A SINGLE-INJECTION ANALYSIS OF PESTICIDES AND OTHER POPS BY GC-ORBITRAP: OPPORTUNITIES AND CHALLENGES

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Introduction

For more than a quarter of century, high resolution magnetic sector mass spectrometers (GC-HRMS) provide the basis of dioxin analysis. Lately these instruments began to be used for other POPs. We have no doubt that in the foreseeable future magnetic HRMS will remain the gold standard in ultra-trace organic analysis. However, rapidly developing methods of "digital" high resolution mass spectrometry technics such as time of flight, Fourier transform and Orbitrap have opened a new era of analytical chemistry. Each of these methods provides chromatograms in the total ion current (TIC) mode with high mass assurance, which gives great opportunities for creating multi-component analysis methods. It is difficult to predict which technology will become the leading one in the long run, but today the Orbitrap technology (Thermo Q Exactive GC) is the most attractive for the end user. On these instruments TIC chromatograms are obtained with 120000 FWHM nominal resolution and sensitivity which, in our experience with real-life dioxin samples, comes close to that of a brand new Thermo DFS and is higher than that of a well-used Waters Autospec Premier instrument. Without a doubt, Q Exactive GC-MS is a phenomenal tool for academic research, but in routine applied research its prospects are not so obvious. Being very expensive, Q Exactive can hardly compete with magnet sector HRMS instruments in confirmatory dioxin analysis, and it is irrational to consider Q Exactive as an alternative to magnet or benchtop MS for routine analyses. But its use can be practicable in case of multicomponent screening studies, which cannot be performed on magnetic instruments due to limitations on the number of mass traces or their mass range or lack of selectivity in case of quadrupoles. Also the Orbitrap technology has limitations that are not immediately obvious for users of magnetic instruments. In this paper, we will consider the possibilities that the Orbitrap gives to us and the problems that need to solved for multi-residue POPs analysis in the single injection.

Materials and methods

Q Exactive GC Orbitrap and DFS instruments by Thermo, native POPs compounds from various suppliers, and ^{13}C and D-substituted surrogates from CIL and Wellington Laboratories.

Results and discussion:

Registration mode: Of the available Orbitrap scan modes, the most interesting for the purpose are TIC and Target SIM (an imitation of SIM/MID registration). The latter one looks more promising, as it has several sub-modes for avoiding the problems with the number of registration tracks, which are typical for conventional MS instruments. But in practice, almost nothing works as described in the manual. The manufacturer (Thermo) promises to release new drivers, but does not give any deadlines. Registration of TIC in a wide interval of masses is useful for library search, but because of the restriction on the total number of ions in Orbitrap detector it is not well suited for target trace analysis, so we decided to use segment TIC scan, i.e. registering in each time interval only molecular ions or, in their absence, the main fragmentation ions. However, here it is necessary to take into account one more Orbitap bug. Near the border of the ranges, the sensitivity is sharply decreased, which is clearly visible with organohalogen clusters (Fig 1).

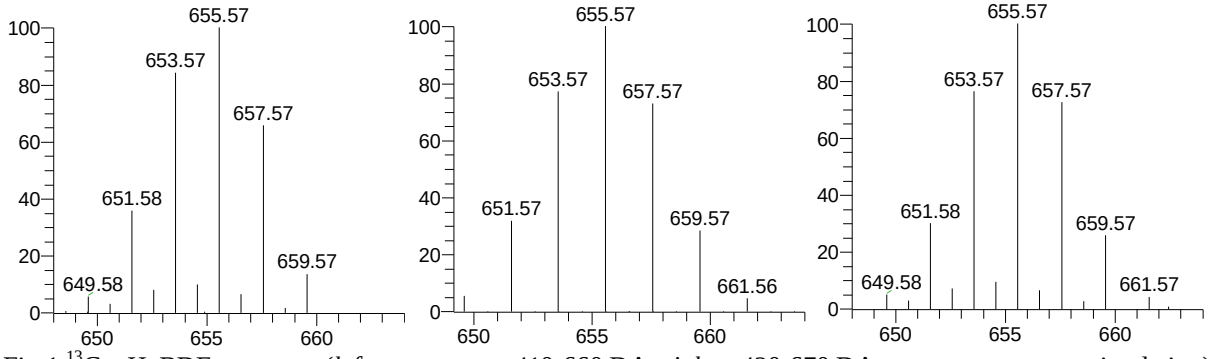

Fig.1 $^{13}C_{12}$-HxBDE spectrum (*left* – scan range 410-660 DA; *right* – 420-670 DA; *center* – computer simulation)

High resolution and accurate mass measurements.

