HRGC-HRMS MULTI-RESIDUAL POPs ANALYSIS METHOD ON A NOVEL AUTOMATED CLEAN UP SYSTEM

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Abstract:

Persistent Organic Pollutants (POPs) are toxic, persistent and lipophilic chemicals. A multi-residual method for 29 organochlorinated pesticides (OCPs), indicator and dioxin-like polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs) in fish has been developed analyzing reference material on a novel automated clean up system. Through in line gel permeation chromatography, acid silica/neutral silica, basic alumina and active carbon columns clean up steps the method provides distinct fractions for multiple run HRGC-HRMS analysis. The recovery of labelled internal standards was in the range indicated by the official methods. The results were in good agreement with reference values. The automated system is equipped with an autosampler and runs in sequential mode up to 9 samples. The method fits the purpose of multi-residual methods.

Introduction:

Organohalogen compounds such as certain organochlorinated Pesticides (OCPs), polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and brominated diphenylethers (PBDEs) are toxic and persistent and have been detected in various environmental compartiments. All these compounds fall under or are candidate to the Stockholm Convention and they are part on United Nations Environment Programme (UNEP) monitoring programme[1].

Often the monitoring of these chemicals is carried out in different specialized laboratories that use different extraction and clean up techniques for the various compound classes, thus combined data from the same location/experiment are scarce.

The purpose of this work is the development of a single analytical protocol involving a single extraction followed by an automated clean up. The clean up shall provide distinct fractions for multiple run HRGC-HRMS analysis covering all the classes of compounds mentioned above.

A new automated clean up system was provided by the laboratory from J2 Scientific (Missouri, USA). A gel permeation chromatography (GPC) with autosampler (AccuPrep MPS^{TM}), an evaporator system (AccuVap^{TM}) and three solid phase module able to manage the acid silica/neutral silica, basic alumina and active carbon columns (Dioxin/SPE module) are in-line. The system processes the samples in sequence.

In this study we analysed one reference (IAEA-406) and one certified (CARP-2) fish materials. Both were analysed for 29 OCPs and the second one also for Dioxin-Like and Indicator-PCBs and PCDD/Fs

Here we present the first data obtained from the new system.

Materials and Methods:

Materials: All organics solvents used were Dioxin analysis grade (Sigma-Aldrich, Buchs SG, Switzerland).
BioBeads SX-3, acid silica/neutral silica, basic alumina and active carbon columns ready to use were obtained from J2 Scientific (Missouri, USA). 68-CVS and 68-LCS were native and ^{13}C-labelled internal standards for 12 congeners DL-PCBs. EPA-1613CVS, EPA1613LCS and EPA-1613ISS were native, ^{13}C-labelled internal and recovery standards for 17 PCDDs/Fs respectively. The standards were obtained from Wellington Laboratories (Guelph, Ontario, Canada). EC-4058 for 7 indicator ^{13}C-labelled PCBs, native and ^{13}C-labelled OCPs internal standards were obtained from Cambridge Isotope Laboratories.
Isotope labelled Aldrin, α - HCH, γ-HCH, Cis-nonachlor, Dieldrin, α -Endosulfan, β-Endosulfan, Endrin, Heptachlor, Heptachlor-endo-epoxide (trans,isomerA), HCB, Mirex, o,p-DDD, o,p-DDT, Oxy-chlordane (gamma), p,p-DDE, p,p-DDT, Trans-nonachlor were used as internal standards.
^{13}C-labelled β – HCH, o,p-DDE, and d8 p,p-DDD were used as recovery standards.

