APPLICATION NOTE #1001

CHROMTECH EVOLUTION GC-QqQ

Analysis of PCB in fish tissue by GC-MS/MS

Fish can accumulate the amount of PCBs in water up to 9 millionfold. Since DDTs and PCBs presence are persisent and ubiquitarious, they can be detected even in the deepest and most remote places on earth. Because of the high amounts of PCBs and Dioxins, especially found in fatty fish (e.g. eel, cod), the BfR (Federal Insitute for Risk Assessment) has published several recommendations for the consumption of fish: for low-fat seafish a toxicologically accepted daily intake of 80-150 g and for fat rich seafish a toxicologically accepted daily intake of 70 g. For fish liver there is no maximum amount defined.

Due to the fact of contaminant accumulation described above, fish can also be used as an excellent indicator for the pollution of water.

Even the smallest amounts of contamination with PCBs can be monitored by analyzing fish tissue (e.g. liver and melt) of fat-rich fish like eel.

Congener-specific analysis of the PCBs in fish tissues is often performed by GC-ECD or GC-MS. However both techniques suffer from the often huge amount of matrix compounds being inherent in the sample extracts, coeluting with the target PCB congeners. Though tedious and costly clean-up procedures eliminated many of these interfering compounds, still enough remained to cause uncertainties for quantitation. To overcome these matrix-related issues the GC-MS/MS technique has proven to be the gold-standard.

Due to the selectivity of the GC-MS/MS technique, matrix effects can be greatly reduced thus enhancing sensitivity. Also the possibility of false positives or false negatives is lowered.

This application note describes the successful use of the CHROMTECH EVOLUTION Triple Quadrupole GC-MS/MS system for the analysis of PCB in fish tissue.

