Detection of Dioxins and Furans PCDDs / PCDFs in marine sediments by Triple Quad GC/MS/MS Instrument

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Introduction

PCDDs and PCDFs pollute the environment primarily or exclusively as a result of anthropogenic activities. These chemicals bioaccumulate and are considered strongly toxic and are able to produce a wide spectrum of adverse health effects in biota and humans. They occur as unwanted products of chemical manufacturing and incineration processes; they enter the acquatic system by atmospheric deposition, direct and indirect discharges and riverine inputs. Once delivered to the water, because their hydrophobic nature, dioxins and furans accumulate in surface sediments.
The purpose of this poster is to illustrate method and results obtained from PCDDs and PCDFs analysis in marine sediments sampled at 3 km off the Lazio's coast. Sediments have been collected from 6 different sampling points at 2 different sampling depths: 0-5cm and 15-20cm depth (**figure1**).

Figure 1: Area map and sampling points

Experimental

GC/MS/MS Method

For unit mass MS analysis the Agilent GC/MS/MS Triple Quad 7000 Series has been used with a 60m HP5-MS Ultra Inert capillary column (**figure 2**).

Method Parameters:

Column: Agilent DB5-MS Ultra inert 60m X 250um X 0.25um
Inlet: PTV Pulsed Splitless mode
Inlet Temperature Program:
100 °C for 0.2 min
700 °C/min to 300 °C for 5 min
30 °C/min to 260 °C for 0 min
Injection Volume: 4uL
Oven Temp: 100 °C for 1 min
40 °C/min to 230 °C for 0 min
2 °C/min to 290 °C for 0 min
20 °C/min to 320 °C for 5 min
MSD transfer line: 320°C
Colum Flow: 1.2 mL/min
Run Time: 40 min

GC/MS/MS MRM:

Targets and Qualifiers MRM transitions are used for labelled and unlabelled compounds. Loss of COCl as fragment and qualifiers ions type M/M+2 and M+2/M+4 are used for M+2 (Tetra, Penta and Hexa compounds) and M+4 parent ion (Hepta and Octa compounds) ; see **Table 1**.

Figure 2: Agilent 7000 Series Triple Quadrupole GC/MS

Experimental

The widest/widest MRM resolution mode has been used for all Target and Qualifiers precursors and products ions in order to achieve higher sensitivity (lower resolution mode at 2.5Da PW).

