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**Laboratorio
Area Vasta Costa
Livorno**

Analysis of Tributyltin (TBT) in superficial and sea water by solid-phase derivatization and GC-MS/MS determination

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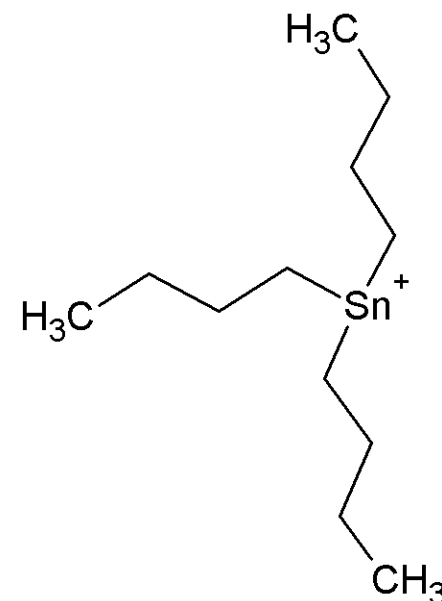
***Settore Laboratorio, Chimica II, Area Vasta Costa**

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TBT

Tributyltin (TBT) compounds conform to the following general formula $(\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2)_3\text{Sn-X}$, where X is an anion (normally a halogen ion such as Cl), or a covalently-bonded functional group.

Tributyltin (TBT) is unique among the organotins in that it is used as a **biocide**. Monobutyltin and dibutyltin don't exhibit these properties.



Tributyltin (TBT) was widely used from the 1950s to 2008 as the active constituent of **organotin antifouling paints** on commercial and military marine vessels, as well as on small recreational watercraft [1].

World production of organotin compounds increased from about 40,000 tons/year in 1985 and peaked at 50,000 tons/year in 1996 [2] .

[1] M. Hoch, Organotin compounds in the environment - an overview, Appl. Geochem. 16 (2001) 719-743.

[2] B. Antizar-Ladislao, Environmental levels, toxicity and human exposure to tributyltin (TBT)-contaminated marine environment. A review, Environ. Int. 34 (2008) 292-308

Biofouling

Ship fouling is the unwanted growth of biological materials, such as barnacles, algae and other marine organisms, on ships' hulls immersed in seawater.



Chthamalus stellatus

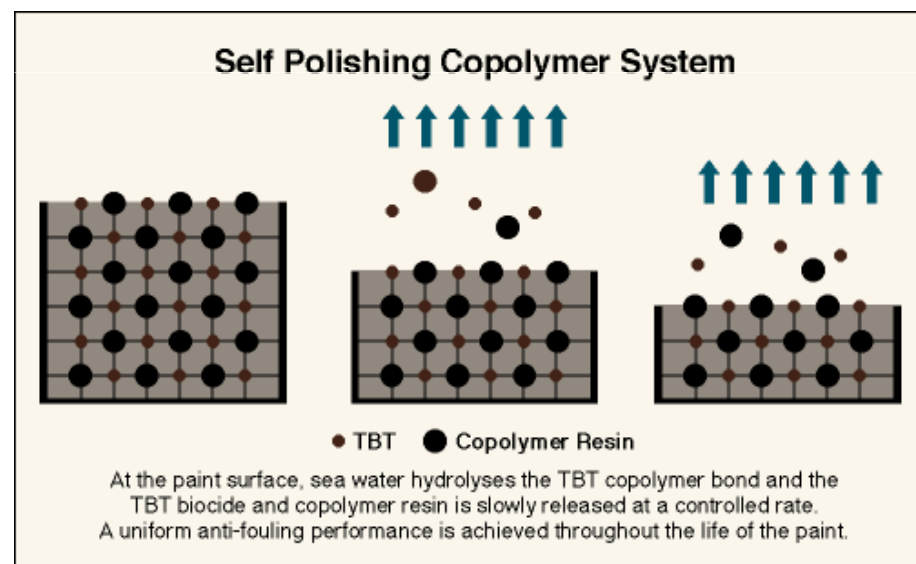
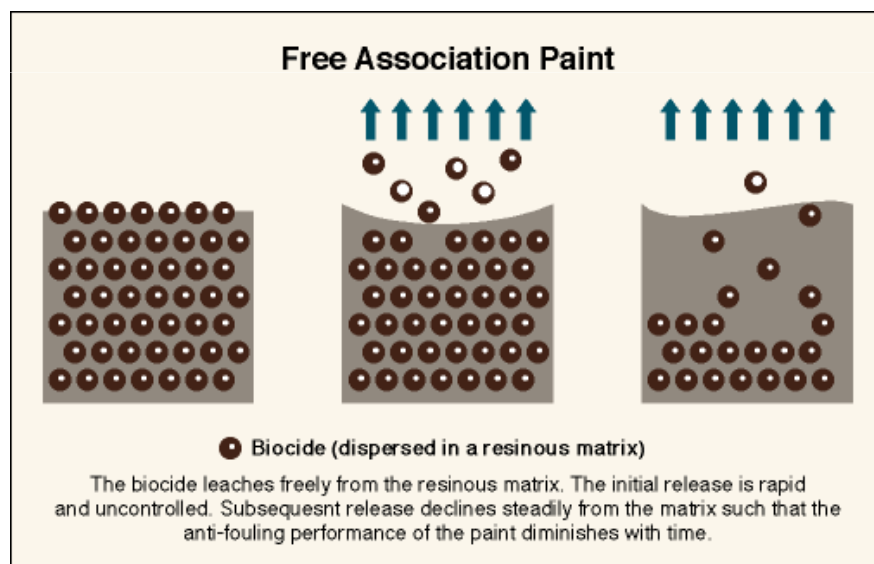


Biofouling has a direct economic impact on marine industry. It has been reported that a 1 mm thick layer of algal slime increases hull friction by 80% and causes a 15% loss in ship speed, whereas a 5% thickness increase in fouling for a tanker of 250,000 tons will increase fuel consumption by 17% [3].

[3] S.M. Evans, A.C. Birchenough, M.S. Brancato, The TBT ban: out of the frying pan into the fire?. Mar Pollut. Bull. 40 (2000) 204-211.

Antifouling paints

Antifouling paints are applied to ships' hulls to prevent the growth of these marine organisms to reduce friction between the hulls of ships and water. Effective antifouling techniques using antifouling paints enable ships to travel more smoothly and faster in water and so reduce fuel costs.



Figures reproduced from Bennett, R.F. 1996. Industrial manufacture and applications of tributyltin compounds. In: Tributyltin: Case Study of an Environmental Contaminant, editor S.J. de Mora. Cambridge University Press, Cambridge.