Magnet sector HRMS instruments are usually tuned to a resolution of about 10000 (5% peak height), mass accuracy is ensured by continuous automatic adjustment using PFK peaks, which leads to increased noise on some traces of low-halogenated compounds.

The Orbitrap resolution is somewhat philosophical, the maximum nominal value is 120000 FWHM, i.e. formally about 5 times higher than in classical dioxin analysis. At the same time, its mass accuracy in the acquisition mode which uses automatic adjustment by background siloxane peaks ($C_3H_9O_2Si_2$, $C_5H_{15}O_3Si_3$, $C_7H_{21}O_4Si_4$, $C_9H_{27}O_5Si_5$, $C_{11}H_{33}O_6Si_6$, $C_{13}H_{39}O_7Si_7$) does not exceed the electron mass (0.55mmu) and is a little higher without this option even for small peaks. To increase the resolution from minimum to maximum does not require retuning of the instrument and does not lead to decreasing peak areas. Only the scanning speed decreases in the process, but, even at the maximum resolution, it is higher than that typical for magnetic HRMS.

In practice, the combination of extra high resolution and accurate mass in organohalogen compounds analysis gives you the opportunity not only to get rid of interference from non-halogenated compounds (which magnet sector HRMS also provide) and other organohalogen compounds (which is not always possible on a magnet sector HRMS), but also to distinguish ^{13}C from CH, as is clearly seen in the reduction of artifacts on Aroclor 1254 chromatogram (Fig.2). Unfortunately, it is impossible to completely get rid of artifacts due to the hydrogen adduction (Fig 3). Fig 4 shows PeCD/PeCDF chromatograms, the high side peak on native PCDFs traces is from fragmentation ions of ^{13}C-Mirex, different peak positions are due to stationary phase difference. This means that the role of sample preparation in dioxin analyses has changed: earlier, it was necessary to separate PCDDs/PCDFs as efficiently as possible from other halogenated compound. With Orbitrap it is no longer necessary, but other issues need to be addressed. Users of magnetic HRMS usually give no consideration to column background peaks or residual components of the matrix that do not cause distortion of chromatographic peaks. But these factors are critical on Exactive GC due to the limited ion capacity of the Orbitrap unit and a less robust, rapidly contaminated ion source. Increased background noise not only makes it difficult or impossible to search for minor quantities of non-target substances, but it reduces the sensitivity or leads to the disappearance of ions upon target analysis.

Sample preparation.

The physical-chemical properties of PCDDs/PCDFs and PCBs/PBDEs make it possible either to collect them in one fraction or to separate them into different fractions. If others POPs are to be analyzed, the clean-up procedure is radically complicated. Some pesticides are not stable under sulfuric acid treatment; others are difficult to elute even from silica gel; and using either activated basic aluminum oxide or Florisil PR is also impossible due to problems with elution or destruction of analytes. One possible solution is to use separate sample preparation protocols for stable, easy eluting compounds and for all others. Specifically, in this paper we tried to find a soft, nondestructive clean-up method for analysis of fat samples. In the first stage it is proposed to use a column with potassium silicate with elution by 25% DCM, next the sample is evaporated to dryness, fat residue is emulsified in MeCN and passed through activated (400°C, 12h) neutral aluminum oxide with elution by MeCN. If necessary, the last stage is repeated for separating the fat that was deposited during concentration stage. By this method, we obtained acceptable chromatograms (Fig. 5), which show the peaks of all GC-amenable POPs. However, in some cases (for example for PCDD/Fs), recovery drops below 50% which indicates that the method needs further optimization and it is necessary to use as many labeled standards as possible.

