Quantitation and Identification of Organotin Compounds in Food, Water, and Textiles Using LC-MS/MS

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Overview

Organotin compounds are chemicals composed of tin linked to hydrocarbons, used in industrial materials and various biocides and fungicides. As a result, organotin compounds can enter the environment through a number of channels, and can often be found in seawater, seafood, fruits and vegetables, and consumer goods. Due to the toxicity of these compounds, there is a need for analytical methods allowing accurate quantitation and identification. Here we present an LC-MS/MS method to measure tributyltin, fentin, cyhexatin, and fenbutatin oxide in different matrices. Triphenyl phosphate was used as the internal standard.

Spiked apple, potato, synthetic seawater, and textile samples were prepared using a quick and easy acetonitrile extraction. Organotin compounds were detected using an AB SCIEX 4000 QTRAP® system with Electrospray Ionization (ESI) using Multiple Reaction Monitoring (MRM). Detection limits were determined to be well below regulated levels, enabling extra dilution of the sample extract to minimize possible matrix effects.

Introduction

Organotin (organostannic) compounds are chemical compounds comprised of tin with hydrocarbon substituents. Organotin compounds are widely used as additives in plastic material, wood preservatives, marine biocides, and agricultural pesticides.

Tri-substituted organotin compounds were previously widely used as antifouling agents in paints on ships. However, such paints were found to release organotin compounds into the aquatic environment, where they can accumulate in sediments and organisms or degrade to less substituted toxic compounds. Studies have shown that trace amounts of organotin compounds can have significant detrimental effects on aquatic organisms. For instance, tributyltin (TBT), present in sea water at ng/L levels, has been identified as an endocrine disruptor promoting harmful effects on aquatic organisms. Therefore, the use of organotin compounds in antifouling paints is prohibited or restricted in many countries.[1-3]

The use of organotin compounds in consumer products, such as textiles, footwear, wall and floor coverings, etc., has been found

to pose a risk to human health, particularly for children. Therefore, the use of tri-substituted and di-substituted organotin compounds, including TBT, tributyltin (TPhT), dibutyltin (DBT), and dioctyltin (DOT) in consumer products is restricted.[4-5]

Finally, organotin compounds enter the human diet through contaminated seafood and the use as agricultural pesticides. International maximum residue limits (MRL) have been established by Codex Alimentarius and the EU for many food commodities, with some MRL as low 50 µg/kg.

Traditionally gas chromatography coupled to mass spectrometry (GC-MS) was used for analysis of organotin compounds. However, the analysis by GC requires time consuming derivatization, because of poor compound volatility, and long chromatographic run times. Liquid chromatography with tandem mass spectrometry (LC-MS/MS) allows simplifying sample preparation and shortening run times due to increased selectivity and sensitivity and, thus, is evolving as a preferred technique for the analysis of organotin compounds.

Method Details

Sample Preparation

TBT chloride, fentin acetate, cyhexatin and fenbutatin oxide were purchased from Sigma-Aldrich and spiked into four matrices (apple, potato, synthetic seawater (drinking water with 35 g salt per liter), and textile material). Triphenyl phosphate (TPP) was used as the internal standard.

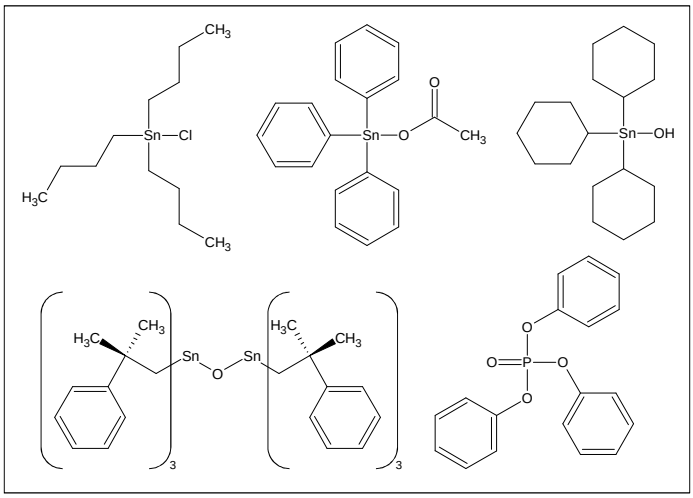

Figure 1. Target organotin compounds: TBT chloride, fentin acetate, cyhexatin, fenbutatin oxide, and internal standard triphenyl phosphate (top left to bottom right)

Spiked samples were extracted using acetonitrile and diluted 10x with LC grade water prior to LC-MS/MS analysis. The spiked synthetic seawater was directly injected for detection of organotin compounds. Note that additional dilution is possible depending on required limits of detection to reduce possible matrix effects (Figure 2).

