ANALYSIS OF ORGANOTIN COMPOUNDS TRIBUTYLTIN, DIBUTYLTIN AND MONOBUTYLTIN IN FISH AND SHELLFISH FROM GALICIA, NW SPAIN, BY GAS CHROMATOGRAPHY-ION TRAP TANDEM MASS SPECTROMETRY

Blanco SL, Martínez A, Álvarez A, González V, Porro C, Vieites JM.[1]

[1]Centro Técnico Nacional de Conservación de Productos de la Pesca (ANFACO-CECOPESCA); Carretera Colexio Universitario nº 16, 36310, Vigo, Pontevedra, Spain

Introduction

Tributyltin (TBT) has been extensively used as biocide in antifouling paint causing extensive damage to non-target organisms at very low concentration levels and accumulating in sediments and biota. Organotin compounds (OTCs) have entered various ecosystems in considerable amounts; in the marine environment TBT is present together with its dealkilation products, dibutyltin (DBT) and monobutyltin (MBT), which are less toxic to aquatic organisms. For these reasons, restrictions on TBT use were introduced since the mid-1980s; nevertheless, butyltins persist in many areas at levels considered to be chronically toxic to the most susceptible organisms. As a result, the European Union (EU) has included TBT and its degradation products in the list of priority pollutants[1]. Development of reliable and fast methodologies capable of analysing these contaminants in a routine basis by analytical laboratories is a need. Moreover, the presence of these compounds in marine organisms needs to be investigated, in order to evaluate the ingestion of these compounds by the consumers.

The aim of the present paper was the development of a GC-MS ion-trap method, in fish and shellfish, to evaluate levels of TBT, DBT and MBT in marine organisms from the Galician Rías of Arosa and Vigo, especially the most consumed edible species..

Materials and methods

Different matrixes samples were used: fish, shellfish and cephalopods.

Monobutyltin (MBT, 98.8%) trichloride, dibutyltin (DBT, 99.0%) dichloride, tributyltin (TBT, 98.5%) chloride and tripropyltin (TPrT, 98.8%) chloride from LGC Standards GmbH (Germany) were used as standards. TPrT was used as internal standard. Standard solutions were prepared in methanol weekly.

Gas chromatograph Varian CP-3800 coupled to an ion trap tandem mass spectrometer Saturn 2000 GC/MS from Varian was used. BPX-5 column (SGE, 60m×0.25mm ID, 0.25 µm Film), was used. Helium Alphagaz He-2 (purity≥99.9999%) purchased from Air Liquide (Spain) was used as carrier gas. Isooctane, n-hexane, acetone and methanol of residue analysis grade, and silica gel 60 (0.063-0.200mm) and sodium acetate were purchased from Merck. Acetic acid was obtained from Scharlau. Tetramethylammonium hydroxide (TMAH), aluminum oxide, activated, acidic and anhydrous sodium sulphate were from Sigma-Aldrich. Sodium tetraethylborate 97% was from Acros-Organics.

Detection was performed in full scan mode. Quantification was based on the internal standard method. Calibration was performed in the range 25-1100 µg mL^{-1}. Linearity was obtained for all the compounds.

Results and discussion:

Extraction method was a modification of a previously described method[2], using tetramethylammonium hydroxide (TMAH, 25% in water). The optimized extraction conditions were: 0.5 g of freeze-dried sample and 15 mL of TMAH 25% in water, with vigorous agitation for 10 min. and "a plus"of 15 min. in ultrasounds bath, and 4 hours of incubation at 60ºC. This step is crucial to obtain a good recovery of the compounds of interest.

Derivatization is also an important step, specially in GC-based methods[3], furthermore sodium tetraethylborate (STEB), is easily degraded. A solution containing 20%(w/v) of STEB in water was stored under inert gas at 4ªC and out of direct light. As referenced[4], this solution is stable for at least three months, and this way, the handling is easier. The digested samples are neutralized with acetic acid, and buffered with sodium acetate-acetic acid solution, pH 5.0. Then, 2 mL of n-hexane and 100 µL of STEB solution are added, and vigorous agitation is carried out for ten minutes. This step achieves simultaneously derivatization by ethylation and solvent extraction of the analytes, simplifying the analytical procedure, as stated by others[3]. After centrifugation, organic phase (n-hexane) is taken for further sample processing.

