

PCBs (Arochlor™ 1242, 1254 and 1260) in water and mussels by GC-MS-MS (Varian - SATURN 2000)

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ABSTRACT: The Polychlorinated biphenyls (PCBs) are synthetic organic chlorinated compounds that are widely used as fluids in transformers and capacitors. They are also called Askarels. In the 80's PCBs were confirmed to be Persistent Organic Pollutants (POPs), toxic and containing reasonable contamination of dioxins. In this decade, about 70 to 90 congeners of PCBs were already present in the environment. In despite of the prohibition of PCB utilization in newer equipments many old equipments are still in use, being gradually substituted. The lipophilic nature and persistence of these compounds contribute to their high potencial of bioacumulation. Mussels as good indicators of the presence of PCBs, were used in this study. The analytical method most used for the PCBs analysis is the GC-ECD (Gas Chromatography with Eletron Capture Detector) and the purification is done in open column chromatography using florisil, alumina or silica as stationary phases and with the collection of three fractions for analysis. In this study we optimized a GC-MS-MS (Gas Chromatography with Mass Spectrometer working with double ion selection) to quantify PCBs in mussels. The PCBs were quantified as the most common commercial products used: Arochlor™ 1242, Arochlor™ 1254 and Arochlor™ 1260. A purification method was optimized in a single fraction to better suit instrumental determination by GC-MS-MS, more selective then GC-ECD, making it rapid and economical. This purification technique was tested for PCBs and also for organochlorinated pesticides and policyclic aromatic hydrocarbons, all by GC-MS-MS with recoveries $\geq 80\%$ for most of the analytes. Detection limit (3σ) of 0.8-1.2 ng/g and quantification limit (10σ) of 2.7-4.0 ng/g were obtained for the three Arochlor™. The GC-MS-MS Technique has the advantage of being a confirmatory technique; that is the detected peaks can be confirmed by the comparison of the obtained spectra. The disadvantages are the great work of the method optimization and the risk of not getting good detection limits as the GC-ECD method. Besides this is necessary the verification and analysis of each peak spectrum after analysis. The optimized technique was applied in the analysis of nine samples of mussels *Anomalocardia brasiliiana* (papa-fumo) collected in distinct places of the Todos os Santos Bay, state of Bahia, Brazil. Values ranged from <1 to 42 ng/g and were similar to results obtained in 1988 in this Bay. These results show reasonable PCBs contamination in three urban sites two of each near industrial areas and one near the former Salvador city waste dumping area.

MATERIALS

- a) GC-MS-MS Saturn 2000 with 8200 autosampler from VARIAN Instruments.
- b) Arochlor 1242, 1000 μ g/mL in Methanol from Absolute Standards, # 70018
- c) Arochlor 1254, 1000 μ g/mL in Methanol from Absolute Standards, # 70021
- d) Arochlor 1260, 1000 μ g/mL in Methanol from Absolute Standards, # 70020
- e) Internal Standard: Acenaphtene d10, 1000 μ g/mL in Methanol from Absolute Standards, # 79002
- f) Surrogate: Fluorene d10, 1000 μ g/mL in Methanol from Absolute Standards, # 71490
- g) SPE column with 5g of Florisil (Varian 1225-6030).
- h) Sodium Sulfate anidrous.
- i) Petroleum Ether Pesticide Grade.
- j) Methylene Chloride Pesticide Grade.
- k) Ultrasonic Bath.
- l) Solution of Internal Standard, Acenaphtene d10 at 100 μ g/mL in Chloroform (dilution of e)).
- m) Solution of PCBs at 10 μ g/mL in Chloroform (dilution of b), c) and d)).
- n) Solution of Surrogate, Fluorene d10 at 100 μ g/mL in Chloroform (dilution of f))
- o) Vials of 2mL for autosampler with TriSpring Inserts of 200 μ L.