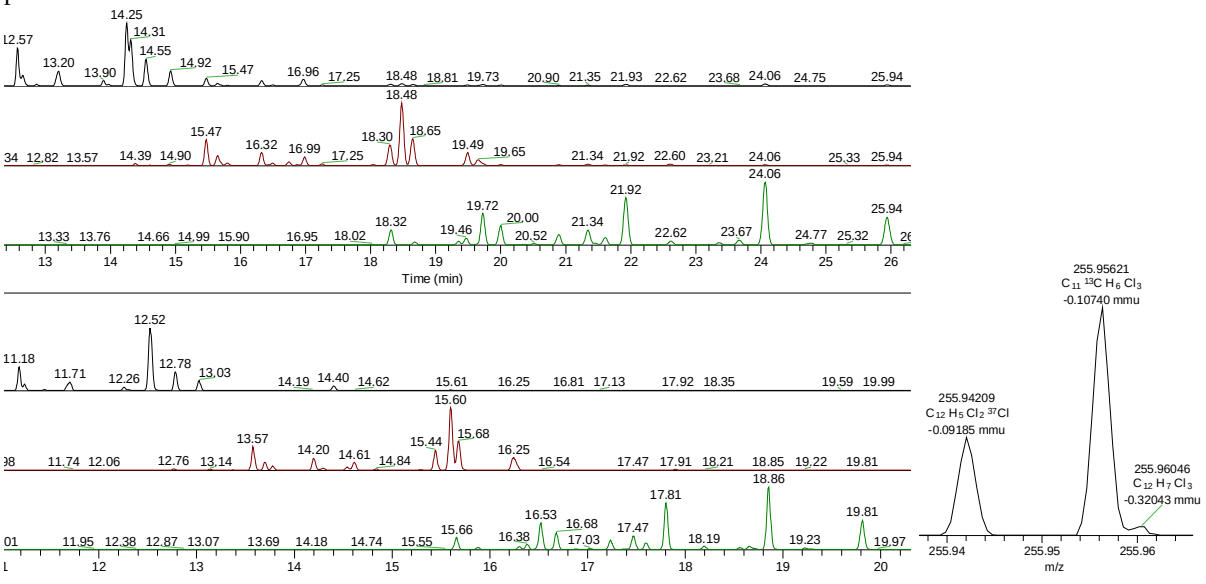

Fig.2 Mass Chromatogram for tri-, tetra- and penta PCB in Aroclor 1254 (*top* – DFS R=20000, column Thermo TG-Dioxin; *bottom* – Q Exactive GC R=120000, mass tolerance 1 mmu, column Thermo TG-5SILMS.

Fig. 3. Fragmentation ions in the spectrum of individual PCB-52

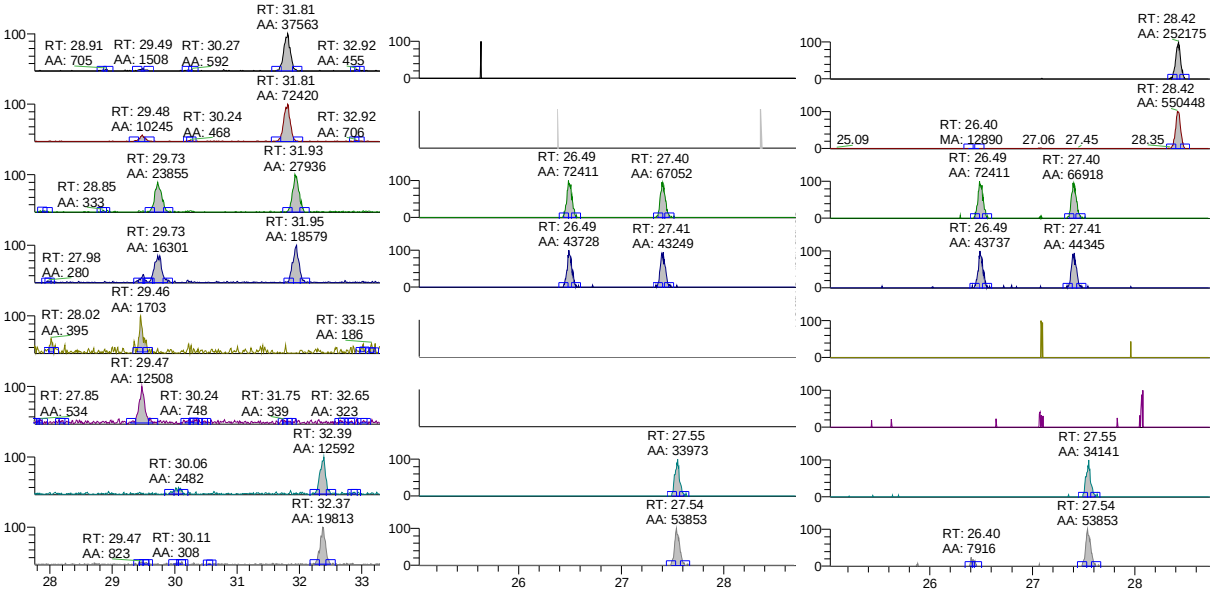

Fig.4 Chromatograms of PeCD/PeCDF in cheese fat after soft non-destructive clean-up, *left* – DFS R10000, column – Thermo TG-Dioxin; *middle* and *right*- Q Exactive GC R=120000, column J&W VF-Xms, mass tolerance 2 and 20, respectively.