Sources of TBT contaminant in the marine environment

In the last 40 years scientific studies have shown that TBT-based anti-fouling paints release highly toxic TBT into aquatic systems from relatively diffuse sources such as TBT-based antifouling paint surfaces and from intense point sources such as shipyards and hardstand areas within commercial harbors and marinas [4].

Main ways in which TBT can enter the marine environment

TBT leached into seawater from antifouling paints applied to the hulls of ships

TBT-based antifouling paints scraped off from ship's hulls

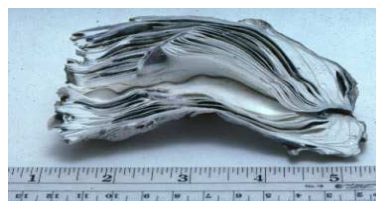
Cleaning activities in shipyards

Removal of paints and discard of paint remnants

[4] E.D. Burton, I.R. Phillips, D.W. Hawker, In-situ partitioning of butyltin compounds in estuarine sediments, Chemosphere 59 (2005) 585-592.

Toxicity

Adverse effects of TBT on oyster growth were observed in oyster farms on the Atlantic coast of France in 1980s [5] and it raised serious concerns on the toxicity of TBT to marine ecosystems and waterways, even at very low concentrations.



A normal oyster shell... and one exposed to TBT

ACUTE

Nervous System

GABAergic transmission in the immature neurons

Immune System

Immunosuppression

Cellular Metabolism

Changes in energy metabolism

Oxidative stress

Induction of ACP and AKP
Activation of SOD and CAT

CHRONIC

Reproductive

Superimposition of male characters as females gastropods with repercussions on the reproductive cycle (imposex)

[5] M. Hoch, Organotin compounds in the environment - an overview, Appl. Geochem. 16 (2001) 719-743.

TBT in water and sediments

Removal processes of TBT in **water column**



Half life is in the range of several days to several weeks.

Temperature
pH
Depth
Salinity
Microbial
Concentration
Suspended Particulate

TBT shows a tendency for both **bioconcentration** and **sediment accumulation**.



Half life in sediments is in the range of several years.

Sediments are potential environmental sinks for TBT and can also act as a store for renewed contamination. As a result, elevated concentrations of TBT are often found in effluents from shipyards and docks or in sediments around harbours, shipyards or docks.

Degradation processes of TBT

Item	Characteristics
<i>Breaking of Sn-C</i>	UV irradiation, chemical agent, thermal cleavage, biotic processes
<i>Degradation</i>	Progressive removal of the organic groups attached to the tin atom $\text{Bu}_3\text{SnX} \rightarrow \text{Bu}_2\text{SnX}_2 \rightarrow \text{BuSnX}_3 \rightarrow \text{SnX}_4$
<i>Toxicity</i>	Maximum biological activity in any alkyltin, $\text{R}_n\text{SnX}_{4-n}$, occurs when $n=3$

International regulations

Europe	Year	Regulations
France	1982	Prohibited the use of TBT-based paints on vessels less than 25 m LOA, except for aluminium-hulled vessels.
United Kingdom	1985 1987	Sale of TBT-based paints restricted, effective ban on TBTO free-association paints. Prohibited the use of TBT-based paints on vessels less than 25 m LOA and on fish-farming equipment. TBT antifoulants available only in 20 L containers. All antifoulants to be registered as pesticides; Advisory Pesticides Committee must approve sale and use. Washing/blasting slurry treated as hazardous.
Norway	1989	Prohibited the use of TBT-based paints on vessels less than 25 m LOA.
Sweden	1989 1992	Prohibited the use of TBT-based paints on vessels less than 25 m LOA. Maximum leaching rate of $4\mu\text{g}/\text{cm}^2/\text{day}$ for vessels greater than 25 m LOA. All antifoulants must be registered
Germany	1990	Prohibited the use of TBT-based paints on vessels less than 25 m LOA. Ban on retail sale. Ban on its use on structures for mariculture. Regulation for the safe disposal of antifouling paints after removal.
Netherlands	1990	Prohibited the use of TBT-based paints on vessels less than 25 m LOA. Washing/blasting slurry used to prepare TBT antifoulants may be treated as hazardous waste TBT antifoulants available only in 20 L containers All antifoulants must be registered

Directive 89/677/EEC: introduce the organotin compounds in the list of hazardous substances subjected to restrictions and unified Europe-wide discipline of antifouling products containing TBT.

The prohibition of the use of TBT compounds



International Convention on the Control of Harmful
Anti-fouling Systems on Ships

(AFS 2001)



European Commission Parliament

included the recommendations
of IMO and adopted the:

Regulation 782/2003 on the prohibition of organic compounds on ship

Article 4

Prohibition of the application of organotin compounds which act as biocides (2003)

Article 5

Prohibition of the bearing of organotin compounds which act as biocides (2008)

Environmental regulations

Directive 2000/60/EC (Water Framework Directive)
of the European Parliament and of the council of 23 October 2000
establishing a framework for Community action in the field of water
policy (OJ L 327, 22.12.2000, p. 1)

Environmental quality standards (**EQS**) for *priority substances* and certain
other pollutants (as provided for in Article 16 of Directive 2000/60/EC as laid
down in European Parliament legislative resolution of 17 June 2008)

Name of substance	AA-EQS Inland surface waters	AA-EQS Other surface waters	MAC-EQS Other surface waters	AA-EQS Sediments <i>D.M. 260/2010</i>
Tributyltin compounds	0.0002 µg/l	0.0002 µg/l	0.0015 µg/l	5 µg TBT / kg d.w.

AA: Annual Average

MAC: Maximum Allowable Concentration

CEN TC 230 received Mandate M 424 from the European Commission, DG Environment.

