ANALYSIS OF PESTICIDES AND OTHER ORGANIC CONTAMINANTS ON A "Q EXACTIVE" GC-MS WITH QUECHERS SAMPLE PREPARATION

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Introduction

The ever growing number of detectable analytes is characteristic of modern applied analytical chemistry, as toxic properties are found in numerous substances that have been previously considered harmless for industrial and agricultural applications. At the same time, the reliability of identification and quantitative determination of pollutants have to keep up with the new challenges. So far, the most reliable methods for trace analysis of organic pollutants are those based on high-resolution chromato-mass-spectrometry. However, the use of "classic" magnetic sector instruments for multi-component analysis is limited by the number of mass peaks that can be registered in a time window and by the practicable mass ranges ^{1,2}. The new generation of GC-HRMS instruments are free from these limitations and can register mass chromatograms in total ion current (TIC) mode in a wide range of ion masses with very high mass accuracy (HRAM MS). Such instruments can use different technologies and mass detector designs. They include TOF GC-MS instruments, which have been available for quite some time already; the Exactive series by Thermo Scientific, which is available since 2015³, is using Orbitrap ion traps and Fourier transform (FT); there are some other examples of FT-MS coupled with GC⁴. Each of these techniques has its own advantages, but Thermo Q Exactive GC currently provides the end user with the best analytical options. In order to fully realize its potential, sample preparation should be thoroughly optimized. The so-called QuEChERS method is currently very popular in various applications, including HRAM-MS ^{5,6}. Although the use of QuEChERS for dioxin analysis is questionable, it is quite applicable for simultaneous determination of analytes that are widely different in their physicochemical characteristics, and it does not require chemical decomposition of the matrix. Here we report a QuEChERS sample preparation for the analysis of PCDD/PCDF, PCBs, PBDEs and pesticides classified as POPs on a Thermo Q Exactive GC-MS instrument.

Materials and methods

The QuEChERS protocol was based on the sample preparation method used in our laboratory for the analysis of insecticides in plant-derived and other biological samples. Isotope-labelled internal standards of PCBs (Wellington Laboratories) and POP pesticides (Cambridge Isotope Laboratories) and 10 ml acetonitrile were added to 1 g of the raw sample (0.25 g if lyophilized) in a 40-ml glass vial and mixed on a Heidolph Multi Reax shaker at 2000 rpm, then 6 g of MgSO₄ and 1.5 g NaOAc (Capure) added and shaked again. After sedimentation, 1 ml of the extract was transferred into an Eppendorf tube, a mixture of 150mg MgSO₄, 50 mg PSA (Primary and secondary amine exchange material) and 50 mg C18 added, shaked for 5 mins at 2000 rpm, and centrifuged for 10 mins at 14 000 rpm. The organic layer was evaporated and re-dissolved in isooctane with 10 ul undecane added.

PCBs and POPs pesticides were analyzed on an SGE HT-8 column (25m x 0.25 x 0.25). Injection: 1.5 µl in splitless mode; initial oven temperature 135 °C for 1.5 min, 15 °C/min ramp to 170 °C, followed by ramp of 4 °C/min to 270 °C and by ramp of 5 °C/min to 305 °C for 17 min hold; injector temperature, 280 °C; constant flow of carrier gas (He), 0.8 ml/min. The mass spectra were obtained at 120000 resolution (FWHM) in TIC mode, using slightly wider time windows to avoid the peak intensity distortions at the range limits that are characteristic for Exactive GC (Table 1). To increase the mass accuracy, we used automated adjustment against alkylsiloxane peaks (Table 2). This method works well both with DB-5ms columns (5% of 1,4-bis(dimethylsiloxy)phenylenomethylpolysiloxane stationary phase) and with phenylpolycarbonate siloxane stationary phase. The values for singly charged positive ions were calculated using the Ame2016 atomic mass evaluation ^{7,8}.