Quantifiers				Quantifiers			
Name	Transition	Type	RT	Name	Transition	Type	RT
2,3,7,8-TCDFs C13	315.8 -> 252.0	ISTD	17.8	1,2,3,4,7,8-HxCDD C13	401.8 -> 338.0	ISTD	27.6
qualifier	317.8 -> 254.0			qualifier	403.8 -> 340.0		
2,3,7,8-TCDFs	305.8 -> 243.0	Target	17.8	1,2,3,4,7,8-HxCDD	389.8 -> 327.0	Target	27.6
qualifier	303.8 -> 241.0			qualifier	387.8 -> 325.0		
1,2,3,4, TCDD C13	331.8 -> 268.0	Surrogate	17.9	1,2,3,6,7,8-HxCDD C13	401.8 -> 338.0	ISTD	27.7
qualifier	333.8 -> 270.0			qualifier	403.8 -> 340.0		
2,3,7,8-TCDD C13	331.8 -> 268.0	ISTD	18.3	1,2,3,6,7,8-HxCDD	389.8 -> 327.0	Target	27.7
qualifier	333.8 -> 270.0			qualifier	387.8 -> 325.0		
2,3,7,8-TCDD	321.8 -> 259.0	Target	18.3	1,2,3,7,8,9-HxCDD C13	401.8 -> 338.0	Surrogate	28.2
qualifier	319.8 -> 257.0			qualifier	403.8 -> 340.0		
1,2,3,7,8-PCDFs	339.8 -> 277.0	Target	21.4	1,2,3,7,8,9-HxCDD	389.8 -> 327.0	Target	28.2
qualifier	337.8 -> 275.0			qualifier	387.8 -> 325.0		
2,3,4,7,8-PCDFs	339.8 -> 277.0	Target	21.5	1,2,3,7,8,9-HxCDFs	373.8 -> 311.0	Target	28.7
qualifier	337.8 -> 275.0			qualifier	371.8 -> 309.0		
2,3,4,7,8-PCDFs C13	351.8 -> 288.0	ISTD	22.5	1,2,3,4,6,7,8-HpCDFs C13	419.8 -> 356.0	ISTD	31.1
qualifier	349.8 -> 286.0			qualifier	421.8 -> 358.0		
1,2,3,7,8-PCDD C13	367.8 -> 304.0	ISTD	22.8	1,2,3,4,6,7,8-HpCDFs	407.8 -> 345.0	Target	31.1
qualifier	365.8 -> 302.0			qualifier	409.8 -> 347.0		
1,2,3,7,8-PCDD	355.8 -> 293.0	Target	22.8	1,2,3,4,6,7,8-HpCDD C13	435.8 -> 372.0	ISTD	33.1
qualifier	353.8 -> 291.0			qualifier	437.8 -> 374.0		
1,2,3,4,7,8-HxCDFs C13	385.8 -> 322.0	ISTD	26.3	1,2,3,4,6,7,8-HpCDD	423.8 -> 361.0	Target	33.1
qualifier	383.8 -> 320.0			qualifier	425.8 -> 363.0		
1,2,3,4,7,8-HxCDFs	373.8 -> 311.0	Target	26.3	1,2,3,4,7,8,9-HpCDFs	407.8 -> 345.0	Target	34
qualifier	371.8 -> 309.0			qualifier	409.8 -> 347.0		
1,2,3,6,7,8-HxCDFs C13	385.8 -> 322.0	ISTD	26.5	OCDD	459.8-> 397.0	Target	37.1
qualifier	383.8 -> 320.0			qualifier	457.8 -> 395.0		
1,2,3,6,7,8-HxCDFs	373.8 -> 311.0	Target	26.5	OCDD C13	469.7 -> 406.0	ISTD	37.1
qualifier	371.8 -> 309.0			qualifier	471.7 -> 408.0		
2,3,4,6,7,8-HxCDFs C13	385.8 -> 322.0	ISTD	27.3	OCDFs C13	441.7 -> 378.6	Target	37.3
qualifier	383.8 -> 320.0			qualifier	455.7 -> 390.0		
2,3,4,6,7,8-HxCDFs	373.8 -> 311.0	Target	27.3	OCDFs	441.7 -> 378.6	Target	37.3
qualifier	371.8 -> 309.0			qualifier	443.7 -> 381.0		

Table 1: MRM transitions list and Retention Time for screened compounds

Sample Preparation

The quantitative method is based on isotopic dilution ($^{13}C_{12}$ internal standards) with low resolution MRM acquisition for higher sensitivity.
Regarding sample preparation (**Figure 3**) a Soxhlet automatic extraction system has been used, loading the sediment sample spiked with labelled mix of dioxins/furans.
10g of sample, kept at 40ºC for 48hours, are placed in a thimble for Soxhlet. The thimble is housed in the instrumentation and submitted to automatic Soxhlet extraction process by 50ml of a mixture of hexane: acetone 4:1

Extraction Diving: 130ºC for 60min
Extraction to relapse: 130ºC for 60min
Solvent recovery: 130ºC for 10min.

The obtained 1 mL extracted volume is purified as follows:

Multi-layer column, internal diameter 2cm, packed from bottom to top by 0.5 cm anhydrous Na2SO4, 0.5cm silica gel, 1.5cm NaHCO3/anhydrous Na2SO4 9:1, 8cm Celite545 wetted with ACOD sulfuric acid concentrated, 1.5cm sodium sulfate anhydrous. The extract, located at the top of the column, is eluted with 70ml of hexane.

Basic Alumina column, internal diameter 1cm, filled by stationary phase for 10cm height. The eluted fraction from the multi-layer column is concentrated to 1mL by Rotavapor, is placed in the top of the column and then eluted with 10mL of hexane (hexane eluate is discarded). The column is washed with 40mL of hexane/dichloromethane 98:1 v/v (this eluate is also discarded). The final elution of the analytes is done with 40mL of hexane/dichloromethane 1:1 v/v. This eluate is collected, concentrated in a Rotavapor and dried under a flow of nitrogen. The residue is reconstituted in 100µL of iso-octane including syringe standards.
Four µL of this sample is injected into the GC/MS/MS.