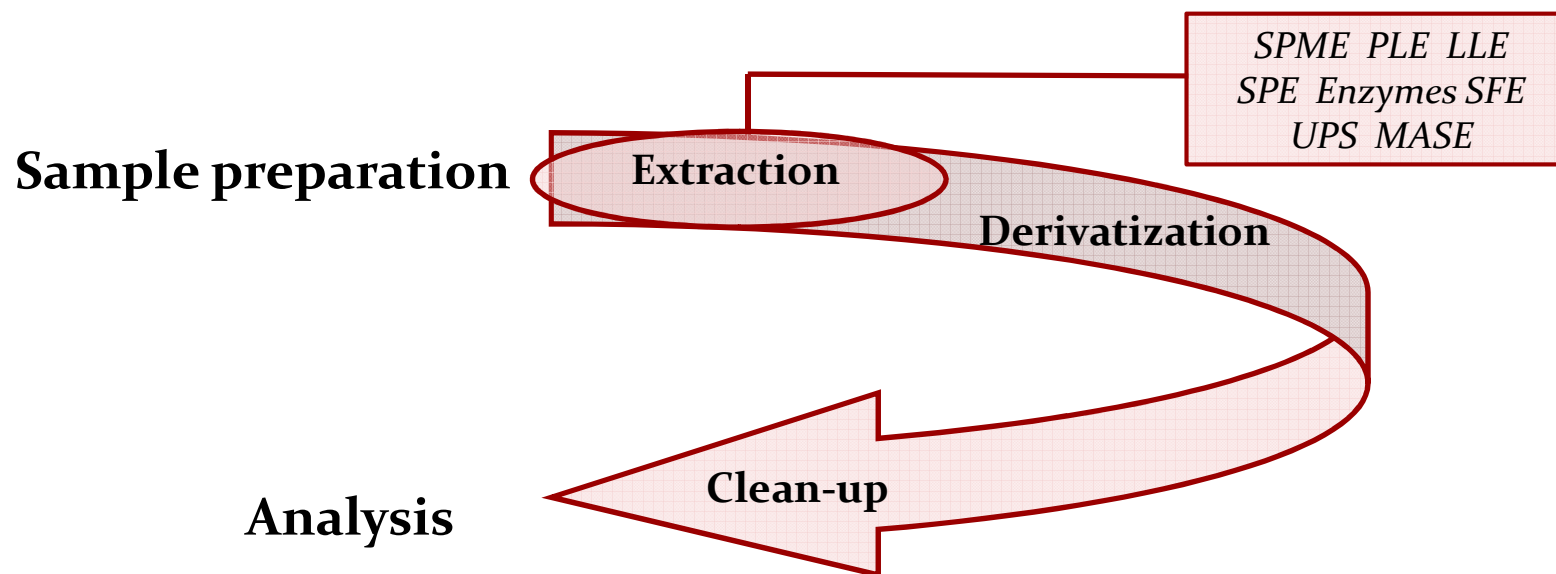
The objective of M 424 is to develop European standards in support of the ecological and chemical status monitoring requirements of the **Water Framework Directive 2000/60/EC (WFD)**, following the design of the monitoring programs carried out and reported by the Member States in March 2007.

With the aim of achieving good surface water chemical status and in accordance with the provisions and objectives of **Article 4 of Directive 2000/60/EC**, the environmental quality standards (**EQS**) for priority substances and certain other pollutants (as provided for in **Article 16 of Directive 2000/60/EC** as laid down in European Parliament legislative resolution of **17 June 2008**) form the basic requirements to be fulfilled by the methods to be developed for standardization purposes.

These requirements are:

- The analysis of whole water samples, i. e. methods shall enable the analysis of samples containing up to **0,5 g/L of suspended solids**;
- The **limit of quantification (LOQ)** shall be equal or less than **30 % of the EQS**; in order to meet the very low LOQ requirements, larger intakes, smaller final volumes, larger injection volumes and/or more sensitive detectors shall be applied.
- The measurement **uncertainty shall be equal or less than 50 %**;
- The methods shall be fully in-house validated and tested for ruggedness prior to interlaboratory validation;
- A European interlaboratory comparison study shall be conducted according to ISO 5725.

Analysis of TBT



<i>Separation techniques</i>	<i>Revelation techniques</i>
GC	AAS, AES, FPD, ICP-MS
HPLC	UV, FI, Elettrochemistry, OES, AAS, AES, MS

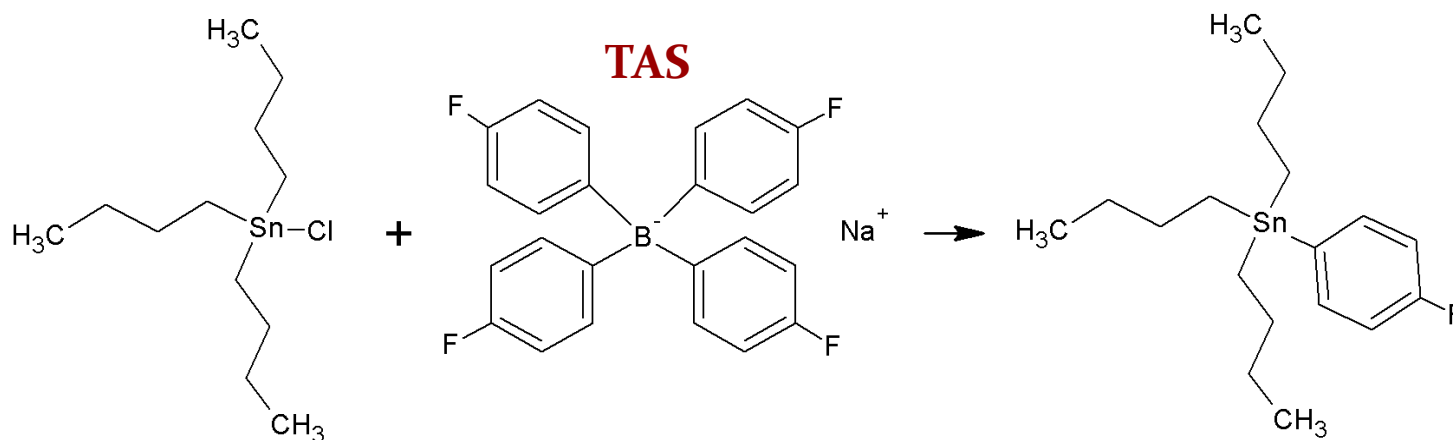
The tributyltin is not a volatile compound, therefore the analysis in GC requires a stage of derivatization of the sample

Derivatization reaction with sodium tetrakis fluorophenylborate (TAS)

Electronegative ligands X on tin frequently undergo ready exchange with electronegative ligands Y on other metals or on tin, providing a method of preparing other halides and pseudohalides from the chlorides which are more readily available.



