THE USE OF GC×GC-NCI-QTOF FOR THE QUANTITATIVE ANALYSIS OF ORGANOCHLORINE PESTICIDES (OCPs) AND POLYCHLORINATED BIPHENYLS (PCBs).

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Organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) are synthetic chlorinated compounds widely used in the past respectively as pesticides and insecticides and as dielectric and coolant fluids in electrical apparatus. In spite of having been banned or restricted due to their toxicity, OCPs and PCBs are widely present as residues in the environment (soil, water, food etc.), leading to their classification as persistent organic pollutants. Since their presence even at very low level brings significant risks for human health and environmental toxicity, their identification and quantification is very important for environmental and food safety.

The most common technique used for the analysis of these compounds at trace level is gas chromatography coupled to an electron capture detector (GC-ECD). ECD detection grants excellent sensitivity and good selectivity. However, for very complex matrices interference can become an issue. In the last years, interested has been shows towards the use of GC coupled to tandem mass spectrometry (GC-MS/MS). This technique allows reliable quantification at very low concentrations with excellent selectivity, once the method is carefully optimized to fit the compounds of interest. The main limitation of these approaches is their applicability exclusively for targets.

Comprehensive two-dimensional gas chromatography (GC×GC) benefits from the coupling of two different separation mechanism in a single analysis. This provides enhanced resolution and peak capacity, leading to superior separation power and much less chance for co-elution. These features make GC×GC a unique analytical tool for targeted and untargeted analysis of highly complex matrices. The use of GC×GC can deliver adequate selectivity for the analysis of OCPs and PCBs with straightforward method development and reduced need for extensive sample clean-up while allowing for in depth characterization of the sample composition.

In this preliminary study we investigate the performance of GC×GC with thermal modulation coupled to a QTOF detector for quantification of OCPs and PCBs at low concentrations. The QTOF allows for acquisition in Election Ionization (EI) and Chemical Ionization (positive and negative modes available). Here we select the negative chemical ionization mode (NCI) because this is expected to grant higher sensitivity for halogenated compounds.

Experimental details

The sample is a standard solution containing several OCPs and PCBs with individual concentrations in the range 150-300 ng/mL (or ppb, w/v) in petroleum ether. A series of dilutions are prepared using n-hexane as solvent for a general concentration range of approximately 2-300 ng/mL (detailed values are listed in Table 1).

All measurements are performed on an Agilent 7890B GC equipped with a split/splitless injector, a Zoex ZX2 cryogen-free thermal modulator and an Agilent 7200B QTOF Detector. The QTOF is operated in NCI mode with methane as reagent gas, Extended Dynamic Range (EDR) acquisition mode and the maximum acquisition speed of 50 Hz. All 2D data are visualized and processed using the *GC Image HR* software package.

Results and discussion

1. Separation and identification

Fig. 1 shows the 2D plot for the OCPs and PCBs standard mixture. The two-dimensional separation provides enhanced resolution for all the target compounds. This is beneficial also to avoid matrix interference when analyzing complex samples. Identification is achieved based on the molecular ion and on the elution pattern. An analysis in EI mode is used to confirm correct identification and assign unclear blobs. Thanks to the Removable Ion Source (RIS) option, switching between EI and CI source is a quick procedure that does not require venting the instrument.
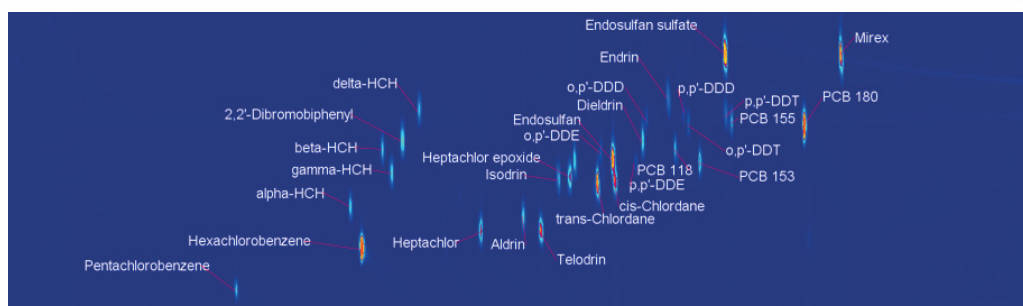
Fig. 1
GC×GC-NCI-QTOF 2D chromatogram for the OCPs and PCBs standard mix.