Figure 3: Sample preparation equipment

Results and Discussion

In order to calibrate the quantitative method a set of 5 calibration levels has been prepared and injected. The lower calibration mix at 0.026pg/µl for 2,3,7,8-TCDD (**table 2**) has been used to evaluate the LOQ and LOD using the 9:1 and 3:1 S/N ratio (noise peak-to-peak) respectively (**table 3**).
All results for Toxic Equivalent (TEQ) have been calculated referring to "Upper Bound" calculation and are reported in ng/Kg.
All the screened compounds have been chromatographically resolved by a 40min run with a long term RT stability (**figure 5 and 6**) and calibration curves show good linearity (**figure 4**).

Compounds	lower calibration std pg/µL	
	Native	Labelled
2,3,7,8-TCDD	0.026	0.8
1,2,3,7,8-PeCDD	0.052	0.8
1,2,3,4,7,8-HxCDD	0.052	0.8
1,2,3,6,7,8-HxCDD	0.052	0.8
1,2,3,7,8,9-HxCDD	0.052	0.8
1,2,3,4,6,7,8-HpCDD	0.104	1.6
OCDD	0.104	1.6
2,3,7,8-TCDF	0.026	0.8
1,2,3,7,8-PeCDF	0.052	0.8
2,3,4,7,8-PeCDF	0.052	0.8
1,2,3,4,7,8-HxCDF	0.052	0.8
1,2,3,6,7,8-HxCDF	0.052	0.8
1,2,3,7,8,9-HxCDF	0.052	0.8
2,3,4,6,7,8-HxCDF	0.052	0.8
1,2,3,4,6,7,8-HpCDF	0.104	1.6
1,2,3,4,7,8,9-HpCDF	0.104	1.6
OCDF	0.104	1.6
1,2,3,4-TCDD C13		3.2
1,2,3,7,8,9-HxCDD C13		3.2
	TEQ 1.22	

Lower calibration std mix	S/N ratio		LOD fg on column	
	Target MRM	Qualifier MRM	Target MRM	Qualifier MRM
2,3,7,8-TCDD	7.1	5.4	**43**	**58**
1,2,3,7,8-PeCDD	8.5	5.9	**74**	**104**
1,2,3,4,7,8-HxCDD	20	6	**32**	**104**
1,2,3,6,7,8-HxCDD	20	6	**32**	**104**
1,2,3,7,8,9-HxCDD	20	6	**32**	**104**
1,2,3,4,6,7,8-HpCDD	17	15	**73**	**83**
OCDD	12	5.5	**104**	**231**
2,3,7,8-TCDF	3.2	3.3	**104**	**104**
1,2,3,7,8-PeCDF	8.9	7.4	**69**	**90**
2,3,4,7,8-PeCDF	9.9	7.4	**63**	**90**
1,2,3,4,7,8-HxCDF	7	2	**89**	**208**
1,2,3,6,7,8-HxCDF	7	2	**89**	**208**
1,2,3,7,8,9-HXCDF	7	2	**89**	**208**
2,3,4,6,7,8-HxCDF	7	2	**89**	**208**
1,2,3,4,6,7,8-HpCDF	11	7	**104**	**170**
1,2,3,4,7,8,9-HpCDF	9	5	**112**	**181**
OCDF	10	6	**126**	**208**