Alwyn G. Davies. Organotin Chemistry Second, Completely Revised and Updated Edition. 2004
 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim



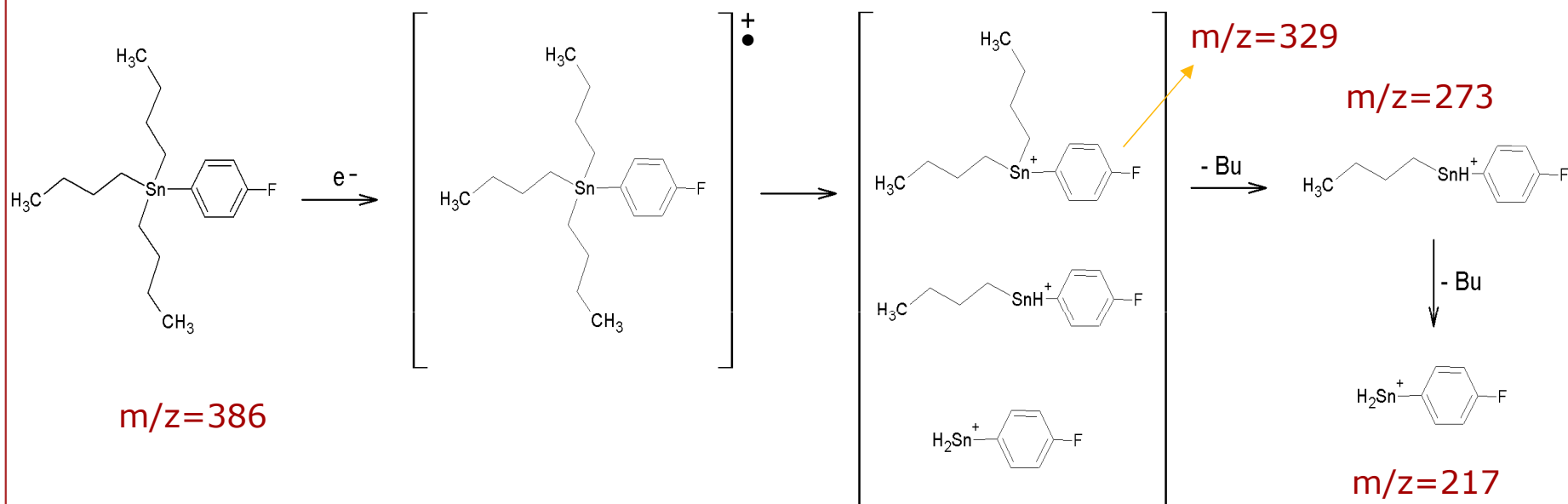
in situ reaction (aqueous solution)

Comparison of methods

	Literature Method (*)	Method A	Method B
Derivatization	50 mL aqueous sample + TBT-D27 (unspecif. quantity) + 60 mg $\text{NaB}(\text{C}_6\text{H}_4\text{F})_4$	250 mL aqueous sample + TBT-D27 (5 ng) + 100 mg $\text{NaB}(\text{C}_6\text{H}_4\text{F})_4$	100 mL aqueous sample + TBT-D27 (5 ng) + 10 mg $\text{NaB}(\text{C}_6\text{H}_4\text{F})_4$
Extraction	Single stage: 10 mL toluene, mechanical shaker for 60 min	Two stage: with 10 + 10 ml of toluene, mechanical shaker for 40 + 40 min.	Single stage: 0,3 ml of trifluorotoluene + 10 ml of acetone, mechanical shaker for 40min. Centrifugation 3000 rpm for 5 min
Evaporation And Redissolution	of the organic extract to 0,1 mL + 0,4 mL di trihexyltriphenyltin (unspecif.icated concentration)	of the organic extract to dryness. Redissolution of residue with 0,1 ml of $\text{PCB}138\ 12\text{C}^{13}$ (20ng/ml in toluene)	of the organic extract to dryness. Redissolution of residue with 0,1 ml of $\text{PCB}138\ 12\text{C}^{13}$ (20ng/ml in toluene)
Revelation	GC-IT	GC-QQQ	GC-QQQ
Conc. Factor	100	2500	1000
LOD	0.07 pg Sn	0.001 $\mu\text{g/l}$	0,0006 $\mu\text{g/l}$

* S. Tsunoi, H. Shioji, M. Tanaka, 2004. *Derivatization of tributyltin with sodium tetrakis (4-fluorophenyl)borate for sensitivity improvement of tandem mass spectrometry*, Analytical Sciences.

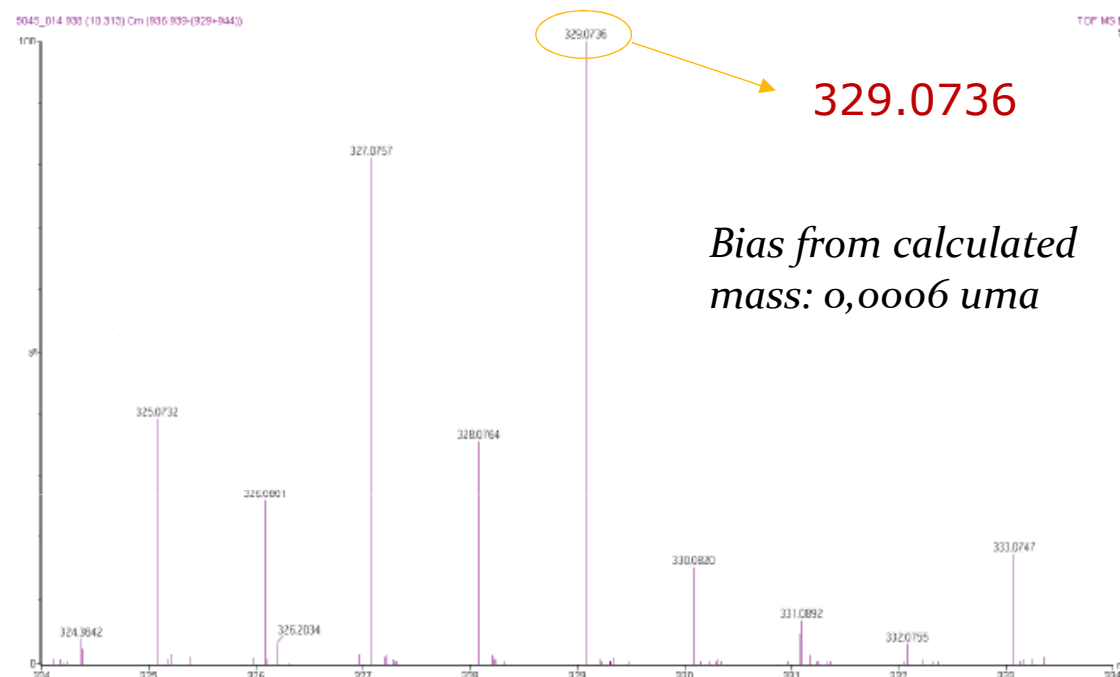
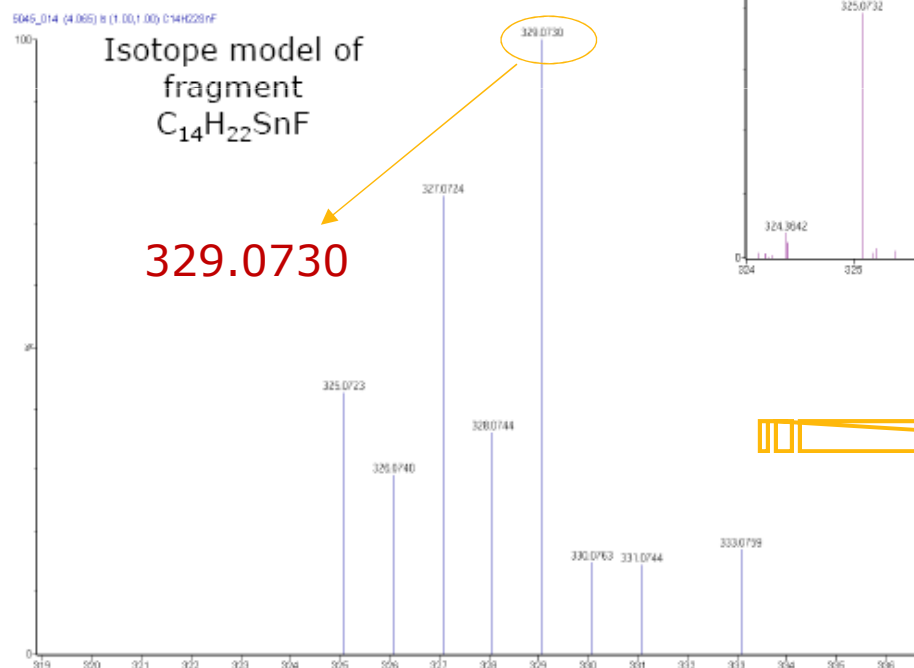
EI Fragmentation of TBT-Fluorophenyl