2. Repeatability

The repeatability of retention times in the two dimensions and quantifier ion response is shown in Table 1 for 8 replicate analyses of the standard solution at nominal concentration 12 ppb. The RSDs are <0.2% and <1.2% for the retention times in the first and second dimension, respectively. For the response the repeatability is always RSD is always <6%, with an average of 3.1%.

Table 1
List of the compounds with results for repeatability (calculated at a nominal concentration of approximately 12 ppb) and linearity.

| Compound | RT I (min) | RT II (sec) | Quantifier (m/z) | RSD % (n=8) | | | Linearity | |
				RT I (min)	RT II (sec)	Response	Concentration range (ppb)	R²
Aldrin	32.00	2.35	237	<0.01	0.67	2.17	2.1-209.3	0.99997
alpha-HCH	24.42	2.50	71	<0.01	0.93	2.64	1.4-143.5	0.99989
beta-HCH	25.83	3.22	71	<0.01	0.41	3.48	2.5-249.5	0.99992
cis-Chlordane	36.00	2.82	410	<0.01	1.11	3.21	2.5-251.0	0.99998
delta-HCH	27.43	3.74	71	0.10	0.59	4.42	1.8-179.4	0.99927
Dieldrin	37.25	3.35	237	<0.01	1.19	1.92	3.3-333.7	0.99993
Endosulfan	35.92	3.12	407	<0.01	1.12	3.69	2.8-284.7	0.99994
Endosulfan sulfate	40.84	4.47	386	<0.01	0.88	3.42	2.5-254.3	0.99997
Endrin	38.34	3.89	237	<0.01	1.05	3.28	3.5-352.9	0.99996
gamma-HCH	26.25	2.91	71	<0.01	0.76	2.43	2.0-202.8	0.99977
Heptachlor	30.17	2.14	266	<0.01	0.72	2.28	3.1-313.5	0.99997
Heptachlor epoxide	34.08	2.83	237	<0.01	0.52	1.39	2.5-255.1	0.99998
Hexachlorobenzene	24.92	1.97	285	<0.01	0.37	1.86	3.2-324.8	0.99990
Isodrin	33.58	2.86	237	<0.01	1.01	2.05	3.1-312.8	0.99999
Mirex	45.92	4.37	404	<0.01	0.25	1.21	3.3-328.9	0.99997
o,p'-DDD	37.42	3.58	246	<0.01	0.51	4.98	3.1-317.9	0.99997
o,p'-DDE	35.42	3.16	246	<0.01	1.53	4.74	2.0-197.9	0.99995
o,p'-DDT	39.25	3.60	246	<0.01	0.43	3.72	3.3-335.4	0.99992
p,p'-DDE	36.93	3.07	281	0.07	0.92	5.28	3.2-327.2	0.99999
p,p'-DDT	40.92	3.65	71	<0.01	1.01	5.78	3.3-331.7	0.99965
PCB 118	38.67	3.21	327	<0.01	0.94	4.10	2.9-292.0	0.99998
PCB 153	39.75	3.02	361	<0.01	0.88	3.07	2.9-292.0	0.99993
PCB 180	44.33	3.57	395	<0.01	0.74	3.28	2.9-292.0	0.99997
Pentachlorobenzene	19.42	1.42	250	<0.01	0.93	1.95	1.7-168.0	0.99999
Telodrin	32.75	2.19	237	<0.01	0.44	2.29	2.9-297.0	0.99999
trans-Chlordane	35.25	2.77	410	<0.01	1.16	1.50	2.5-253.2	0.99998