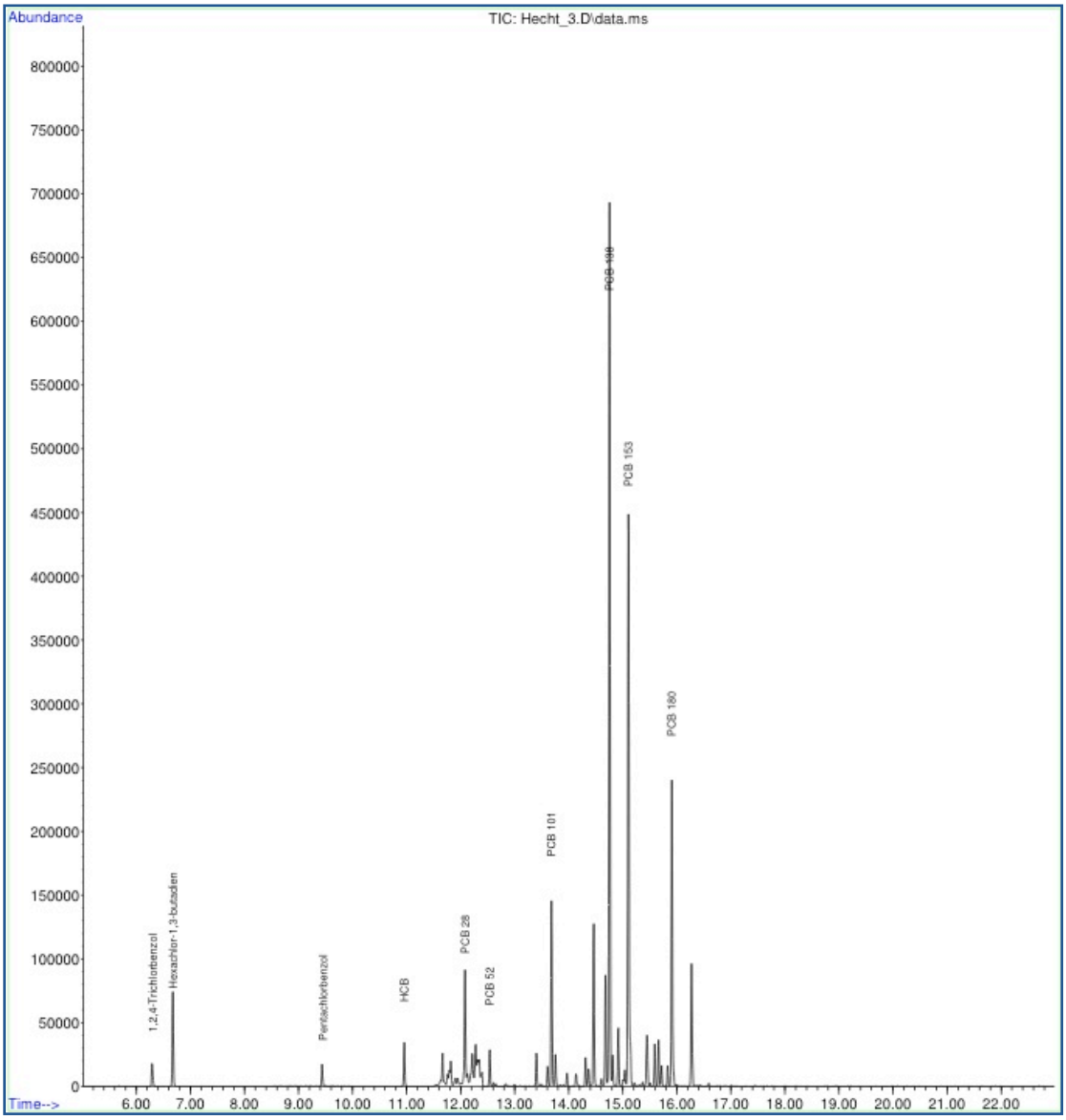

Fig. 1: SRM-TIC, pike liver, isotope dilution quantitation with ^{13}C-labeled internal standards, 1 µL injection volume

GC-QqQ Instrument Parameters:

GC Column:	Agilent 19091S-433, HP-5ms, 30 m x 250 µm x 0.25 µm Constant Flow 1.0 mL/min, Average velocity 36 cm/sec
Inlet:	Splitless injection, 280 °C, Purge time 1 min, Helium
Oven Program:	50 °C, 1.1 min, 100 °C/min, 70 °C, 15 °C/min 320 °C, 9 min
Transfer Line:	300 °C
CID gas:	Argon
MRM Scan time:	0.33 sec/group
Q1+Q3 resolution:	1.0
MS Ion source temp:	230 °C
MS Quadrupole temp:	150°C

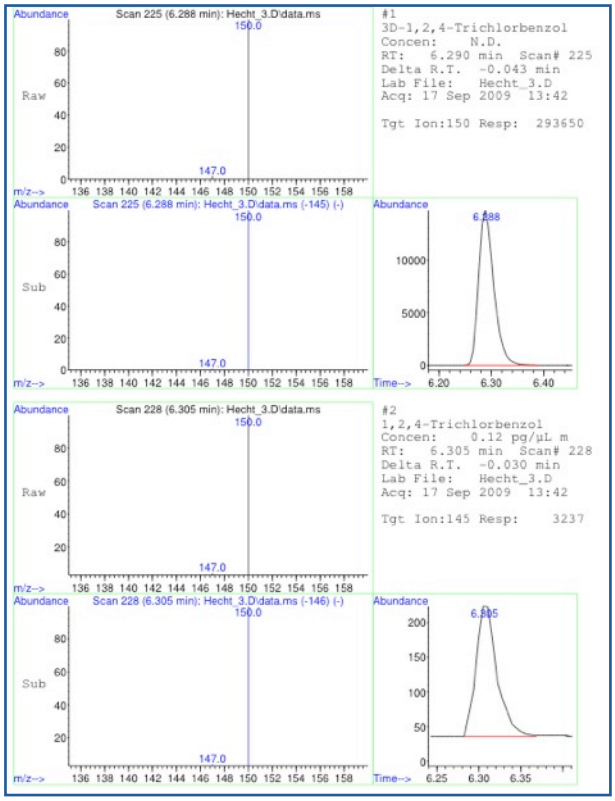

Fig.2: 1,2,4-Trichlorobenzene, quantitation result,
calculated amount 0.12 pg/µL
m/z 180 > m/z 145 @ -15 V CE,
m/z 182 > m/z 147 @ -15 V CE

