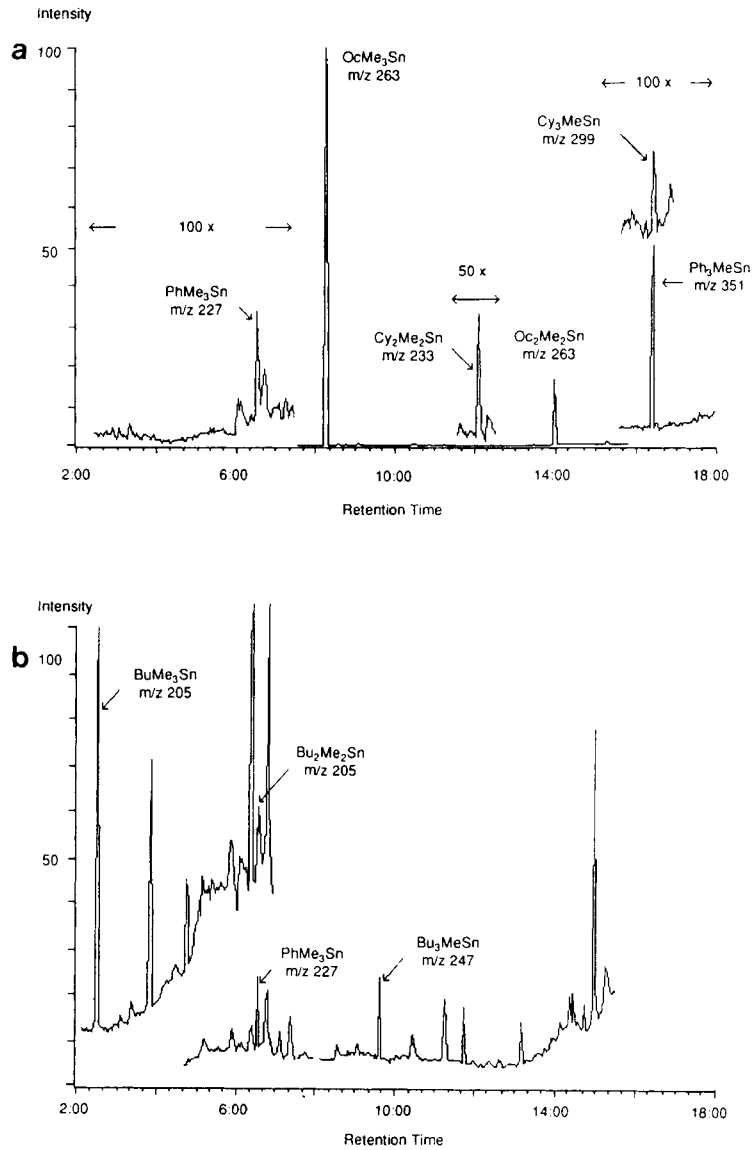

Figure 4 GC MS confirmation of (a) kiwi drink and (b) fruit punch containing monobutyltin {as $BuMe_3Sn$ (m/z 207 $[M_{120}-CH_3]^+$)}, dibutyltin {as Bu_2Me_2Sn (m/z 207 $[M_{120}-C_4H_9]^+$)}, tributyltin {as Bu_3MeSn (m/z 249 $[M_{120}-C_4H_9]^+$)}, monophenyltin {as $PhMe_3Sn$ (m/z 227 $[M_{120}-CH_3]^+$)}, mono-octyltin {as $OcMe_3Sn$ (m/z 263 $[M_{120}-CH_3]^+$)}, dioctyltin {as Oc_2Me_2Sn (m/z 263 $[M_{120}-C_8H_{17}]^+$)}, dicyclohexyltin {as Cy_2Me_2Sn (m/z 233 $[M_{120}-C_4H_9]^+$)}, tricyclohexyltin {as Cy_3MeSn (m/z 299 $[M_{120}-C_6H_{11}]^+$)} and triphenyltin {as Ph_3MeSn (m/z 351 $[M_{120}-CH_3]^+$)}.

pH below 1. Recovery studies with pentane, diethyl ether, methylene chloride or ethyl acetate as tropolone solutions (Fig. 2a, b, c, d) indicated that the best overall recoveries from the four tested juice matrices were obtained with diethyl ether (Fig. 2c). However, as diethyl ether caused emulsions with some samples, pentane/diethyl ether mixtures were tested to improve phase separation after tumbling: 25% pentane/diethyl ether produced better phase interfaces (less emulsion) and recoveries than 50% pentane/diethyl ether in the majority of tested juice samples (Fig.

3a, b). Therefore, 25% pentane/diethyl ether was selected as the solvent system used in the recovery studies (Table 2). Only tricyclohexyltin recoveries remained variable, with much better recoveries from apple, passion fruit juice blend and grape punch than from citrus blend or grapefruit juice (Fig. 3).

Fruit juice analyses

Butyl-, cyclohexyl- and phenyltin were not present in most of the tested fruit juice samples at levels exceeding the GC AA method detection limits (Table 3). Several samples contained low (0.1–0.2 ng cm^{-3}) levels of monobutyltin and one sample contained 0.3 ng tributyltin cm^{-3}. Some samples had 4.8–16.3 ng cm^{-3} and 0.9–4.3 ng cm^{-3} levels of mono-octyl- and dioctyltin respectively (Table 3). GC MS analysis of five fruit samples confirmed the presence of monobutyltin {as $BuMe_3Sn$ (m/z 207; $[M_{120}-CH_3]^+$)}, tributyltin {as Bu_3MeSn (m/z 249 $[M_{120}-C_4H_9]^+$)}, mono-octyltin {as $OcMe_3Sn$ (m/z 263 $[M_{120}-CH_3]^+$)}, and dioctyltin {as Oc_2Me_2Sn (m/z 263 $[M_{120}-C_8H_{17}]^+$)}. Dibutyltin {as Bu_2Me_2Sn (m/z 207 $[M_{120}-C_4H_9]^+$)}, monophenyltin (as $PhMe_3Sn$ {m/z 227 $[M_{120}-CH_3]^+$)}, dicyclohexyltin {as Cy_2Me_2Sn (m/z 233 $[M_{120}-C_4H_9]^+$)}, tricyclohexyltin {as Cy_3MeSn (m/z 299 $[M_{120}-C_6H_{11}]^+$)} and triphenyltin {as Ph_3MeSn (m/z 351 $[M_{120}-CH_3\}]^+$)} residues were also detected by GC MS at levels below the GC AA detection limits (Fig. 4a, b). The calculated molecular ion was based on the tin isotope ^{120}Sn.

Octyltins were present in fruit juice samples sold in containers constructed of poly(vinyl chloride) but not in those made from poly(ethylene terephthalate) (Table 3). Therefore, the likely source of the octyltin was the PVC container material. Canadian food regulations permit no more than 1 μg total octyltin g^{-1}.[14] The very low levels of butyltins found in these samples indicate that either the fruit juice concentrates were not in contact with non-food-grade PVC during bulk shipping or that the juices do not extract butyltins from liners efficiently.

CONCLUSIONS

Recoveries of organotins from apple and from a passion fruit juice blend were very high using 0.05% tropolone in 25% pentane/diethyl ether. Fruit juice samples purchased at the retail level contained low or undetectable levels of butyl-, phenyl- and cyclohexyl-tin compounds. Several samples, purchased in PVC containers, had low ng cm^{-3} levels of mono- and dioctyl-tin present.

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