Fig. 2 provides additional evidence of the good repeatability with the overlay of the modulated peaks (TIC and Extracted Ion Chromatogram of the quantifier) for Aldrin in the raw data for 8 individual runs.
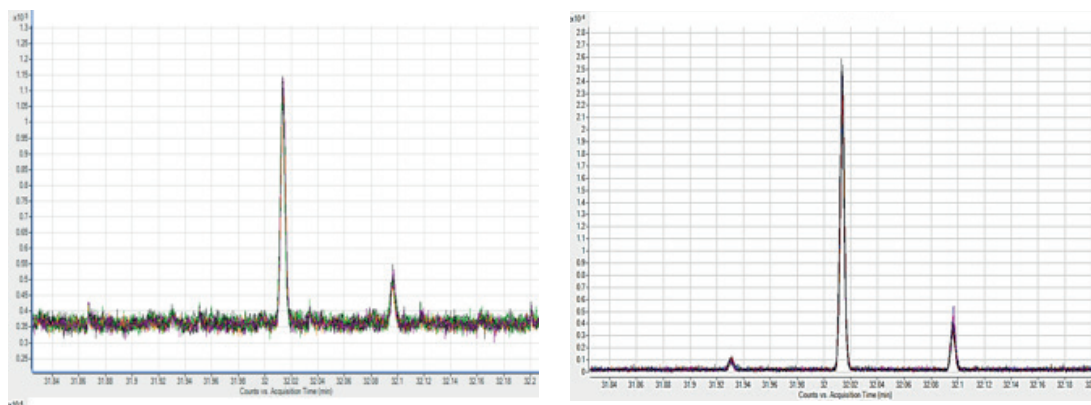
3. Linearity

The linearity results in the nominal range 3-300 ppb are summarized in Table 1. This is evaluated for the individual quantifier ion response (nominal mass). The R^2 values for a linear fit range from 0.99977 to 0.99999, for an average of 0.99991, confirming good linearity. Examples of calibration lines are produced in Fig. 3.
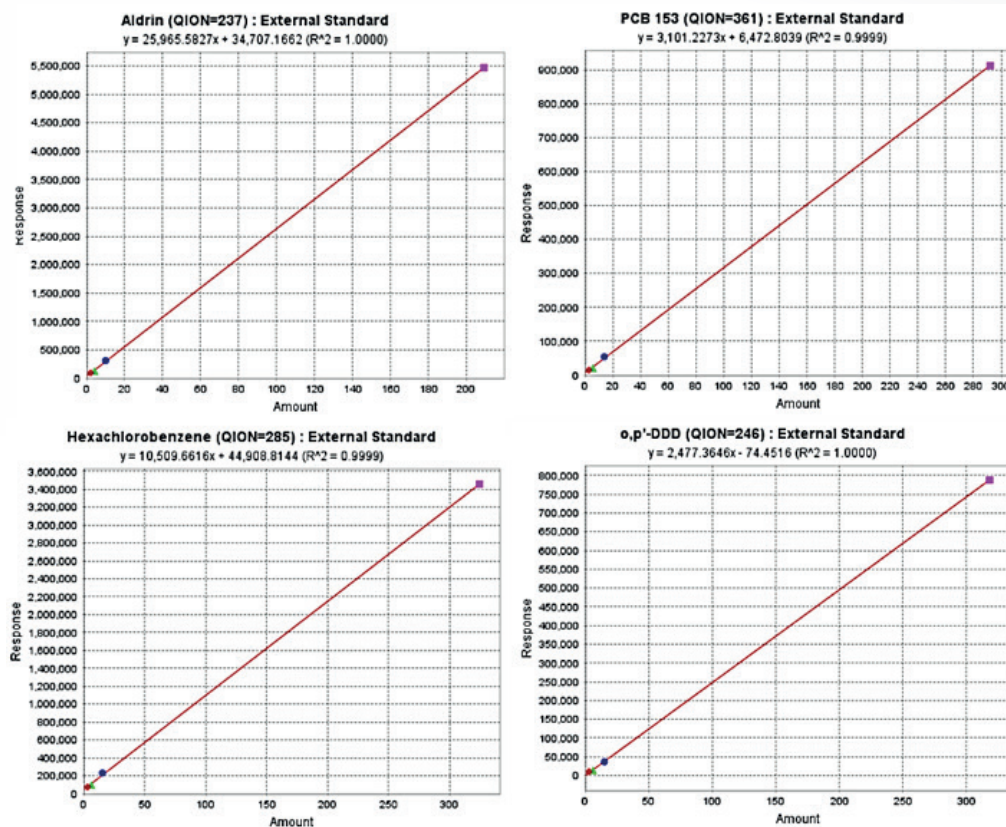
4. Sensitivity

The TIC signal-to noise (S/N) ratio for the standard mix at the lowest concentration level measured (nominal 2.5 ppb) ranges from 33 to 186, suggesting detection limits with the current method at mid ppt range (pg/mL or µg/L). This value refers to the injected solution, pre-concentration steps normally employed for this application are not yet considered.