Table 1: Time and mass windows

Time, min	m/z
7-14.5	184-304
14.5-27	230-430
27-31.2	334-510
31.2-35.8	367-590
35.8-41	400-670
41-55	700-800

Table 2: Lock ions and mass

Ion	m/z
$C_3H_9O_2Si_2$	133.01356
$C_5H_{15}O_3Si_3$	207.03235
$C_7H_{21}O_4Si_4$	281.05114
$C_9H_{27}O_5Si_5$	355.06993
$C_{11}H_{33}O_6Si_6$	429.08872
C13H39O7Si7	503.10752

Table 3: Exact masses of selected chlorinated pesticides

Compound (formula)	ion	m/z (native)	m/z (¹³ C)		
$\mathbf{H}\mathbf{C}\mathbf{C}\mathbf{H}(\mathbf{C},\mathbf{H},\mathbf{C}\mathbf{L})$	[M HC1.1+	216.91339	222.93412		
		218.91104	224.93117		
$HCB(C_{1}C_{1})$	[M]+	283.80962	289.82975		
$\operatorname{HCD}\left(\operatorname{C}_{6}\operatorname{CI}_{6}\right)$		285.80667	291.82680		
Pontachloroonisola (C-H-Cl-O)	[M]+	279.85915	286.88264		
rentacinoroanisole (C7113C15O)		281.85620	288.87969		
$DDE(C_{1},H_{2}C_{1})$	[M]+	315.93746	327.97772		
$DDE\left(C_{14}H_{8}C_{14}\right)$		317.93451	329.97477		
DDT/DDD	$[M_{DDT}-CCl_3]^+$	235.00758	247.04784		
$(C_{14}H_9Cl_5 / C_{14}H_{10}Cl_4)$	$[M_{DDD}-CHCl_2]^+$	237.00463	249.04489		
$D_{ad} \rightarrow DDD (C_{ad}H_{a}D_{a}CL)$	[M CHCl.]+	243.05780			
$D_{8(ring)}$ - DDD (C14112 D_{8} C14)		245.05485			
	[M CL]+	401.74732	411.78087		
Mirey (CroCho)		403.74437	413.77792		
1000000000000000000000000000000000000	[M, C, C]	271.80962	276.82639		
		273.80667	278.82344		
"drins"	$[C_{1}C_{1}]^{+}$	236.84076	241.85754		
unins		238.83781	243.85459		
Aldrin ($C_{12}H_8Cl_6$)	$[M_{Al}\text{-}C_5H_6Cl]^+$	262.85641	269.87990		
Dieldrin/Endrin (C ₁₂ H ₈ Cl ₆ O)	$[M_{Diel/End}\text{-}C_5H_6ClO]^+$	264.85346	271.87695		
Endrin aldehyde (CyaHaClaO)	[M C1]+	344.89828	356.93854		
		346.89533	358.93559		
Hentachlor (CueHcCla)	$[M-C_5H_5Cl]^+$	271.80962	276.82639		
		273.80667	278.82344		
Hentachlor enovide (CueHcCl-O)	[M C1]+	352.84366	362.87721		
		354.84071	364.87426		
Chlordane (CroHcClo)	[M C1]+	372.82542	382.85897		
		374.82247	384.85602		
Oxychlordane (CueHiCloO)	[M H ₂ C1] ⁺	386.80468	396.83823		
Oxychiordane (C10114C18O)		388.80173	398.83528		
Nonachlor (CuaH-Cla)	[M C1]+	406.78645	416.82000		
		408.78350	418.81705		
Endocultan (CoHCLOoS)	[M C1S1 ⁺	338.87246	347.90265		
Endosunan (C916C16O3S)		340.86951	349.89970		
	[M]+	421.80830	430.83849		
Endocultan sulfate (CoHcCl.O.S)		423.80535	432.83554		
Endosuntan suntate (C9116C16O4S)	[M C1]+	386.83944	395.86964		
		388.83649	397.86669		