S. Tsunoi, H. Shioji, M. Tanaka, 2004. *Derivatization of tributyltin with sodium tetrakis (4-fluorophenyl)borate for sensitivity improvement of tandem mass spectrometry*, Analytical Sciences.

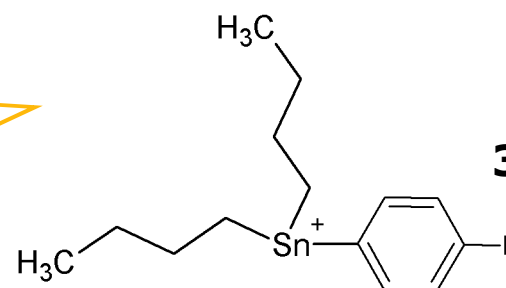
Characterization of 4,FluorophenylTBT

Comparison between calculated spectra (below on the left) and high resolution full scan spectra (GC-TOF)



329.0736

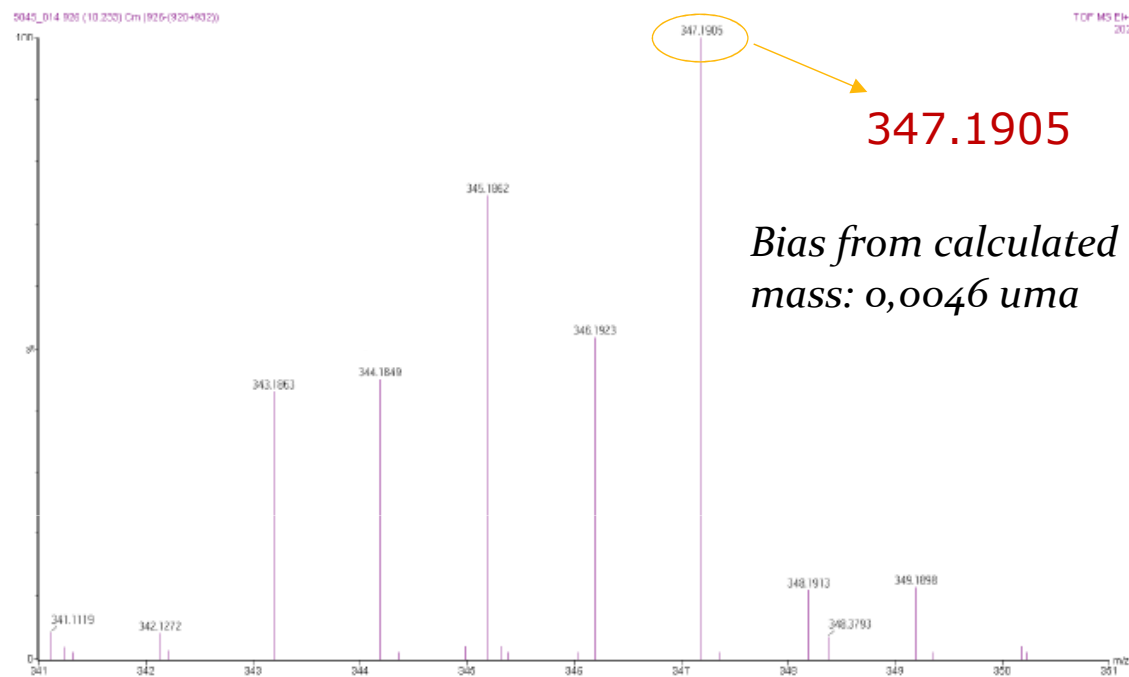
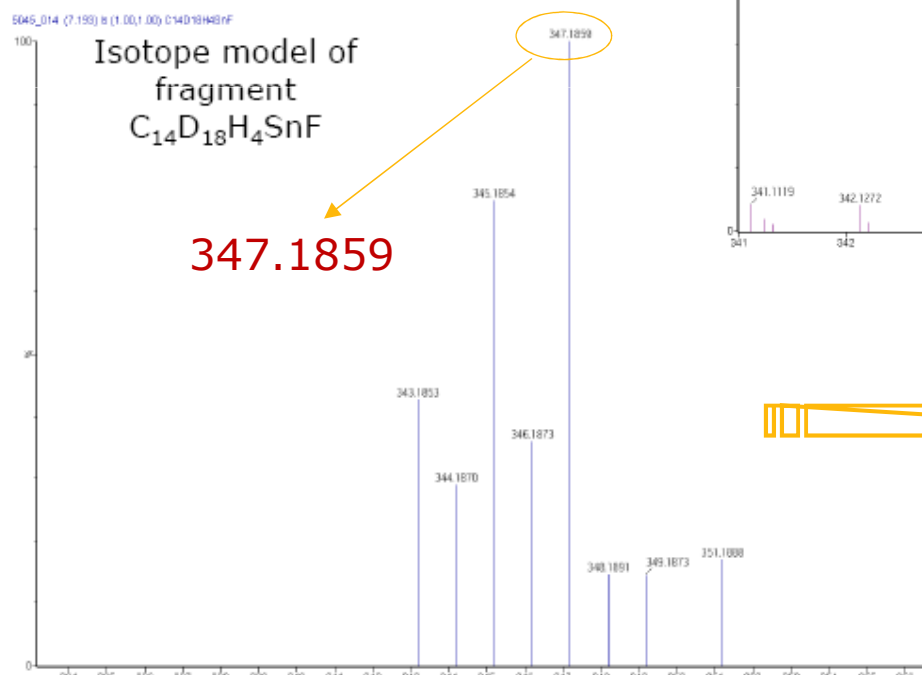
Bias from calculated mass: 0,0006 uma