UHPLC Separation

A Shimadzu UFLC$_{XR}$ system was used with a Phenomenex Kinetex 2.6u C18 50x3mm column at 40ºC. A gradient of water with 2% formic acid + 5 mM ammonium formate and methanol with 2% formic acid + 5 mM ammonium formate at a flow rate of 800 µL/min resulted in a total run time of 12 minutes.

The injection volume was set to 20 µL for apple and potato extracts and 50 µL for textile extracts and synthetic seawater.

Figure 2. Sample preparation protocols for the analysis of organotin compounds in fruit and vegetable, textiles, and water

MS/MS Detection

The AB SCIEX 4000 QTRAP® LC/MS/MS system with Turbo V™ source and ESI probe was used. All the analytes and internal standard were detected in positive polarity using MRM for best selectivity and sensitivity. Two MRM transitions were monitored for each compound to allow quantitation and identification using the characteristic MRM ratio. The *Scheduled* MRM™ algorithm was activated for best data quality (Table 1).

The data was processed in MultiQuant™ software version 2.1.

Table 1. MRM transitions and retention times (RT) of targeted organotin compounds

Organotin compound	Q1 (amu)	Q3 (amu)	RT (min)
TBT 1	291.0	123.0	3.8
TBT 2	291.0	235.1	3.8
Fentin 1	351.0	120.0	3.0
Fentin 2	351.0	197.0	3.0
Cyhexatin 1	369.0	205.0	5.3
Cyhexatin 2	369.0	287.1	5.3
Fenbutatin oxide 1	519.1	351.0	6.2
Fenbutatin oxide 2	517.1	349.0	6.2
TPP (internal standard)	326.9	152.1	4.4

Results and Discussion

Chromatography conditions were important for successful determination of organotin compounds by LC-MS/MS. Organotin compounds are known for strong interaction with reversed phase material resulting in peak broadening. A strong acidic mobile phase was used to reduce this effect and to optimize peak shape.[8]

Two chromatographic interferences were observed for TBT in all matrices. Thus, stable retention times and good separation was important. A core-shell column (Phenomenex Kinetex) was used for improved UHPLC performance while operating at reduced column pressure (Figure 3).

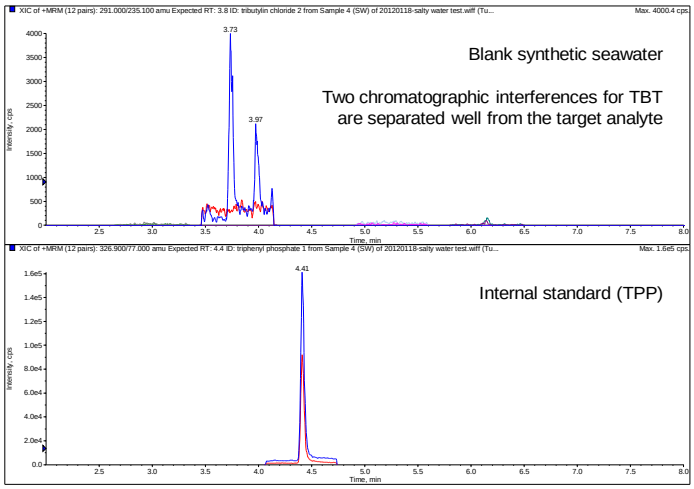

Figure 3. Blank synthetic seawater, two chromatographic interferences for TBT are separated well from the target analyte (top) and internal standard (bottom)

Apple, potato, textile, and synthetic seawater samples were spiked at different concentrations, extracted, and analyzed using the fast LC-MS/MS method. Example chromatograms are shown in Figures 4 and 5.

The achieved Signal-to-noise (S/N) ratios are listed in Table 1. S/N values were measured in MultiQuant™ software after applying a 2x Gaussian smooth. S/N values were used to estimate limits of quantitation (LOQ) for all analytes in each matrix.