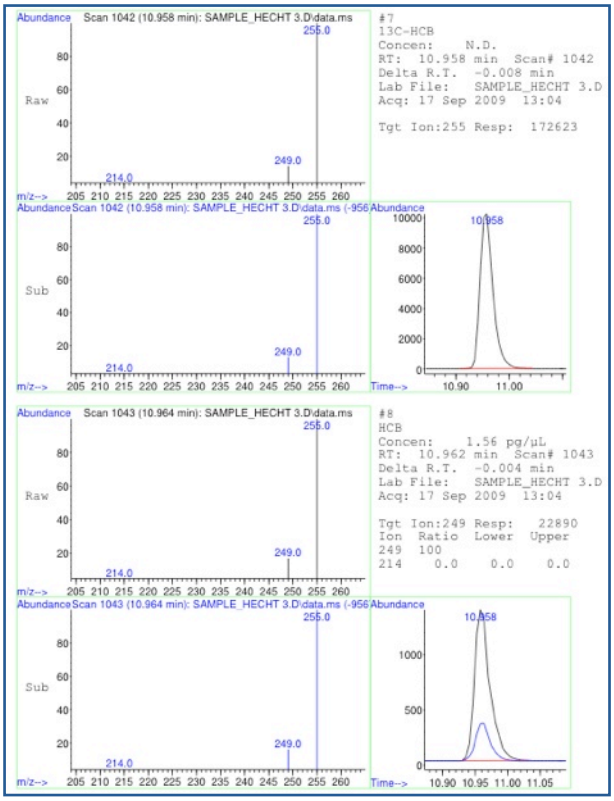

Fig.3: Hexachlorobenzene, quantitation result,
calculated amount: 2.41 pg/µL
m/z 284 > m/z 249 @ -18 V CE,
m/z 249 > m/z 214 @ -13 V CE

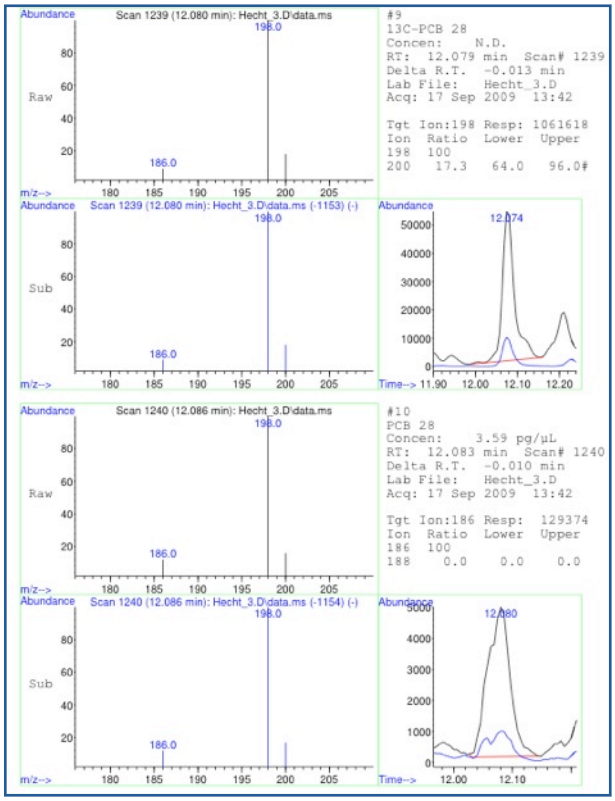

Fig.4: 2,4,4'-Trichlorbiphenyl (PCB 28)in pike liver,
calculated amount: 3.59 pg/µL
m/z 258 > m/z 188 @ -17 V CE,
m/z 256 > m/z 186 @ -17 V CE