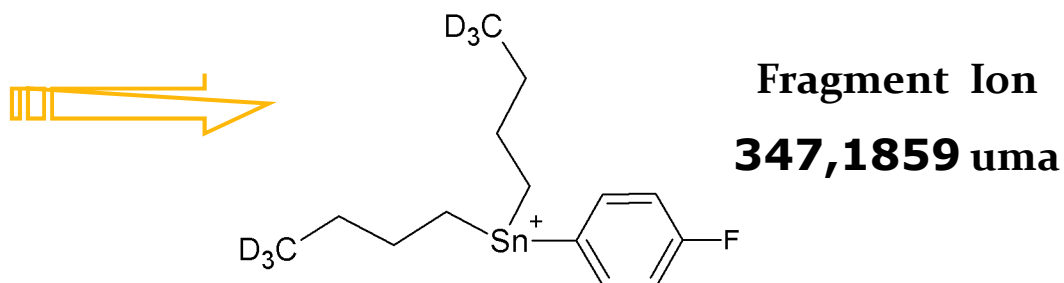
Fragment Ion
329,0730 uma

Characterization of 4,FluorophenylTBT-D₂₇

Comparison between
calculated spectra (below on
the left) and high resolution
full scan spectra (GC-TOF)



*Bias from calculated
mass: 0,0046 uma*



Characteristic of MS/MS method

We chose to investigate two transitions:

4, FluorophenyTBT	329 → 273	273 → 217
4, Fluorophenyl TBT-D₂₇	347 → 283	283 → 219
PCB₁₃₈ ¹²C¹³	372 → 337	372 → 301

Compliance with Decision 2002/657/CE, Table 6

Examples of the number of identification points earned for a range of techniques and combinations thereof

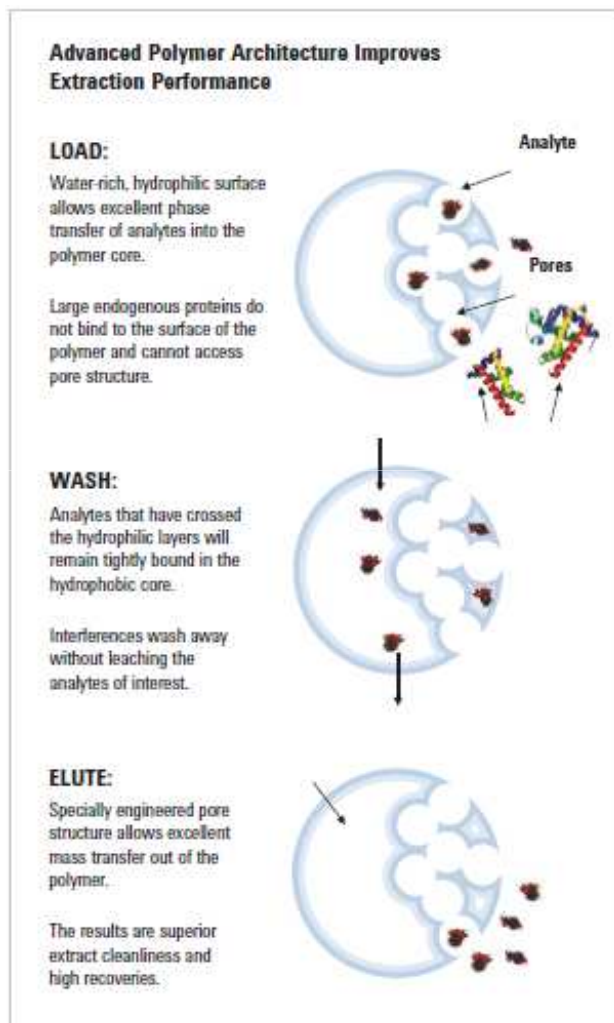
Technique(s)	Number of ions	Identification points
GC-MS-MS	2 precursor ions, each with 1 daughter	5

ARPAT current method with SPE extraction

Conditioning of the cartridge	5 ml CH ₂ Cl ₂ 5 ml Acetone 5ml Methanol 2 x 5 ml H ₂ O/Methanol 95/5
Loading the sample	475 ml aqueous sample +25 ml Methanol + 0,3 ng TBT-D27
Derivatization	2 ml NaB(C ₆ H ₄ F) ₄ 4000 ppm, 60 minutes
Washing	2 x 5 ml H ₂ O/Methanol 95/5
Elution	6 ml Acetone/Toluene 3/1
Evaporation	of the organic extract to dryness
Clean-up	3 x 1 ml toluene and filtration with 1.0 µm glass fiber filter
Evaporation and Redissolution	of the organic extract to dryness. Redissolution of residue with 100µl of PCB138 12C ¹³ (20 µg/l in toluene)
Revelation	GC-QQQ
Conc. Factor	5000
LOD	0,0001 µg/l

SPE features

Bond Elut Plexa: non-polar divinylbenzene-based neutral polymeric sorbent



- Fast flow, reproducible performance and ease-of-use
- Improved extract cleanliness minimizes matrix interferences

Typical properties

Parameters	Bond Elut Plexa
Pore size (nominal)	100 Å
Particle size	45 µm
Specific surface area m ² /g	450 m ² /g
pH range stability	1-14
Chemical resistance (solvents)	Broad range of protic and non-protic polar and non-polar solvents
Retention capacity / Ion exchange capacity	10% of sorbent mass

Cartridge type:
 200mg,
 3 ml - 6 ml

Figures reproduced from Agilent Bond Elut brochure

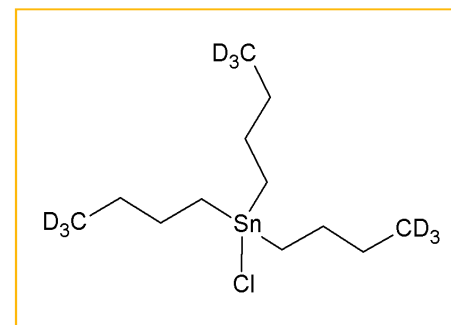
Isotope dilution

- Introduction in the process of derivatization-extraction of a substance with a chemical-physical behavior similar to the compound researched;
- Good gas-chromatographic separation level between the two isotopes;
- Partial loss of analyte during processing is compensated and chemical or physical interferences affect each isotope identically;
- Most accurate and precise method for trace analysis.