A clean-up step was introduced after a number of injections since chromatographic quality, and stability and robustness of the procedure was significantly improved. Different approaches were assayed: only aluminum oxide clean-up, and combined activated silica-gel and aluminum oxide clean-up. The best quality was obtained combining both adsorbents, the column was prepared with 2 g of aluminum oxide on the bottom, and 2.5 g of activated silica-gel on top, and conditioned with 30 mL of n-hexane. 1 mL of the sample n-hexane extract was passed through and analytes were collected by elution of 7.5 mL of n-hexane. Evaporation was carried out, and finally, isooctane (50 μL) was used as keeper solvent to avoid dryness of the solution. The extracts were ready for injection in GC-MS system.
This purification scheme was adequate to prevent the contamination of the column and especially of the injector, improving the reproducibility, and robustness of the method. Performance of the whole analytical method was evaluated.

Different seafood samples including shellfish, cephalopods, crustaceans and fish were analyzed: mussels, cockles, scallops, octopus, cuttlefish, spider crab, plaice and red mullet from Ría de Vigo and Ría de Arousa were sampling from southern Galician. Sampling locations are shown in Fig 1.

Certain levels of Monobutyltin (MBT) have been detected in cephalopods, bivalves, crustaceans and fish samples. Amounts of dibutyltin (DBT) were detected in cephalopods and shellfish. Tributyltin (TBT) levels were also found in shellfish and some fish. Results will be presented during the Symposium.

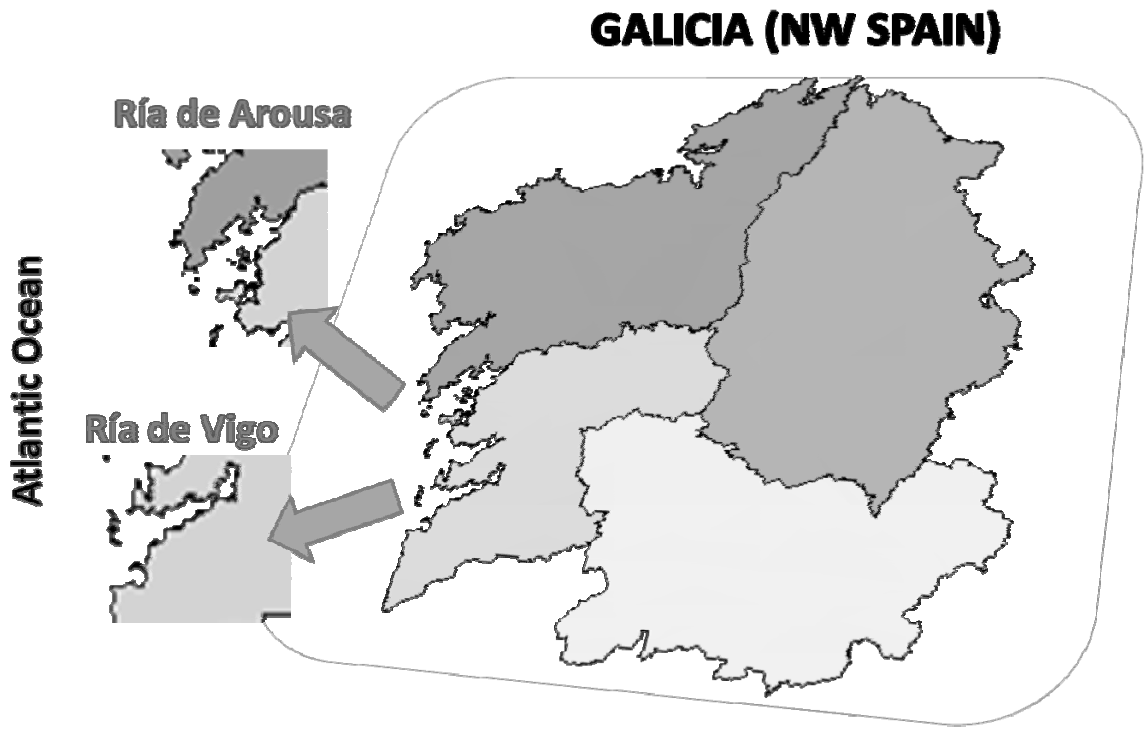

Fig.1. Map showing the sampling area: (Ría de Arousa and Ría de Vigo) in Galicia, northwest Spain.

Acknowledgements:
This work was funded with Grant TAL 2 IN827F from Xunta de Galicia, Spain.

References:
1. Decision 2455/2001/EC amending the Water Framework Directive 2000/60/EC.
2. Díez S, Lacorte S, Viana P, Barceló D, Bayona JM. (2005); E*nvironmental Pollution* 136: 525-536
3. Magi E, Liscio C, Di Carro M (2008); *J Chromatogr A* 1210: 99-107.
4. ORTEP STF Analytical Method (2006),in *http://www.ortepa.org/stabilizers/pages/PDFS/ORTEPA%20-%20Water%20extraction.pdf*