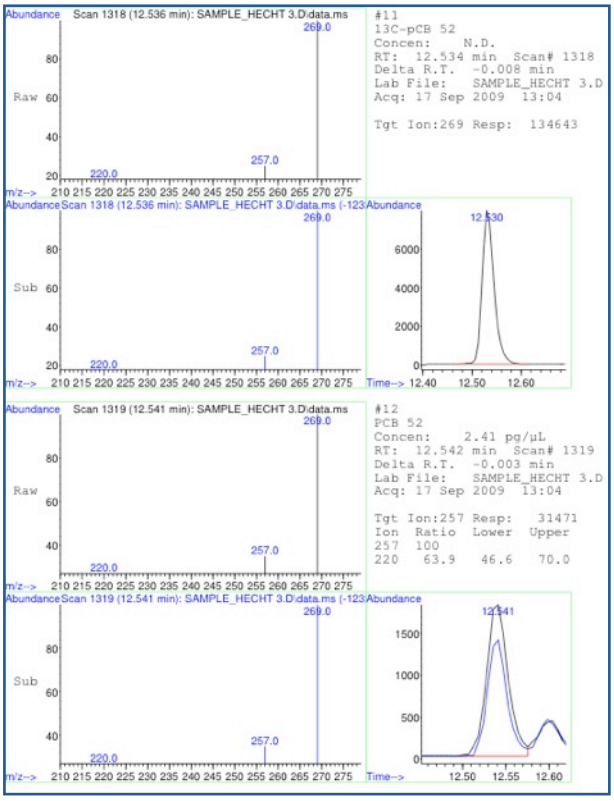

Fig.5: 2,2',5,5'-Tetrachlorbiphenyl (PCB 52) in pike liver,
calculated amount: 2.41 pg/µL
m/z 292 > m/z 257 @ -14 V CE,
m/z 292 > m/z 220 @ -17 V CE

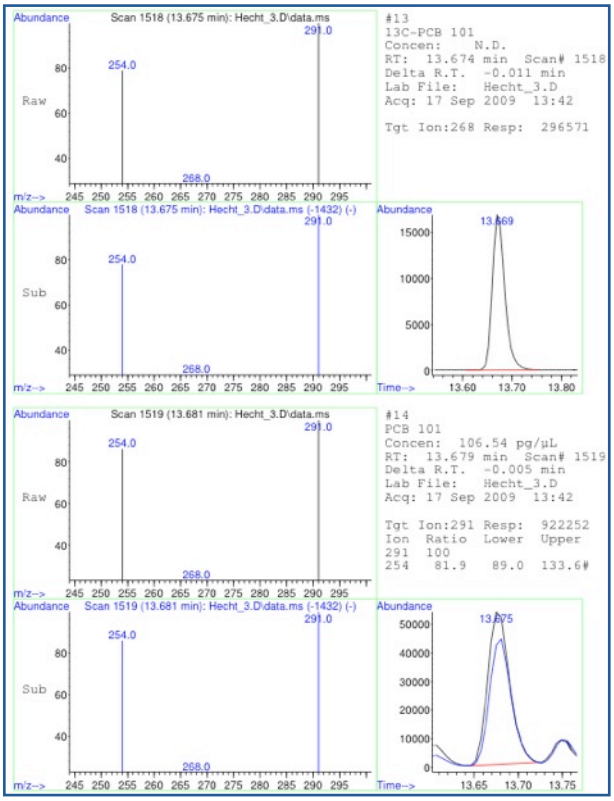

Fig.6: 2,2',4,5,5'-Pentachlorbiphenyl (PCB 101) in pike liver, calculated amount: 106.54 pg/µL
m/z 326 > m/z 291 @ -13 V CE,
m/z 326 > m/z 254 @ -18 V CE

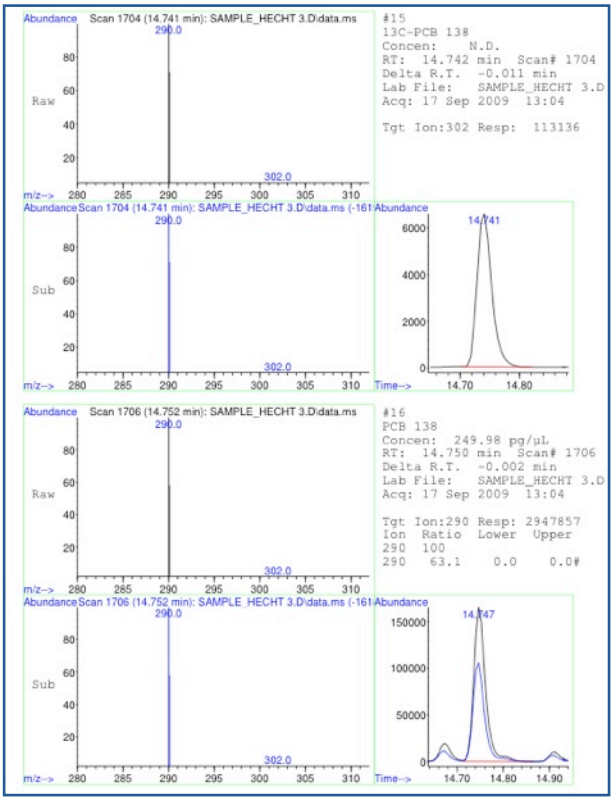

Fig.7: 2,2',3,4,4',5'-Hexachlorbiphenyl (PCB 138) in pike liver, calculated amount 249.98 pg/µL
m/z 360 > m/z 290 @ -17 V CE,
m/z 262 > m/z 290 @ -17 V CE