This performance is achieved by using a split ratio of 1:30, passing to splitless injection would also grand an additional order of magnitude.

Most likely lower detection limits would be possible by targeted fine-tuning of the NCI parameters. On the other hand, for all the compounds here listed the sensitivity is already significantly enhanced compared to EI.

The S/N ratio also get higher when the quantifier ions are selected, as already seen in Fig. 2. Even better sensitivity is possible when accurate mass is used. The absolute response will get smaller but noise is filtered out, leading to cleaner baselines. This is illustrated by the example in Fig. 4. Based on these consideration, a detection limit in the low ppt (µg/L) is plausible.
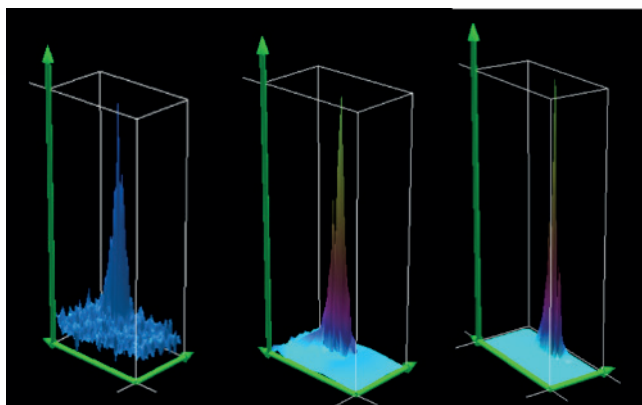

Fig. 4
Aldrin 3D peak at 2.1 ppb in TIC (left) and in EIC for the quantifier in nominal mass 237±0.5 m/z (middle) and accurate mass 236.8416±0.001 m/z (right).

5. Mass accuracy

Fig. 5 shows the example of HRMS spectrum for Pentachlorobenzene. The formula expected (C6HCl5) is suggested by *GC Image HR* for the M$^-$ fragment with excellent mass accuracy (error <0.8 ppm). An additional tool that can be used is the isotope ratio. This is very useful especially for characteristic, well-known isotopic patterns typical in the presence of multiple chlorine atoms, like for OCPs and PCBs. For this case the agreement between the experimental data and the theoretical distribution pattern of both the accurate masses and their relative abundance is, as can be seen, excellent.