Tables 2-3: Lower calibration standard mix; S/N ratio and LOD

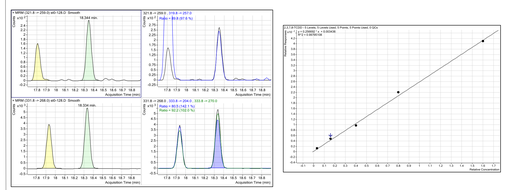

Figure 4: 2,3,7,8-TCDD calibration curve from 0.026pg/µL to 1.28pg/µL

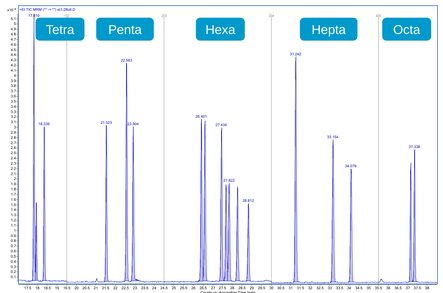

Figure 5: Chromatographic overview for all screened compounds

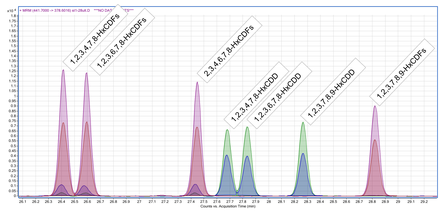

Figure 6: Chromatographic results for Hexa Compounds MRMs in std mix

Results and Discussion

Sample preparation, compound identification, triple quadrupole MRM selectivity and quantitative analysis have been successfully evaluated using Certified Reference Material WMS-01 provided by Wellington Lab (**table 4**). Labelled compounds %Recovery is always above 70% (usually between 70% and 85%).

WMS-01 Wellington Lab.	TEQ - EPA	Experimental Results ng/Kg	Certified values ng/Kg
2378-TCDD	1	16.1	17.7±5.6
12378-PeCDD	0.5	6.9	7.96±2.8
123478-HxCDD	0.1	10.0	8.66±2.7
123678-HxCDD	0.1	20.6	20.8±4.8
123789-HxCDD	0.1	21.3	17.3±8.0
1234678-HpCDD	0.01	222.0	293±63
OCDD	0.001	1549.0	1899±456
2378-TCDF	0.1	47.8	52.5±16
12378-PeCDF	0.05	16.9	12.6±5
23478-PeCDF	0.5	20.3	18.5±6.1
123478-HxCDF	0.1	71.8	67.3±24
123678-HxCDF	0.1	28.9	20.3±8.7
234678-HxCDF	0.1	23.7	16.0±8.0
123789-HxCDF	0.1	2.9	2.7±4.0
1234678-HpCDF	0.01	335.0	299±73
1234789-HpCDF	0.01	27.4	15.1±4.6
OCDF	0.001	472.0	509±157

Table 4: Certified sediment quantitative results and EPA TEQ values

2,3,7,8-TCDD is identified by RT and by two MRM transitions ratio: for low resolution mode, the 2,3,7,8-TCDF shows signals for the same MRM transitions at different RT and different ratio (**figure 7**).

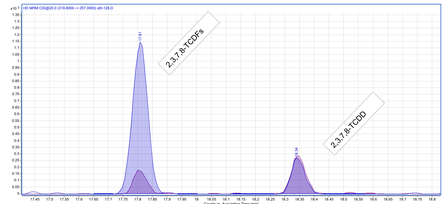

Figure 7: TCDD MRM Target and Qualifier Transitions at 0.128pg/µL

In all sediment samples, highest TEQ factor compounds (i.e. 2,3,7,8-TCDD) are always below LOQ and total TEQ values for samples have been estimated using the "Upper Bound" calculation. The PCDDs/PCDFs distribution within samples reveals values according to other scientific papers where HRMS has been used (see references). The estimated amounts are close to the LOD of this analytical method. The expected fingerprint of marine sediments sampled close to urban and industrial area is confirmed (**figure 8 and 9**).
Most significant TEQ sample is showed in **figure 10** and all results are summarized in **table 5**.