GC-MS-MS OPTIMIZATION

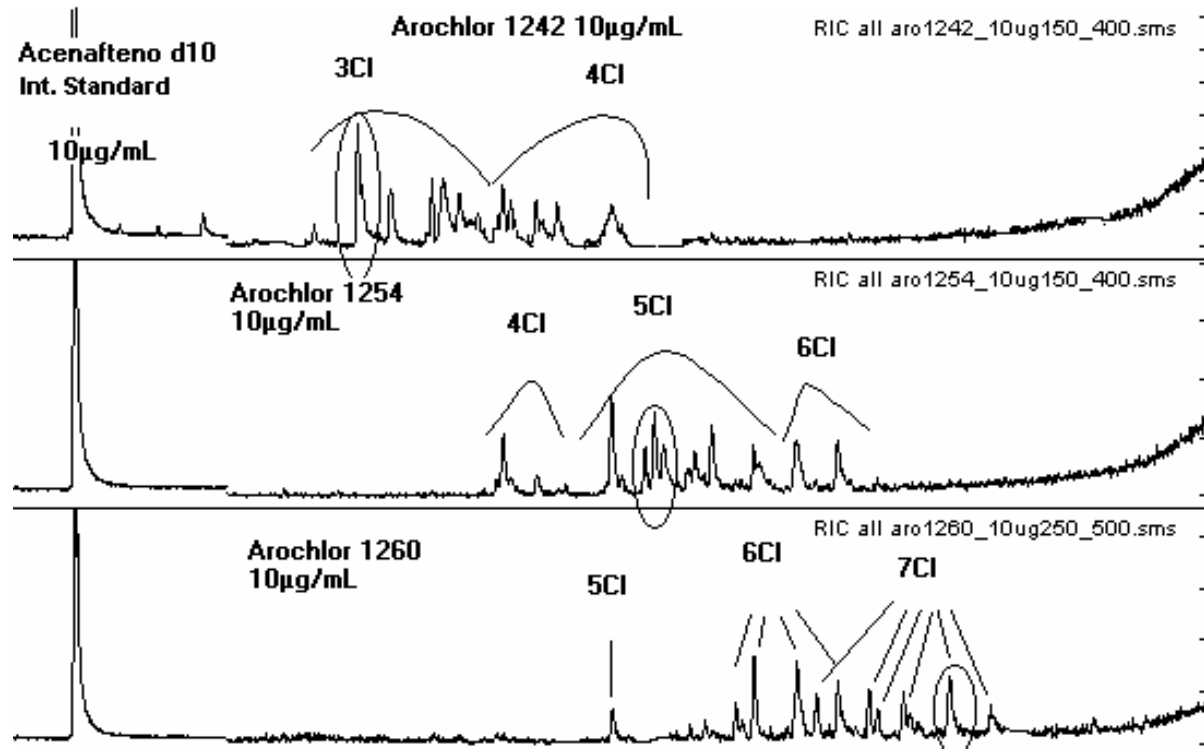
The GC in all the tests was programmed as shown in Table-1

Table-1: Cromatograph Program

Injected Volume:	2 μ L						
Column pressure:	2 psi						
Column:	CP-SIL 8 CB Low Bleed/MS, 30m, 0,32mm ID, 0,25 μ m film						
Injector Temperature:	250°C						
Oven Program							
50°C	15°C/min.	110°C	6°C/min.	270°C	15°C/min.	300°C	2,33min.
Splitless injector (1177)							
Split rate: 1:10							
Time (min.)	Status						
Inicial	ON						
0,00	OFF						
0,50	ON						

At first a standard solution containing 10 μ g/mL of each Arochlor mix in Methylene Chloride was injected as "Total Ion" in the GC-MS-MS system. The obtained chromatograms are shown in Figure-01.

Figure-01: Total Ion (GC/MS) Chromatograms of the Arochlors



The Figure-01 shows what peaks were chosen to be used in the quantification process (calibrations curves preparing). We can observe in this figure that the peaks chosen for each mix is not present in the others in concentration that could cause interference.

The optimization process was concluded developing a GC-MS-MS program specific for these analytes. The use of MS-MS program caused a serious noise reduction and about 30 times reduction in detection limits. The figures 2, 3 and 4 shows optimized chromatograms for the three Arochlors and the MS-MS spectrum obtained.