Table 2. Signal-to-noise (S/N) in different matrices

Organotin compound	Apple (2 µg/kg)	Potato (2 µg/kg)	Textile (0.1 mg/kg)	Seawater (50 ng/L)
TBT 1	105	71	93	53
Fentin 1	355	315	209	186
Cyhexatin 1	240	197	51	133
Fenbutatin oxide 1	339	377	66	176

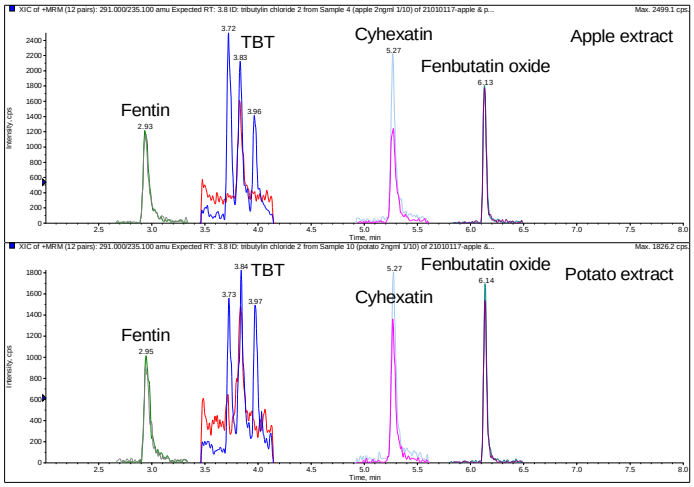

Figure 4. Apple (top) and potato (bottom) sample spiked at 2 µg/kg and diluted 10x after extraction

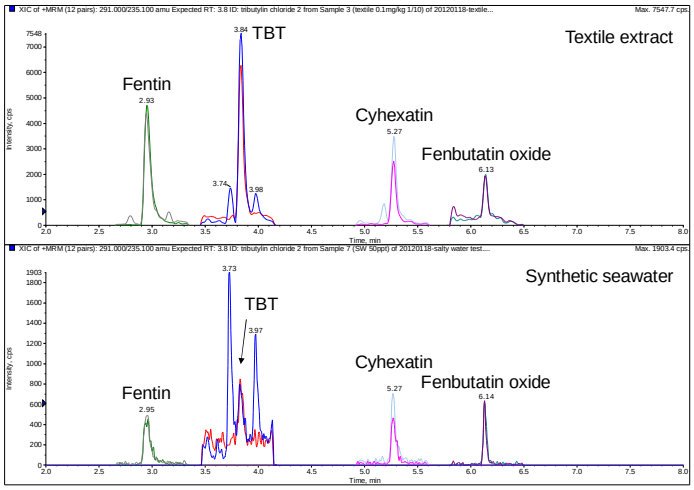

Figure 5. Textile material spiked with 0.1 mg/kg and diluted 10x after extraction (top) and synthetic seawater spiked at 50 ng/L and analyzed by direct injection (50 µL)

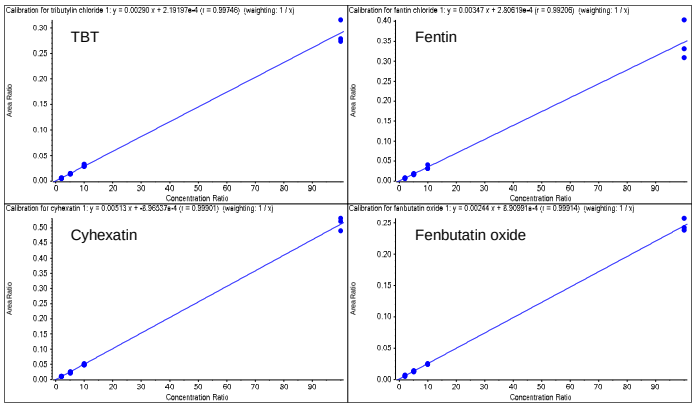

Figure 6. Calibration lines of organotin compounds in apple matrix (2 to 100 µg/kg)

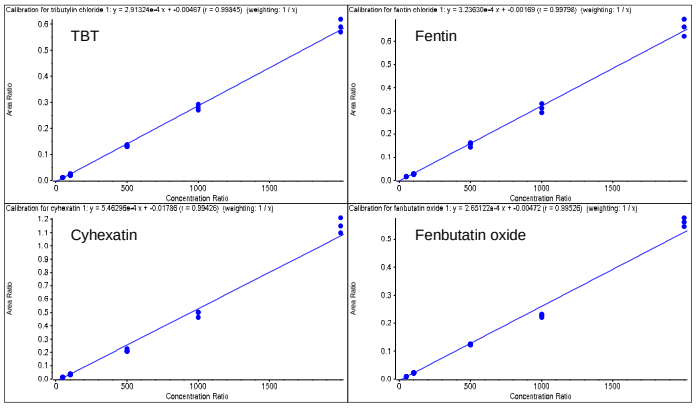

Figure 7. Calibration lines of organotin compounds in synthetic seawater (50 to 2000 ng/L)