To check the analytical procedure it was used a deuterium-labelled standard (TBT-D₂₇).



Determination of the Response Factor RF



Response Factor

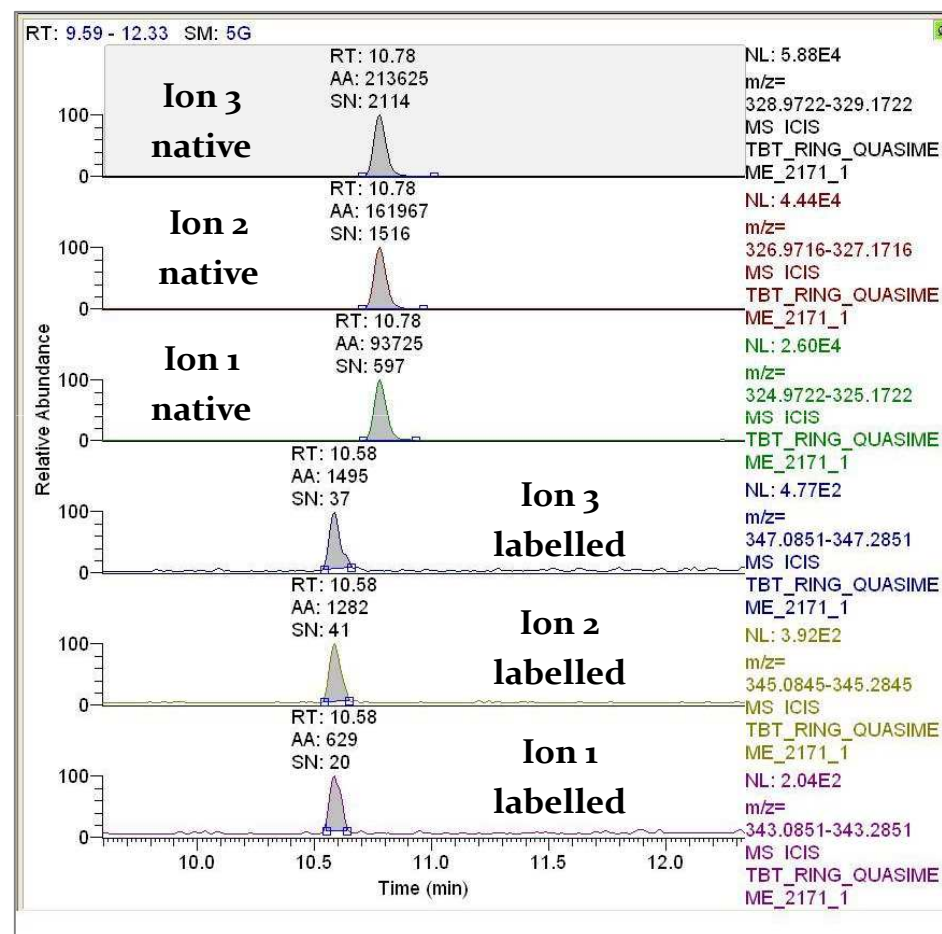
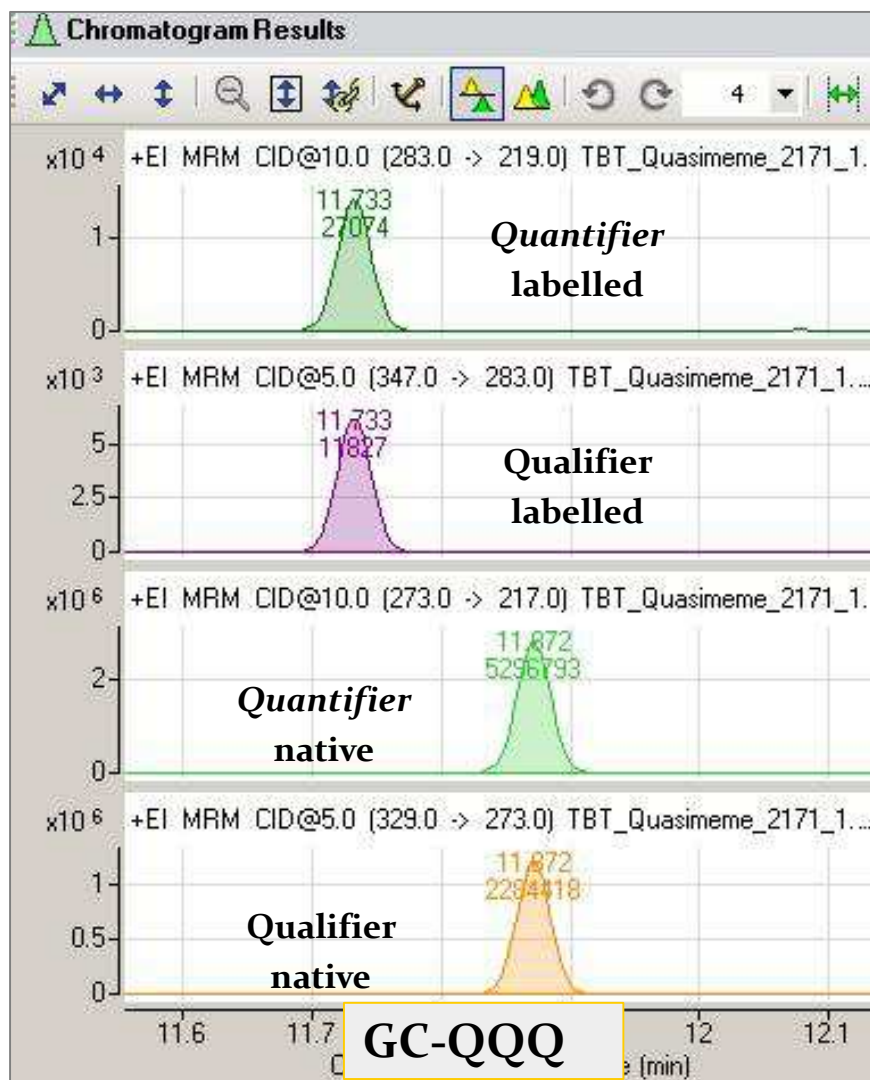
The determination of the average Response Factor (RF) was carried out by subjecting the entire analytical procedure over 40 blanks spiked with TBT native in the range of concentration from 1,5 ppb to 4 ppb and deuterium-labelled TBT-D₂₇ at concentration of 3 ppb.