Figure-2: GC-MS-MS Arochlor 1260 optimized chromatograms at 0,2µg/mL range

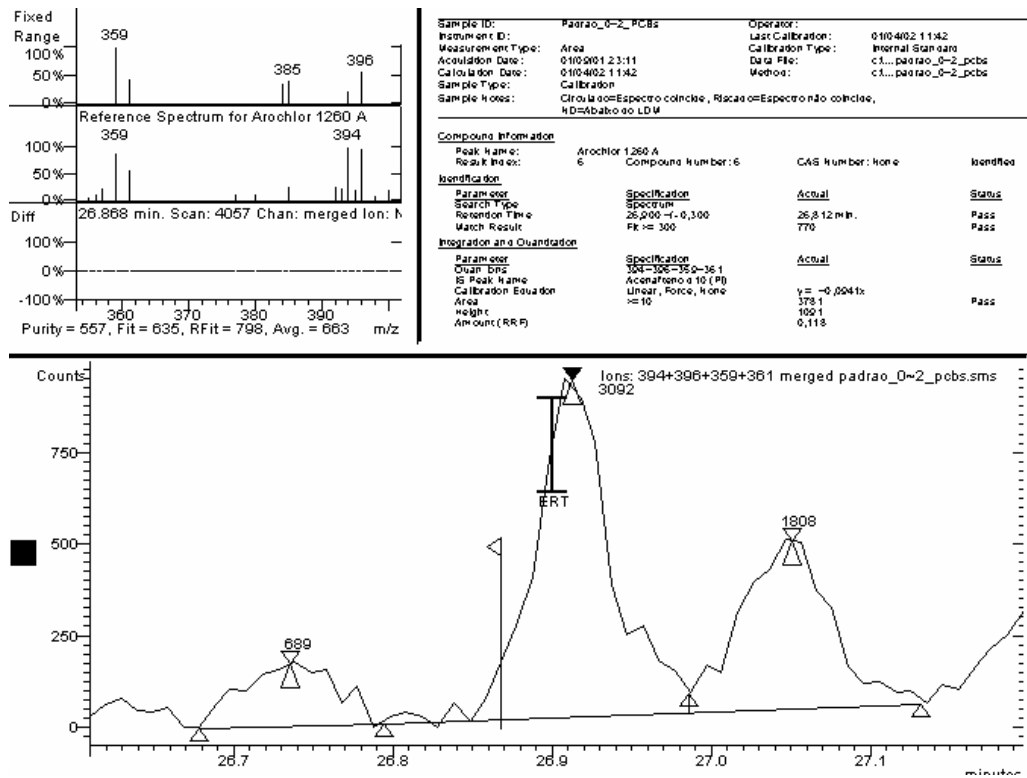


Figure-3: GC-MS-MS Arochlor 1254 optimized chromatograms at 0,2µg/mL range

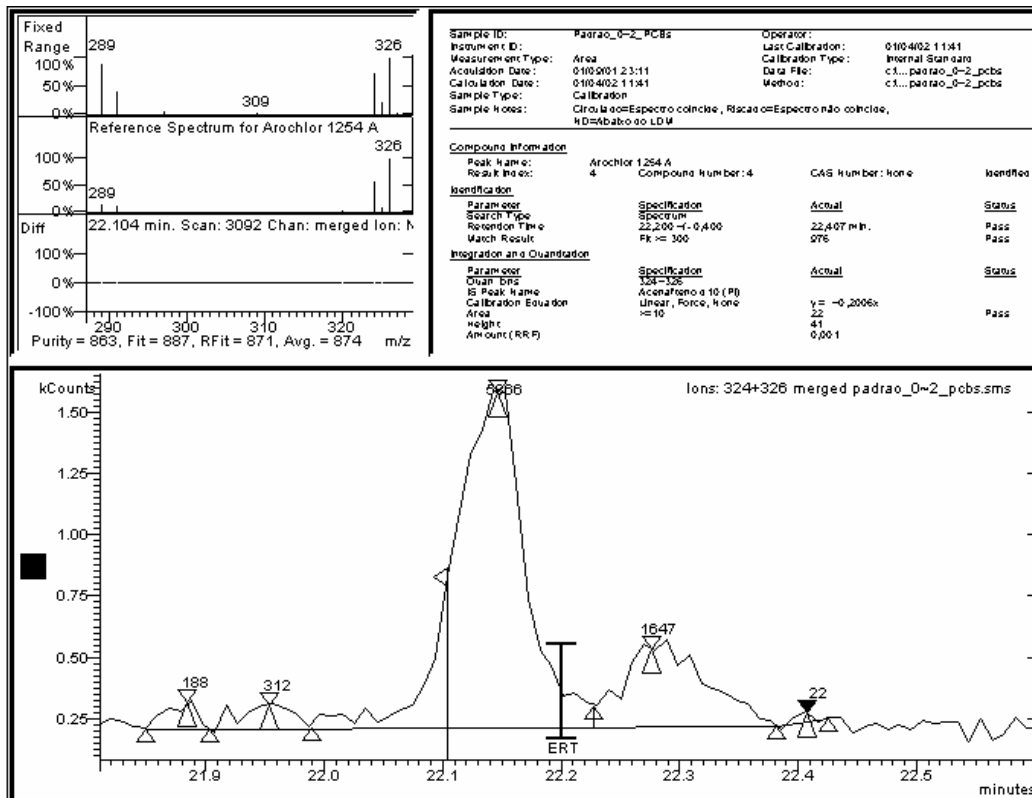
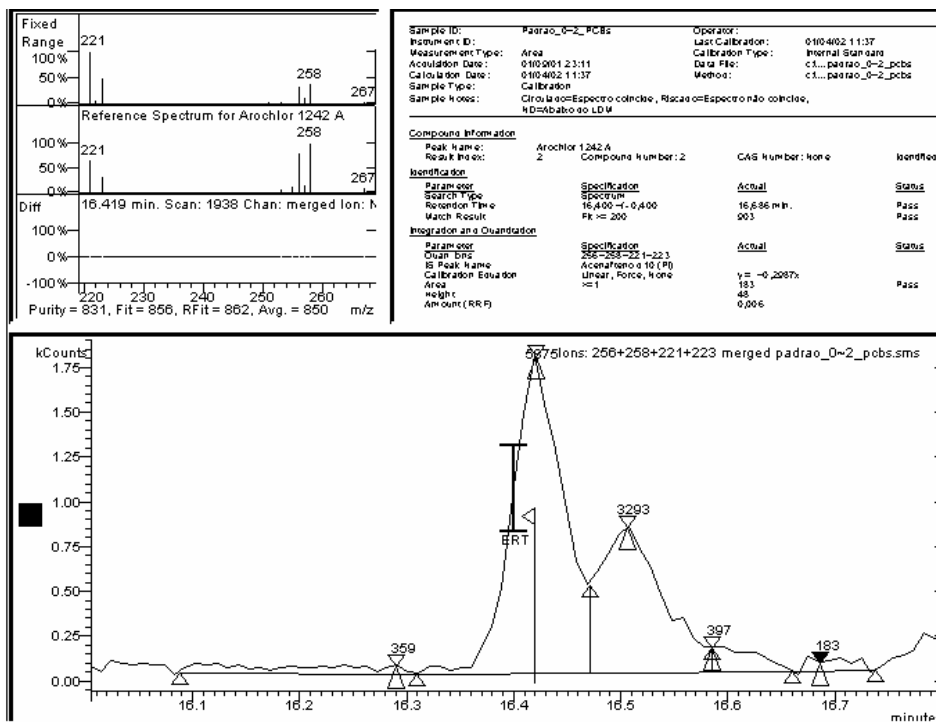


Figure-4: GC-MS-MS Arochlor 1242 optimized chromatograms at 0,2µg/mL range



The Table-2 shows the peaks used in the detection and quantification by GC-MS-MS

Table-2: Peaks used in the detection and quantification by GC-MS-MS

Compound	Retention time (min.)	Relative Retention time to Acenaphthene d10	Ions used in the quantification
Acenaphthene d10 (IS)	11,0	1,000	162+164
Fluorene d10 (SRG)	12,7	1,154	174+175
Arochlor™ 1242	16,4	1,491	256+258
Arochlor™ 1254	22,2	2,018	326+324
Arochlor™ 1260	27,0	2,454	396+394

The Table-4 and the paragraphs that follows it shows the MS-MS program utilized for the PCBs analysis.

Table-4: MS Detector Program in MRM Mode

MRM Segment (minutes)	Monitored Compounds	Scan range	Ions ± Range	Excitation Storage Level
0 - 4.00	Solvent	--	None	--
4.00 - 14.00	Acenaphthene d10 (IS) and Fluorene d10 (SRG)	100-200	164±3 175±3	100 100
14.00 - 20.00	Arochlor™ 1242	220-270	256±1 258±1	110 110
20.00 - 24.00	Arochlor™ 1254	280-340	324±1 326±1	130 130
24.00 - 30.00	Arochlor™ 1260	350-400	394±1 396±1	180 180

Constant Conditions in all Segments

EI (Electron Ionization): Auto (automatic)
Scan Time: 0,3 seconds
Emission Current: 50µA
Target TIC: 5000 counts
Max. Ionization time: 25000µseg.
Pre-Scan time: 20µseg.

Constant conditions for all the selected ions

Ion Preparation: MRM (Multiple Reaction Monitoring)
Waveform Type: Ressonant
Excitation amplitude: 0.3V

The Method Detection Limits were evaluated by spikes in mussels in the 4 ng/g concentration and calculations by 3 standard deviations of 7 analysis. The LDM was 1ng/g in mussels and were almost equal for the three Arochlors™.