Table 3. Estimated limits of quantitation (LOQ) in different matrices based on S/N of 10

Organotin compound	Apple µg/kg	Potato (µg/kg)	Textile (µg/kg)	Seawater (ng/L)
TBT	0.2	0.3	10	10
Fentin	< 0.1	< 0.1	< 10	< 10
Cyhexatin	0.1	0.1	20	< 10
Fenbutatin oxide	< 0.1	< 0.1	15	< 10

The linear dynamic range was evaluated from 2 to 100 µg/kg for apple and potato, from 0.1 to 1 mg/kg for textiles, and from 50 to 2000 ng/L for seawater. Example calibration lines of all four organotin compounds in apple and synthetic seawater are shown in Figures 6 and 7.

Repeatability was found to be less than 15% coefficient of variation (%CV) and accuracy between 85 and 115% for all compounds at all concentrations (Table 4).

Table 4. Repeatability (%CV) and accuracy of organotin compounds at the lowest point of the calibration line

	Apple (2 µg/kg)		Potato (2 µg/kg)		Textile (0.1 mg/kg)		Seawater (50 ng/L)	
Organotin compound	**%CV**	**Accuracy (%)**	**%CV**	**Accuracy (%)**	**%CV**	**Accuracy (%)**	**%CV**	**Accuracy (%)**
TBT	10.0	97.0	13.9	86.4	7.3	95.6	6.3	113.1
Fentin	9.9	101.4	12.4	96.8	4.7	95.8	7.9	112.6
Cyhexatin	5.9	108.5	2.4	88.4	3.6	93.3	4.2	115.0
Fenbutatin oxide	11.4	104.4	11.8	99.5	13.2	97.3	3.6	107.4

Compound identification was achieved using the 'Multicomponent' query in MultiQuant™ software. This query automatically calculates and compares MRM ratios for identification and highlights concentrations above a user specified residue level. Examples of the result table and peak review after running the query file are shown in Figures 8 and 9.

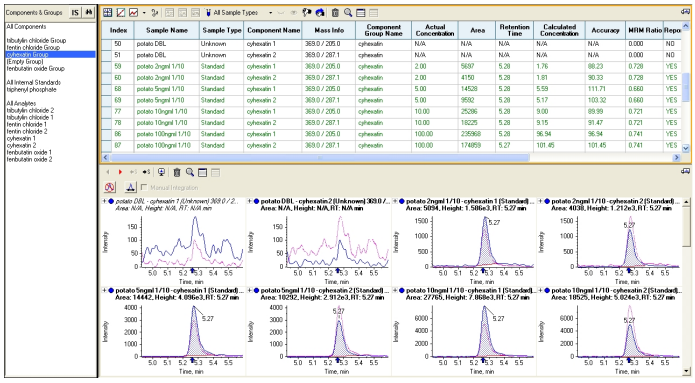

Figure 8. Automatic compound identification using the 'Multicomponent' query (example cyhexatin in potato)

Figure 9. Automatic compound identification using the 'Multicomponent' query (example fentin in textile)

Summary

A quick, easy, and robust LC-MS/MS method for the determination of different organotin compounds in food, seawater, and textile materials was developed. The method allows accurate and reproducible quantitation using the selectivity and sensitivity provided by the AB SCIEX 4000 QTRAP® system operated in MRM mode. Detection limits well below regulated levels allow sample extract dilution to minimize possible matrix effects. Confident compound identification was achieved through the automatic calculation of MRM ratios using the 'Multicomponent' query in MultiQuant™ software.

References

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2 E. Gonzalez-Toledo et al.: 'Detection techniques in speciation analysis of organotin compounds by liquid chromatography' Trends Anal. Chem. 22 (2003) 26-33

3 Regulation (EC) 'on the prohibition of organotin compounds on ships' No 782/2003

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5 International Association for Research and Testing in the Field of Textile Ecology: OEKO-TEX Standard 100, Edition 4 (2012)

6 http://www.codexalimentarius.org/standards/pesticide-mrls/en/

7 Council Directive 'maximum levels for pesticide residues' 96/32/EC

8 EU Reference Laboratory for Single Residue Methods: 'Analysis of Organotin Compounds via QuEChERS and LC-MS/MS – Brief Description' www.crl-pesticides.eu

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