The High Resolution (HR) data can be used for additional identity confirmation or, as seen, to increase selectivity and thus sensitivity. Fig. 3 and Table 2 show the mass accuracy stability evaluation performed for a few selected compounds. For consistency, mass accuracy is in all cases calculated at the blob apex. Over a period of 8 measurements, for all test compounds the mass accuracy error is always <4 ppm and with an average <2.2 ppm, indicating good stability and reliability.
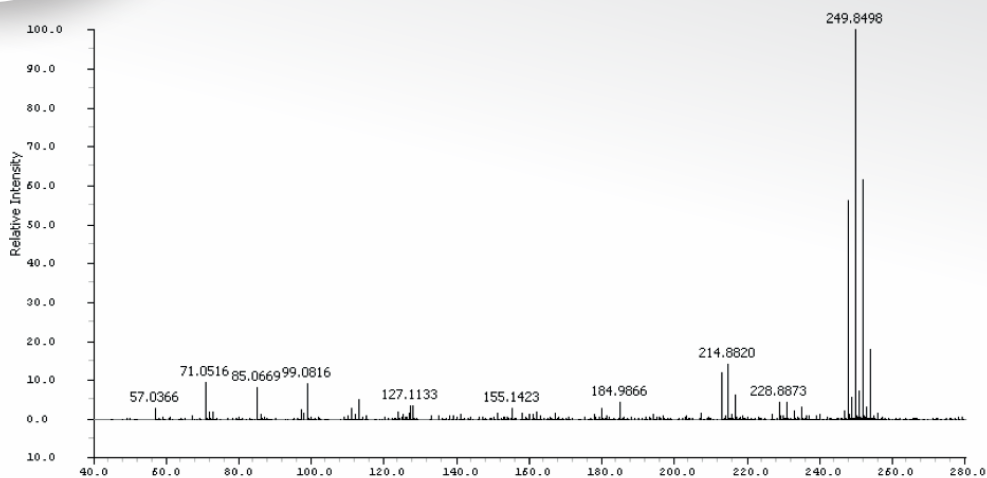
Fig. 5 – NCI-HRMS spectrum (top) and detail of the molecular ion isotopic pattern (bottom) for Pentachlorobenzene at 3.3 ppb.
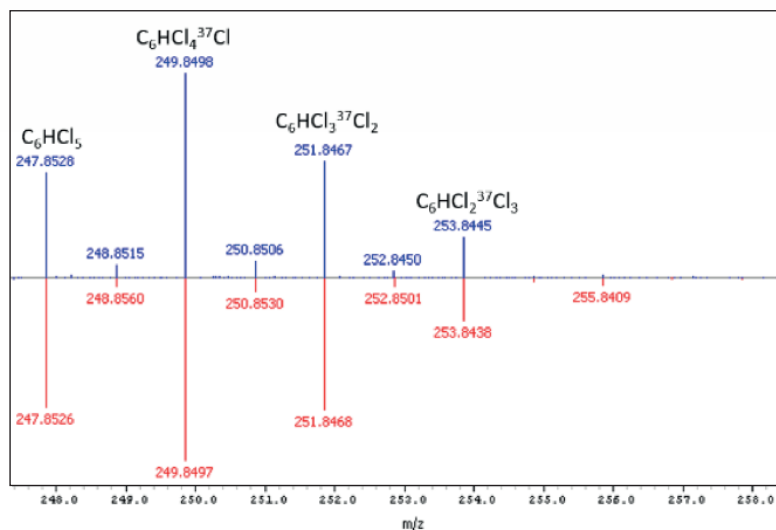
Fig. 6 – Evaluation of mass accuracy and its stability in time for selected compounds. Mass accuracy is calculated for the molecular ion fragment at a nominal concentration of approximately 10 ppb.
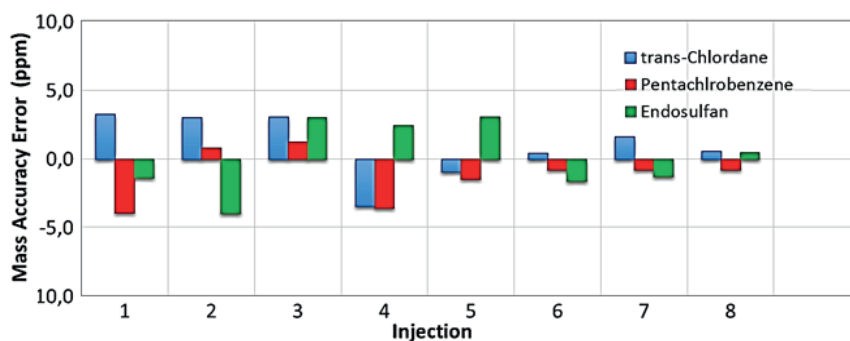
Table 2
Evaluation of mass accuracy and its stability in time for selected compounds. Mass accuracy is calculated for the molecular ion fragment at a nominal concentration of approximately 12 ppb.

Compound	Formula	Molecular mass (m/z)	Theoretical accurate mass for the M⁻ fragment (m/z)	Average ($n=8$) mass accuracy error (ppm)
trans-Chlordane	$C_{10}H_6Cl_8$	406	405.7983	2.03
Pentachlorobenzene	C_6HCl_5	248	247.8526	1.67
Endosulfan	$C_9H_6Cl_6O_3S$	404	403.8174	2.14

CONCLUSIONS

- GC×GC with thermal modulation provides higher resolution power than standard GC and allows for easier, detailed separation of complex OCPs and PCBs mixtures.

- Thanks to the higher peak capacity, co-elution is much less likely even with very dirty matrices.

- Coupling to MS in scan mode allows simultaneous identity confirmation for targets and identification of unknowns. This is not possible with ECD or a triple quadrupole (MS/MS).

- The QTOF's high resolution and mass accuracy provide additional identification confidence, selectivity and sensitivity.

- The results obtained for linearity and repeatability show that GC×GC with thermal modulation and QTOF are robust and provide performance suitable for reliable identification and quantification.

- NCI mode provides good sensitivity for chlorinated compounds such as OCPs and PCBs.

- The possibility to switch from EI to CI mode, in combination with the MS/MS capability, grant wide operational flexibility.

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