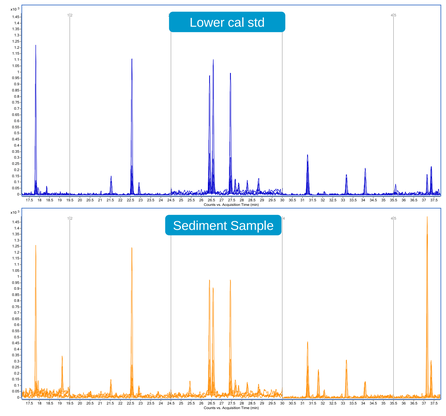

Figure 8: Natives compounds comparison for lower standard calibration mix and 5-20cm sampling depth real sample

Results and Discussion

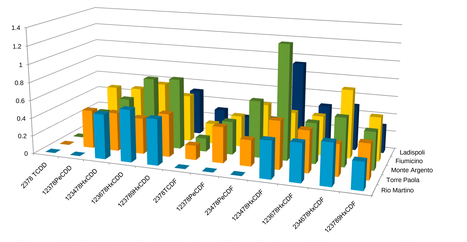

Figure 9: PCDDs/PCDFs distribution (Tetra, Penta and Hexa compounds)

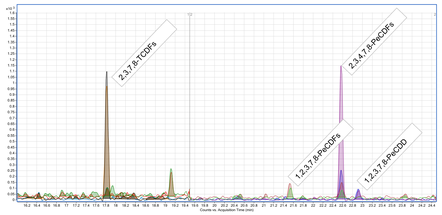

Figure 10: Fiumicino sample at 15-20cm depth, Tetra and Penta compounds

	LOQ	Fiumicino	Fiumicino
	ng/Kg	ng/Kg	ng/Kg
		0-5 cm	15-20 cm
2378-TCDD	0.3	<0.3	<0.3
12378PeCDD	0.5	0.6	0.6
123478HxCDD	0.2	0.6	1.2
123678HxCDD	0.2	0.6	0.6
123789HxCDD	0.2	0.5	0.5
1234678HpCDD	0.5	2.0	1.6
OCDD	0.7	10.4	17.0
2378TCDF	0.6	<0.6	<0.6
12378PeCDF	0.5	<0.5	<0.5
23478PeCDF	0.5	0.5	0.6
123478HxCDF	0.6	<0.6	0.7
123678HxCDF	0.6	<0.6	<0.6
234678HxCDF	0.6	0.8	0.6
123789HxCDF	0.6	<0.6	<0.6
1234678HpCDF	0.7	1.8	1.5
1234789HpCDF	0.7	0.7	1.1
OCDF	0.9	4.4	3.4
TEQ ng/Kg		**1.2**	**1.5**

	Torre Paola	Torre Paola	Rio Martino	Rio Martino	Foce F. Marta
	ng/Kg	ng/Kg	ng/Kg	ng/Kg	ng/Kg
	0-5cm	15-20cm	0-5cm	15-20cm	15-20cm
TEQ	0.4	0.5	0.5	0.5	0.7
	Ladispoli	**Ladispoli**	**Monte Argento**	**Monte Argento**	
	ng/Kg	ng/Kg	ng/Kg	ng/Kg	
	0-5cm	15-20cm	0-5cm	15-20cm	
TEQ	0.6	1.3	0.9	1.1	

Tabe 5: Summarized TEQ results and detailed results for higher level sample

Conclusions

Results, reported as Upper Bound TEQ (Toxic Equivalent), reveal amounts within the 0.4-1.5ng/Kg range. These PCDDs/PCDFs amount levels are analogous to the background values reported in other scientific papers (see references). Those values are aligned with the suggested limits coming from Environmental Canada where 0.85ng/Kg TEQ is used as the sediments guideline reference value.
Amounts from the 15/20cm depth layer are usually slightly higher than 0/5cm depth layer and the 2,3,7,8-TCDD is always below the limit of quantitation (0.3ng/Kg). The LOD has been tested as well and the Agilent GC/MS/MS easily allows detection of 0.1ng/Kg for TCDD using just 10 grams of sample.
In this work the detected PCDDs and PCDFs amounts could be considered as background values from uncontaminated areas, considering the distance from the coast were sediments have been sampled.

References

Ethel Eljarrat et al. 2005, Occurrence of polybrominated diphenylethers, polychlorinated dibenzo-p-dioxins, dibenzofurans and biphenyls in coastal sediments from Spain*; Environmental Pollution 136 (2005) 493-501*

Roberto Miniero et al. 2005, Selected persistent organic pollutants (POPs) in the Italian environment; *Ann Ist Super Sanità 2005;41(4):487-492*