Calibration: the standards were prepared directly in 2,0mL vials with the additions with microseringe of the diluted solutions of the analytes. PCBs were used in four concentrations: 2,0µg/mL; 1,0µg/mL; 0,5µg/mL and 0,2 µg/mL (equiv. to 80ng/g, 40ng/g, 20ng/g and 8ng/g in mussels). In the four calibration standards Acenaphthene d10 and Fluorene d10 were kept ever at 10µg/mL. The final volume was completed to 1,0mL with micro-pipete. By the use of a internal standard the dilution have no direct influence in the results or calibration.

SAMPLE PREPARATION

The mussels samples (*Anomalocardia Brasiliiana*) were collected in the low tide. At least 20 individuals were collected in each of the nine stations in the Todos os Santos bay in Brazil, from 2000, October to 2001, February. The samples were submitted to liofilization in some days after the collection, so they could be analyzed some months after the collection without preservation problems.

Once the mussels or fish samples are not used only for PCBs analysis tests were made to can use the extracts for analysis of PCBs, Organochlorine Pesticides and PHAs all by GC-MS-MS. Spiked samples were pre-purified ever with sulfuric acid and some solvents were tested in the purification process using Florisil and Alumina columns. Table-5 shows the results of the tests.

Table-5: Purification in a single fraction. Spikes of 50 ng/g in mussels

Analyte	Test 1	Test 2	Test 3	Test 4	Test 5
	%Recover	%Recover	%Recover	%Recover	%Recover
PCBs					
Arochlor™ 1242	40	92	80	84	84
Arochlor™ 1254	30	88	78	64	78
Arochlor™ 1260	42	74	70	80	84
PAHs					
Naphtalene	4	8	26	42	102
Acenaphtene	0	0	0	24	62
Fluorene d10 (SRG)	5	4	21	40	81
Fluorene	0	28	50	64	112
Anthracene	0	0	0	2	64
Pest. Organoclorados					
Lindane	32	44	38	46	98
Heptachlor	16	26	4	78	68
Aldrin	12	26	20	72	108
Heptachlor epoxide	24	34	42	36	104
DDE	22	40	54	80	114
DDD+DDT	28	58	66	94	99

Test-1: 30mL of 20% Ethyl Ether :80% of Petroleum Ether. Activated Florisil.

Test-2: 30mL of 20% Ethyl Ether :80% of Petroleum Ether. Florisil with 0,5% water.

Test-3: 30mL of 50% Ethyl Ether :50% of Petroleum Ether. Florisil with 0,5% water.

Test-4: 30mL of 50% Ethyl Ether :50% of Petroleum Ether. Alumina with 1,0% water.

Test-5: 30mL of Methylene Chloride 100%. Florisil with 1% water.

Mussels preparation: 5,0g of net or liofilized sample were mixed with 20g of anidrous Sodium Sulfate and completely powdered using a pistil. 20µL of Surrogate Fluorene d10 at 100µg/mL were added. Extraction was done in two steps of 3 minutes each with 50mL of petroleum ether in an ultrasonic bath in a 250mL erlenmeyer, followed by decantation separation. Most of the fat was eliminated by addition of 5mL of conc. sulfuric acid in the petroleum ether extract and 30 seconds of vigorous shake in a 250mL erlenmeyer. Purification was done after concentration of petroleum ether extract to 5mL using a SPE column with 5g of Florisil (Varian 1225-6030) and slow gravity elution with just 30mL of Methylene Chloride. This final extract was concentrated to 0,2mL in a conic glass tube of 50mL using a purified air or Nitrogen stream. 20µL of the Internal Standard Acenaphtene d10 at 100µg/mL was added to the concentrated extract in the glass tube. The extract with internal standard was transferred using a micro-pipete to a 2 mL vial for autosampler containing a TriSpring Insert of 200µL. 2,0 µL of this extract was injected in a Saturn 2000 GC-MS-MS.

ANALYSIS RESULTS

The Table-6 show the results of the mussels samples from Todos os Santos bay

Table-6: Results obtained in the collected samples

Code	Station	Arochlor™ 1242 ng/g	Arochlor™ 1254 ng/g	Arochlor™ 1260 ng/g	Total PCBs ng/g
T01	Cabrito	7	20	15	42
T02	São Tomé de Paripe	ND	11	16	27
T03-4	Mapele	ND	10	12	22
T04-1	Ilha de Maré	ND	ND	ND	ND
T06	Coqueiro Grande	ND	ND	ND	ND
T12	Dom João	ND	ND	ND	ND
T20	Mutá	ND	ND	ND	ND
T22	Jiribatuba	ND	ND	ND	ND
T25	Saubara	ND	ND	ND	ND

All the results in dry basis. Water content medium: 85,5%.