Analytical determinations: 10g of reference fish sample CARP-2 was lyophilized and submitted to the extraction. 2g of reference fish homogenate sample IAEA-406 was extracted without any pretreatment. Both materials were processed in triplicate. Analytical blank were performed in parallel.
The extraction was carried out by Soxhlet for 24h with a mixture of acetone/n-hexane 1/1 after spiking with internal standards (16 PCDD/Fs ^{13}C-labelled 2,3,7,8-chlorine-substituited congeners with 400 pg each, except OCDD with 800 pg and 12 DL-PCBs and 7 indicators - PCBs ^{13}C-labelled with 2000 pg each) and 50ng of 19 labelled OCPs.
The extract was dried under nitrogen flow and the lipid content was determined gravimetrically.
The lipid sample was diluted to 5ml with a mixture of cyclohexane/ethyl acetate 1/1 and injected into a 5ml loop of automated GPC system. The GPC column was 2.5cm x 32 cm filled with BioBeads SX-3 resin working at a flow rate of 5ml/min using cyclohexane/ethyl acetate 1/1. The eluate was collected between 23:30 and 45 min (107 ml).
The 10 % of sample collected was concentrated under nitrogen flow and spiked with OCPs recovery standards. The final volume of 100µl was submitted to instrumental analysis for OCPs.
The remaining 90% was concentrated under nitrogen flow until 0.5ml and then diluted with n-hexane to 5ml. These 5ml was submitted to an automated clean up using acid silica/neutral silica, basic alumina and active carbon columns.
The sample was loaded on acid silica/neutral silica connected to a basic alumina column and eluted with 75ml of n-hexane. This fraction was discharged. The basic alumina column was eluted with 60 ml of 98/2 n-hexane/dichloromethane and this fraction was collected for PCBs analysis. Then the basic alumina connected to an active carbon column was eluted with 120ml of a mixture of 50/50 n-hexane/dichloromethane. The carbon column was eluted before with 4ml of a mixture of 50/50 ethyl acetate/toluene and then with 10ml of n-hexane. The last three fractions were collected and added to the PCBs fraction in order to improve PCBs recovery.
Finally the carbon column was eluted in reverse flow with 75ml of toluene and collected for non-ortho PCBs and PCDDs/Fs.
PCBs and PCDDs/Fs fractions were concentrated to 100µl and 30µl respectively and spiked with their recovery standards and submitted to instrumental analysis.
The flow rate used for all columns was 6ml/min.
The system, which runs in sequential mode up to 9 samples, was equipped with an autosampler that managed the sample loading during the different clean up steps automatically.
The system was equipped with automated evaporator system in-line, but during this study it has not been used and all solvent concentrations were performed by TurboVap.

Instrumental analysis: The instrumental analysis of PCDD/Fs, and PCBs were based on isotope dilution using HRGC-HRMS (high resolution gas chromatography – high resolution mass spectrometry) for quantification on the basis of EPA1613[2] and EPA 1668[3].

OCPs were analysed using isotope dilution with HRGC-HRMS for the quantification on the basis of an in house method referring to the QA/QC criteria laid down in the methods mentioned above.

Non-ortho PCBs, PCDD/Fs, and OCPs were analyzed on double HRGC (Thermo Trace GC Ultra, Thermo Electron, Bremen, Germany) coupled with a DFS high resolution mass spectrometer HRMS (Thermo Electron, Bremen, Germany) operating in EI-mode at 45 eV with a resolution of >10000. For Non-ortho PCBs, PCDD/Fs the most two abundant ions of the isotopic molecular cluster were recorded for both native and labelled congeners.
For OCPs we selected two ions of the isotopic cluster coming from the fragmentation and selected on the basis of close elution of different OCPs and the dynamic mass range of the HRMS.
The compounds were identified through comparison of retention times of the corresponding standard and the isotopic ratio of the two ions recorded.
Non-ortho PCBs, PCDD/Fs and OCPs were separated on a BP-DXN 60 m long with 0.25 mm i.d. (inner diameter) and 0.25 μm films (SGE, Victoria, Australia). The following gas-chromatographic conditions were applied for non-ortho PCBs and PCDD/Fs: split/splitless injector at 280 °C, constant flow at 1.0 ml min^{-1} of He, GC-MS interface at 300 °C and a GC program rate: 160 °C with a 1 min. hold, then 2.5 °C min^{-1} to 300 °C and a final hold at 300 °C for 8 min.
Gas chromatographic conditions for OCPs were: Split/splitless injector at 250 °C, constant flow at 1.0 ml min^{-1} of He, GC-MS interface at 270 °C and a GC program rate: 100 °C with a 1 min. hold, then 10 °C min^{-1} to 300 °C and a final hold at 300 °C for 9 min.
Mono-ortho PCBs and Indicator-PCBs were analyzed on a GC (HP-6890, Hewlett Packard, Waldbronn, Germany) coupled with a VG Autospec Ultima high resolution mass spectrometer (Micromass, Manchester, UK) operating in EI-mode at 34 eV with a resolution of >10000. The most two abundant ions of the isotopic molecular cluster were recorded for both native and labelled congeners.
The congeners were separated on HT-8 capillary column, 60 m long with 0.25 mm i.d.(inner diameter) and 0.25 μm film (SGE, Victoria, Australia).
Gas chromatographic conditions for Mono-ortho PCBs were: Split/splitless injector at 280 °C, constant flow at 1.5 ml min^{-1} of He, GC-MS interface at 280 °C and a GC program rate: Starting from 120 °C with 20 °C min^{-1} to 180 °C, 2 °C min^{-1} to 260 °C, and 5 °C min^{-1} to 300 °C isotherm for 4 min.