Table 1. Stability of organochlorine pesticides on silica gel impregnated with sulfuric acid and potassium silicate (packed Pasteur pipette, elution with 25%DCM).

Compound	**SiO_2/H_2SO_4**	**K_2SiO_3**
HCB	+	+
α-HCCH	+	+
γ- HCCH	+	+
β- HCCH	+	+
δ- HCCH	+	+
o,p'-DDE	+	+
p,p-DDE	+	+
o,p'-DDD	+	+
p,p'-DDD	+	+
o,p'-DDT	+	+
p,p'-DDT	+	+
Heptachlor	+	+
Aldrin	+	+
Oxyclordane	+	+
Heptachlor epoxide	+	+
cis- Chlordane	+	+
trans-Chlordane	+	+
Trans-Nonachlor	+	+
Dieldrin	–	+
Mirex	+	+
Toxaphene	+	+
Endrin	–	+
Endosulfan	+	+
Endosulfan sulfate	+	+
Endrin aldehyde	–	+

"+" – stable; "-" – unstable

Table 2 Organochlorine pesticide fractionation on an activated silica gel column.

	Recovery *			
	Hexane, 15 ml	25% DCM, 30 ml	60% DCM, 30 ml	DCM 30 мл
HCB	50.6	49.1	0,3	0
α-HCCH	0	96.1	3.9	0
γ- HCCH	0	90.1	9.9	0
β- HCCH	0	93.4	6.6	0
δ- HCCH	0	85.2	14.8	0
p,p'-DDE	0	98.8	1.2	0
p,p'-DDD	0	95.3	4.7	0
p,p'-DDT	0	100	0	0
Heptachlor	0	99.1	0.9	0
Aldrin	1,5	98.5	0	0
Heptachlor epoxide	0	77.9	22.1	0
cis- Chlordane	0	98	2	0
trans-Chlordane	0	98	2	0
Dieldrin	0	0	100	0
Endrin	0	0	61.6	0
Endosulfan A	0	0	43.3	0
Endosulfan sulfate	0	0	100	0
Endrin aldehyde	0	0	72.7	27.3

* for total recovery values within 85-115%, the data are normalized to 100%, otherwise the real values are given

Chromatographic separation

A GC column for separation of all existing POPs isomers does not exist. Any choice will be a compromise, which, however, is acceptable for screening analysis. Until recently, the main choice was between "DB-5ms like" columns providing acceptable PCDDs/Fs isomer separation (though not for all!), but not able to resolve PCB 28 and 31 or 2,4'-DDT and 4,4'-DDD, and "HT-8 like" columns well separating PCBs and pesticides, but useless for PCDDs/Fs. At present, this problem can be considered almost solved. In our hands, a Thermo TG-Dioxin column provided better PCDD/F isomers separation than DB-5ms and showed significantly better results for PCBs (Fig 2, PCB-31 RT14.25, PCB-28 RT14.31) and pesticides. The only remaining problem is with heavily-brominated PBDEs.