Average RF	1,34
Minimum RF	1,08
Maximum RF	1,60
% Variability of calculated concentration	20 %

$$RF = \frac{\text{Area TBT} \times \text{Conc. TBT-D}_{27}}{\text{Area TBT-D}_{27} \times \text{Conc. TBT}}$$

Real Samples

Proficiency Tests

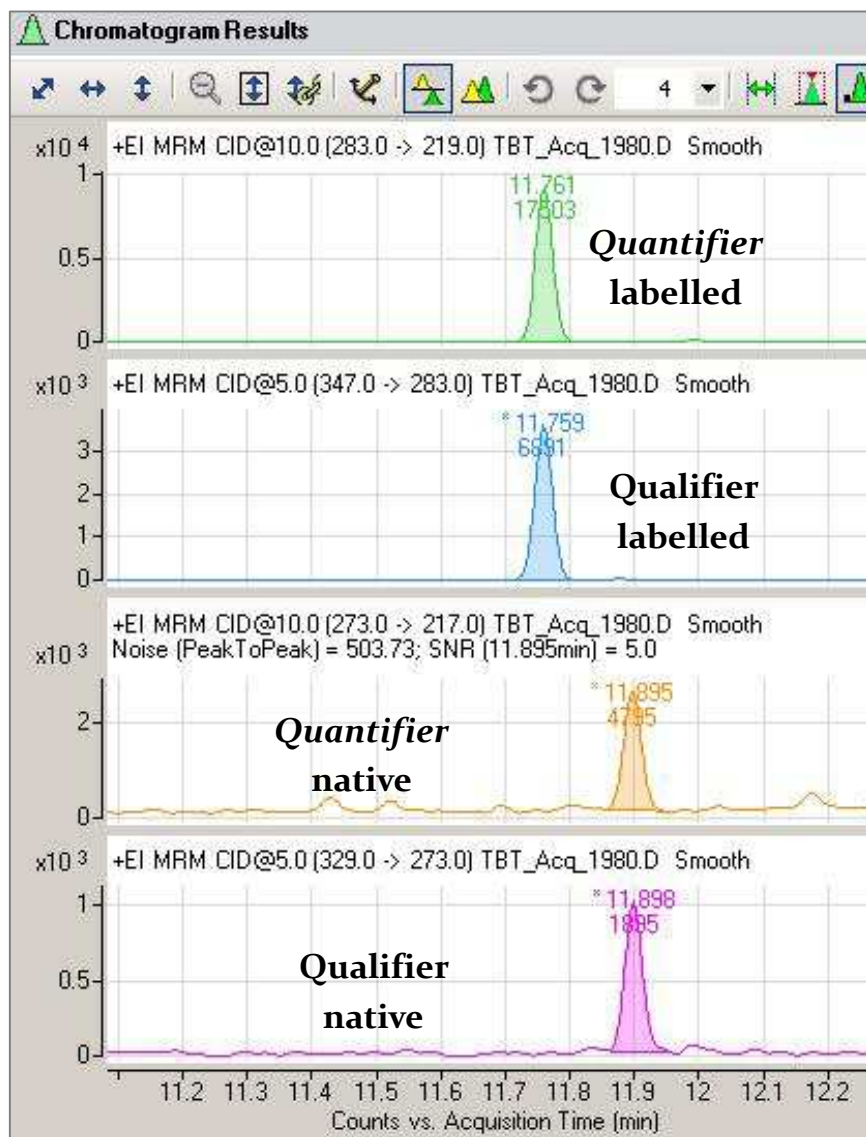


AGLAE Proficiency tests Results

Date	Assigned value, ng/l	Z-score
5/2015	57,6	+1,48
5/2015	6,98	-0,25
5/2015	57,2	+1,17
11/2015	252	+1,84
03/2016	170	+0,08

$|Z| \leq 2$ denotes satisfactory results of analysis

Real Samples



Sample with TBT concentration
 below SQA: $<0,0002 \mu\text{g/l}$ ($<0.2 \text{ ng/l}$)
 Calculated amount: $0,1 \text{ ng/l}$

Area Quantifier native = 4795
 S/N = 5.0

Hypothetical amount in sample
 with S/N = 3 : **0.06 ng/l**



instrumental LOD

ARPAT current method with SPE extraction

- ✓ *Concentration factor: 5000;*
- ✓ *Defined analytical aims (LOD equal to 0,1 ng/L);*
- ✓ *Minimum amount of TAS in solution;*
- ✓ *Knowledge of second ionization fragments;*
- ✓ *Revelation with GC-QQQ in MRM mode;*
- ✓ *Attestation of positive results by GC-HRMS (DFS)*



GC-QQQ in MRM mode



GC-HRMS

Results of enviromental monitoring of TBT up to 2015 in water column

	Costa Versilia	Antignano	Costa Rosignano	Costa Piombino	Carbo-nifera	Foce Bruna	Foce Ombrone	Porto S. Stefano	Isola del Giglio	Isola di Capraia
2010	<0,01	<0,01	N. P.	N. P.	<0,01	<0,01	<0,01	N. P.	N. P.	N. P.
2011	0,0034	N. P.	N. P.	0,0090	<0,005	N. P.	N. P.	<0,005	N. P.	N. P.
2012	<0,005	0,0128	0,0075	N. P.	0,0103	0,0167	<0,005	N. P.	<0,005	<0,005
2013	0,3352	0,0026	0,0013	0,0028	<0,005	0,0167	0,0013	<0,005	0,0015	N. P.
2014	0,0005	0,0006	0,0007	<0,0006	0,0007	0,0048	0,0010	0,0006	0,0008	<0,0006
2015	0,0006	0,0015	0,0006	0,0011	0,0020	0,0008	0,0017	0,0010	0,0016	<0,0006

N. P. : samples unplanned

Concentration TBT in µg/l

“Annuario dei dati ambientali ARPAT 2015”

“Annuario dei dati ambientali ARPAT 2016” ; www.arp.at.it

Thanks