During the samples analysis, Blank proof, Spikes and duplicates were made. The Table-7 shows the results.

Table-7: Results of Analyticals Quality Controls

CQA	Espected results	Obtained results
Blank of Arochlor™ 1242	ND (<1ng/g)	ND
Blank of Arochlor™ 1254	ND (<1ng/g)	ND
Blank of Arochlor™ 1260	ND (<1ng/g)	ND
Spike of Arochlor™ 1242	50 ng/g	29 (58% recovery*)
Spike of Arochlor™ 1254	50 ng/g	33 (66% recovery*)
Spike of Arochlor™ 1260	50 ng/g	46 (92% recovery*)
Duplicate of Arochlor™ 1242	Similar results	7 ng/g e 10 ng/g
Duplicate of Arochlor™ 1254	Similar results	20 ng/g e 24 ng/g
Duplicate of Arochlor™ 1260	Similar results	15ng/g e 18ng/g

* These relatively low recoveries may be attributed to the analysis of the liofilized mussels without a previous humidification.

The Table-8 show the results obtained in a single analysis of a certified sample of fish.

Table-8: Analysis of a certified sample IAEA-406 (Fish)

PCB	Certified values in ng/g		Obtained result in this work
	Medium \pm Standard deviation (from 11 Laboratories)	Lowest value – Higher value of results	
Arochlor 1260™	13 \pm 3	8 - 15,5	11

The Table-9 shows comparison of results of PCBs in mussels obtained in various independent works. Except this work, the results were obtained by GC-ECD method.

Table-9: Comparison of results of PCBs in mussels in a dry basis

Purpose	Total PCBs ng/g
This Work (Todos os Santos bay, Brazil, 2000 - 2001)	ND - 42
Todos os Santos bay, Brazil, 1988 (Tavares, 1988)	ND - 30
Salvador, Brazil, 1995 (Taniguchi, 1995)	6.93 - 50.01
Brazilian coast, 1995 (Taniguchi, 1995)	ND - 143.41
Rio de Janeiro coast, 2001 (Taniguchi, 2001)	12.8 - 141.7

Portuguese coast, 1985 (Benoliel, 1986)	3.0 - 148.7
Almirantado bay, Antarctica, 1991 to 1994 (Penteado, 2000)	ND - 234,1
Mexico Gulf, 1990 (Sericano, 1990)	3.6 - 1740
Mediterranean coast (Spain), 1988 (Pastor, 1988)	10.8 - 1264
Pacific Norwest (Hong-Kong), 1987 (Tanabe, 1987)	20 - 3136

TOXICOLOGICAL EFFECTS OF THE SAMPLES

The limit for ingestion of Arochlor™ 1254 by WHO is **12µg/day** for a 60kg person. The most contaminated sample of this work has 42 ng/g in a dry basis, equivalent to 6.09 ng/g in wet basis (considering 85.5% water in the samples), so, if the populations would ingest 100g by day of these mussels would ingest **0.609µg/day**, very lower than the WHO limit.

CONCLUSIONS

Using GC-MS-MS is possible to obtain detection Limits and quantification limits adequated for PCBs analysis in mussels. The quantification limits obtained, at about 4 ng/g are very lower than the values considered dangerous for ingestion. The GC-MS-MS techniques have the advantage of being qualitative and quantitative, once the detected peaks may be confirmed by the mass spectra what must minimize the occurrence of "false positives".

The techniques of extract purification were simplified and showed to be adequated for analysis of PCBs, organochlorine pesticides and PAHs by GC-MS-MS as was shown in the recoveries of 50ng/g spiked mussels samples.

The analysis of mussels *Anomalocardia brasiliiana* (papa-fumo) collected in distinct places of the Todos os Santos Bay, state of Bahia, Brazil had result values ranging from <1 to 42 ng/g and were similar to results obtained in 1988 in this Bay. These results show reasonable PCBs contamination in three urban sites two of each near industrial areas and one near the former Salvador city waste dumping area.

The comparison of the results of this work with the obtained by Tavares in 1988, in mussels of the same region showed tendency to keep the same contamination of PCBs in mussels from 1988 to 2001 in the Todos os Santos bay.

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