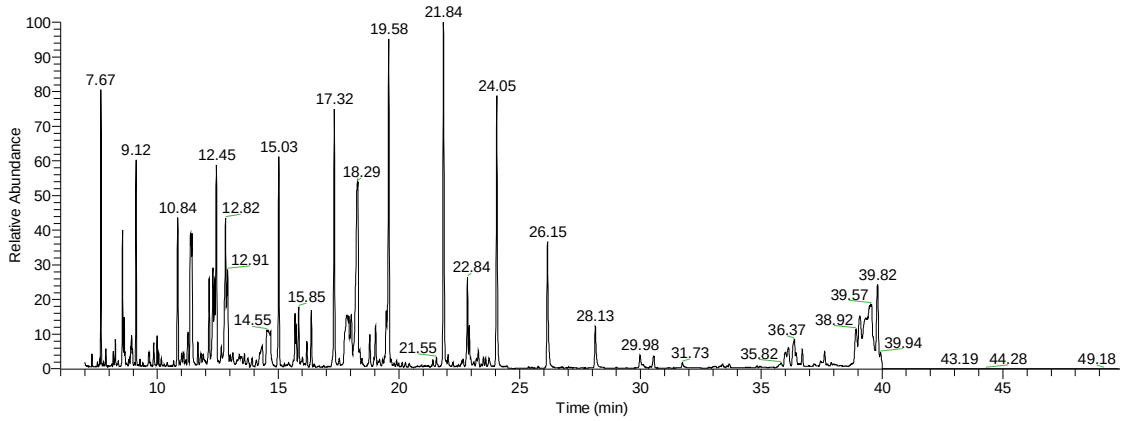

Fig. 5 TIC chromatograms of cheese fat after soft non-destructive clean-up

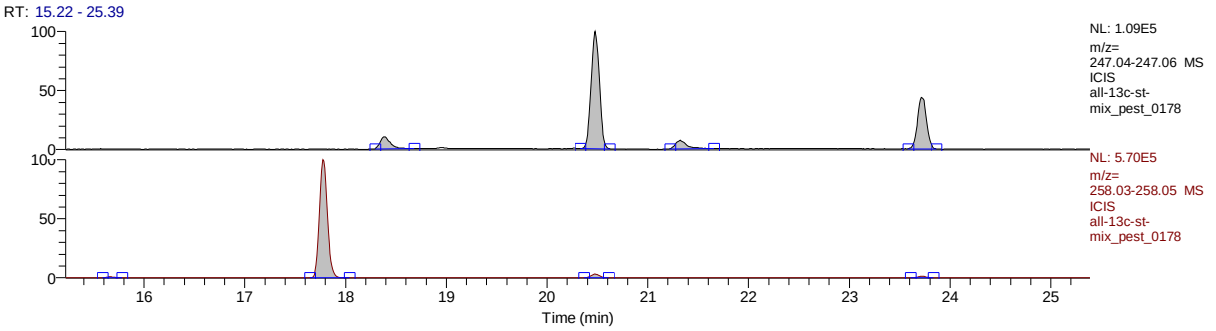

Fig. 6 DDTs, DDDs and DDEs separation on a Thermo TG-Dioxin column

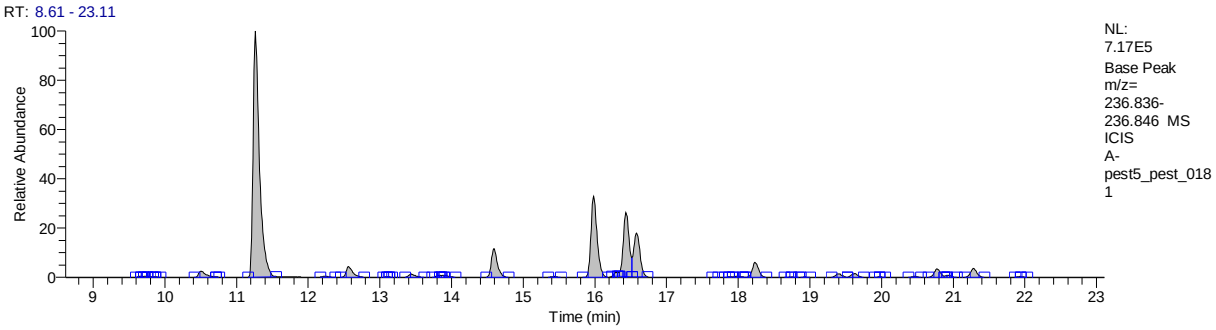

Fig. 7 "drin" pesticides separation on a Thermo TG-Dioxin column

Quantitative calculation

The software by Thermo is user unfriendly with a chaotic arrangement of controls. The user has no choice when setting up and controlling the instrument, but, thankfully, alternative software exists for quantitative target analysis. Raw chromatograms can be converted into CDF format by a built-in Thermo tool, then converted by Waters Databridge into Masslynx format with the subsequent processing in TargetLynx software which has all that is necessary for trace organogalogen analyses, i.e. simultaneous display of several isotopic masses for the target compound and the corresponding standard, manual integration adjustment, the use of relative and absolute retention times. As a bonus, you do not have to be a skillful programmer to use the TargetLynx software.