Cl/Br	PCDDs		PCDFs		PCBs		PBDEs	
	native	$^{13}C_{12}$	native	$^{13}C_{12}$	native	$^{13}C_{12}$	native	$^{13}C_{12}$
1	218.01291	230.05317	202.01799	214.05825	188.03873	200.07899	247.98313	260.02339
	220.00996	232.05022	204.01504	216.05530	190.03578	202.07604	249.98108	262.02134
2	251.97394	264.01419	235.97902	248.01928	221.99976	234.04002	325.89364	337.9339
	253.97099	266.01124	237.97607	250.01633	223.99681	236.03706	327.89159	339.93185
3	285.93496	297.97522	269.94005	281.98031	255.96078	268.00104	405.80210	417.84236
	287.93201	299.97227	271.93710	283.97736	257.95783	269.99809	407.80006	419.84031
4	319.89599	331.93625	303.90108	315.94133	289.92181	301.96207	483.71262	495.75288
	321.89304	333.9333	305.89813	317.93838	291.91886	303.95912	485.71057	497.75083
5	355.85407	367.89433	339.85915	351.89941	325.87989	337.92015	563.62108	575.66134
	357.85112	369.89138	341.85620	353.89646	327.87694	339.9172	565.61903	577.65929
6	389.81510	401.85535	373.82018	385.86044	359.84092	371.88118	641.53159	653.57185
	391.81215	403.8524	375.81723	387.85749	361.83797	373.87823	643.52954	655.5698
7	423.77612	435.81638	407.78121	419.82147	393.80195	405.8422	721.44006	733.48031
	425.77317	437.81343	409.77826	421.81852	395.79900	407.83925	723.43801	735.47826
8	457.73715	469.77741	441.74224	453.78250	427.76297	439.80323	799.35057	811.39083
	459.73420	471.77446	443.73929	455.77955	429.76002	441.80028	801.34852	813.38878
9					461.72400	473.76426	879.25903	891.29929
					463.72105	475.76131	881.25698	893.29724
10					497.68208	509.72234	957.16954	969.2098
					499.67913	511.71939	959.16750	971.20775
				N-DDE D.		719.42441	731.46466	
					NOBL	$\mathbf{p}\mathbf{E}$ - $\mathbf{B}\mathbf{r}_2$	721.42236	733.46261
	7					797.33492	809.37518	
					ревре-вг ₂ 799.33287			811.37313

Table 4: Exact masses of the most abundant ions of PCDDs, PCFDs, PCBs and PBDEs

Results and discussion

Previously we observed that considerable amounts of siloxanes were leaking from QuEChERS materials. They did not interfere with insecticide analysis by MS/MS, but in the case of POPs and pesticides on Q Exactive the increased background not only prevented non-target analysis, but also decreased the sensitivity due to detector overload. These problems are evident, e.g. with p,p'-DDT (Fig. 1), "drins" (Fig. 2, cf. cis-chlordane with RT 19.5), and some PCBs (Fig. 3.). However, the overall quality of chromatograms is acceptable, and the results demonstrate the applicability of QuEChERS sample preparation for multicomponent analysis on a Q Exactive instrument. The following advantages of the method should be highlighted:

- efficient elimination of lipids;

- high recovery values for most analytes, which are at least 50% in difficult cases(γ -HCCH, PBDEs, TCDDs/TCDFs):

- no discrimination for lower-substituted PBDEs, unlike previously reported ⁹;

- registration of ion peaks in a wide range of masses with correct intensities of isotopic cluster peaks.

There are also some obvious problems with QuEChERS sample preparation, including poor limits of detection, as only ca. 10% of the starting material is available for analysis following the purification stage. This is enough for the analysis of PCBs, PBDEs and most common pesticides. However, the monitoring of the full list of POPs including PCDDs/PCDFs at the levels comparable to their MRLs would require no less than 0.5 g of fat. In our opinion, this problem could be approached by combining traditional column-based purification techniques with the QuEChERS methodology.







Fig. 2 "Drins" in canned Baltic sprat (Oxyclordane -17.43, Heptachlor epoxide 17.66; trans-nonachlor 19.3; Dieldrin 20,64, cis-nonachlor 19.3)



Fig. 3, Tri-, Tetra-, Penta- and HxCB in canned Baltic sprat

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