Results and Discussion:

The levels of the analytical blanks obtained during the clean-up process were at least 10 times lower compared to the reported concentrations for all compounds studied or below LOD. The blank level was not subtracted. The reported detection limits were calculated on the bases of a signal to noise ratio of 3/1.
In the Table 1 the recovery for the 19-OCPs labelled internal standard and 29-OCPs concentrations detected compared to the available values of the IAEA-406 and CARP-2 reference materials are reported.
The average recovery obtained for 19 internal standard OCPs ranged from 76% for p-p'-DDT to 146% for Endrin with the exception for cis and trans-nonachlor where the recovery was 40% for both. Nevertheless cis and trans-nonachlor showed concentrations close to the reference values with good reproducibility of 0.86% and 4.1% respectively.
During this study the levels of α-HCH, β-HCH, α and β-endosulfan detected in the IAEA-406 were under the LOD and Heptachlor and Aldrin were detected at very low concentrations (0.02ng/g). These results were in contrast with the recommended/information values reported in the reference sheet. Our recoveries of these pesticides were very good (ranging between 76% and 114%) and no interferences were present on the specific masses recorded. Looking in detail into the analytical methods applied by the participants to the exercise for the material IAEA-406, none used labelled internal standards and most of them applied Electron Capture Detection (ECD) as detector[4]. Our analytical method instead used more severe QA/QC (isotopic dilution method coupled with HRMS), which may explain discrepancies regarding IAEA-406. However additional certified material will be processed in order to check the

results obtained. All the other pesticides were in good agreement with the reference values for both IAEA-406 and CARP-2 material.
Table 2 displays the concentrations of the 17 2,3,7,8-sustituited PCDD/F congeners, DL-PCBs and 6 Indicator-PCBs in CARP-2 Reference Material and the available reference values for some of the compounds analysed.
All results obtained were in good agreement with the reference/certified values for the 7 PCDDs/Fs and 7 PCBs congeners. The automated method showed a very good reproducibility, ranging between 2% and 16% for PCDDs/Fs at low pg/g level and ranging between 0.65% and 6.98% for all PCBs detected at ng/g level. The recoveries were in the range indicated as acceptable in the official EPA1613 and EPA 1668 methods.

Conclusion:

GPC coupled with HRMS is able to perform reliable results for OCPs in biota samples.
The three Automated SPM modules are able to reproduce the method and the performances specified in the official EPA Methods for PCDDs/Fs and PCBs.
The automated methodology was able to fraction OCPs, PCBs and PCDDs/Fs with suitable results when compared to the reference values. The method fits the purpose of multi-residual methods.

The sequential processing of the samples allows reliable overnight processing of the combined GPC/multicolumn clean up.

Next steps:

The evaporator system has not been evaluated in this study. The next steps will include the automated solvent concentration in order to fully automate the procedure.

New references material will be analysed in order to validate this method for the compounds that were not present in the reference materials used. We plan to extend the method to other POPs classes such as polybrominated diphenylethers (PBDEs).

References:
1 - *http://www.chem.unep.ch/pops/*
2 - U.S. EPA., 1994b. *Method 1613*:
3 - U.S. EPA., 1999. *Method 1668, revision A*:
4 - Villeneuve J-P., de Mora S.J. and Cattini C. *IAEA/AL/125 (IAEA/MEL/69)*, IAEA, Monaco, 2000

	Reference Material IAEA-406				Reference Material Carp-2					
Analyte	**Measured average n=3**	**RSD %**	**Recommended or Information Values (median)**	**95% Confidence Interval**	**Measured average n=3**	**RSD %**	**Certify Value (Average)**	**Uncertainty**	**Internal Standards Recovery (%) n=8**	**RSD %**
Concentration	ng/g		ng/g		ng/g		ng/g		78	28
α-HCH	<0.03	53.89	0.79	0.23-1.7	0.73	10.96	---			
β-HCH	<0.07	13.68	0.75	0.2-2.3	0.17	16.08	---			
γ-HCH	0.11	2.54	0.27	0.11-0.80	0.91	14.27	---		78	25
δ-HCH	<0.02	20.29	---		0.02	29.82	---			
ε-HCH	<0.03	14.98	---		0.03	19.40	---			
HCB	2.3	7.35	1.5	0.95-2.0	2.9	13.99	---		76	34
Heptachlor	0.02	19.45	0.32	0.23-0.46	0.03	59.53	---		83	26
Aldrin	0.02	14.47	0.75	0.61-1.2	0.05	9.21	---		76	29
Dieldrin	3.7	9.06	3.5	1.4-7.0	11	7.66	8.3	0.8	89	17
Endrin	0.62	32.36	1.9	0.86-5.1	0.28	22.46	---		146	22
Isodrin	<0.29	33.65	---		<0.08	32.31	---		84	27
Oxychlordane	0.38	9.64	---		1.2	4.90	---		84	27
Heptachlor-exo-epoxide	0.57	6.02	0.99	0.37-1.6	1.9	9.63	---		105	21
Heptachlor-endo-epoxide	<0.06	34.08	---		12	10.28	---			
trans-chlordane	0.45	9.98	0.7	0.63-1.0	2.8	13.16	4.5	0.7		
cis-chlordane	2.8	9.72	2.8	2-4.1	8.2	12.77	---			
trans-nonachlor	4.4	13.25	4.1	3.9-4.1	15	10.51	11	0.9	40	93
cis-nonachlor	1.9	7.33	0.86	0.77-1.4	5.8	9.13	---		40	77
α -Endosulfane	<0.22	53.62	3.5	0.94-4.7	2.2	92.47	---		114	24
β -Endosulfane	<0.06	31.81	1.4	1.0-1.6	0.53	23.06	---		93	17
op-DDE	0.55	7.10	0.76	0.48-1.3	2.2	12.33	2.9	0.5		
pp-DDE	14	7.83	9.2	6.2-11	244.19	9.02	158	14	114	23
op-DDD	1.5	4.92	0.88	0.43-3.0	25	10.35	21.8	0.7	102	21
pp-DDD	2.8	7.20	2.8	2.0-3.7	94	9.29	90.9	8.5		
op-DDT	2.0	10.50	2.9	0.9-4.4	0.04	7.58	---		86	41
pp-DDT	3.0	6.05	3	1.8-5.6	0.21	8.87	---		76	31
Endosulfane-sulphate	<0.02	44.33	---		0.03	14.05	---			
Methoxychlor	<0.04	40.98	---		<0.07	76.33	---			
Mirex	0.31	7.51	---		0.87	10.40	---		91	15

TABLE 1: Concentration of 29 OCPs in Reference Material IAEA-406 and CARP-2

Reference Material Carp-2									
Analyte	**Measured average n=3**	**RSD %**	**Reference Values**	**Uncertainty**	**Analyte**	**Measured average n=3**	**RSD %**	**Certified Values**	**Uncertainty**
Concentration:	pg/g		pg/g		**Concentration**	ng/g		ng/g	
2378-TCDD	9.0	9	7.4	0.7	***DL-PCBs***				
12378-PeCDD	4.7	12	5.3	1.3	**PCB-81**	0.18	2.89	---	
123478-HxCDD	1.6	16	1.6	0.3	**PeCB-105**	65	0.65	53.2	15.6
123678-HxCDD	5.9	1	5.8	0.8	**PeCB-114**	5.8	5.02	---	
123789-HxCDD	0.57	12	0.78	0.12	**PeCB-118**	166	0.35	148	33
1234678-HpCDD	7.4	5	6.4	0.9	**PeCB-123**	4.4	0.64	---	
OCDD	9.4	9	9.4	1.7	**PCB-126**	0.48	1.38	---	
					HxCB-156	10	1.34	---	
2378-TCDF	16	3	18.2	1.6	**HxCB-157**	1.8	3.87	---	
12378-PeCDF	5.3	6	5.6	0.3	**HxCB-167**	5.5	0.87	---	
23478-PeCDF	16	2	---	---	**PCB-169**	0.03	3.00	---	
123478-HxCDF	3.7	6	---	---	**HpCB-189**	1.07	1.47	---	
123678-HxCDF	2.5	4	---	---					
234678-HxCDF	1.6	7	---	---	**1998 WHO-TEQ**	0.08	0.59		
123789-HxCDF	<0.26	48	---	---	**2005 WHO-TEQ**	0.06	1.16		
1234678-HpCDF	4.1	6	---	---					
1234789-HpCDF	<0.15	29	---	---	***Indicator-PCBs***				
OCDF	0.81	14	---	---	**TeCB-52**	153	6.89	138	43
					PeCB-101	149	6.12	145	48
I-TEQ	23.08	6			**HxCB-138**	90	5.73	103	30
1998 WHO-TEQ	25.44	6			**HxCB-153**	115	6.98	105	22
2005 WHO-TEQ	22.12	7			**HpCB-180**	65	1.19	53.3	13

TABLE 2: Concentration of PCDDs/Fs and PCBs in Reference Material CARP-2