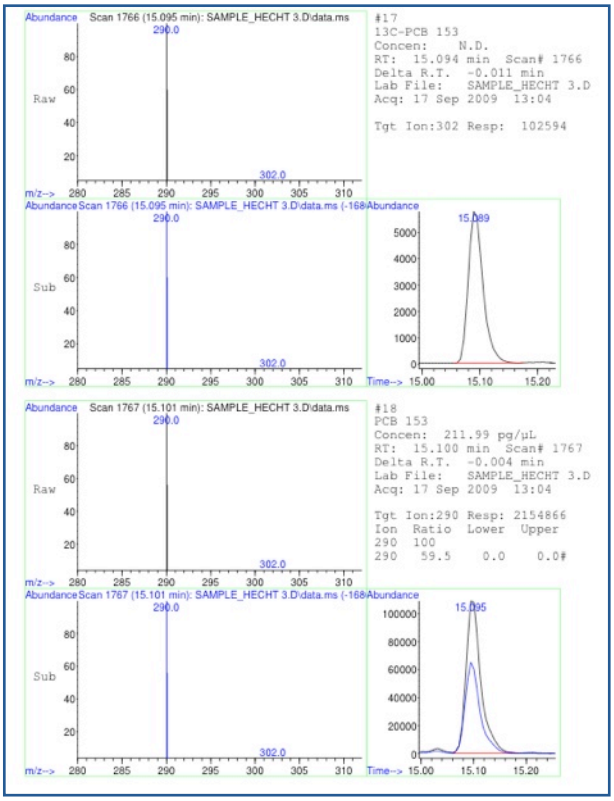

Fig.8: 2,2',4,4',5,5'-Hexachlorbiphenyl (PCB 153) in pike liver, calculated amount 211.99 pg/µL
m/z 360 > m/z 290 @ -17 V CE,
m/z 262 > m/z 290 @ -17 V CE

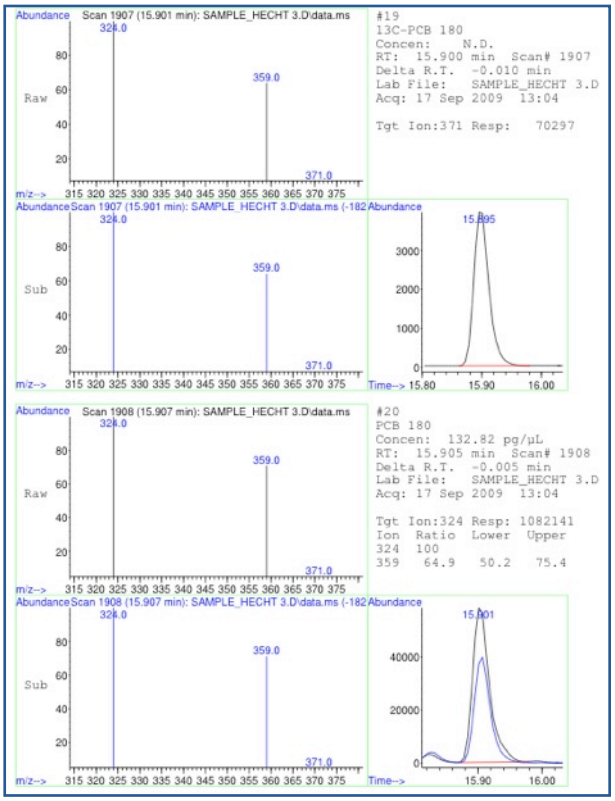

Fig.8: 2,2',3,4,4',5,5'-Heptachlorbiphenyl (PCB 180) in pike liver, calculated amount 132.82 pg/µL
m/z 394 > m/z 324 @ -14 V CE,
m/z 394 > m/z 359 @ -20 V CE

For the detection of each PCB congener two different SRM transitions, each with either a different precursor („parent") or product („daughter") ion from the chlorine isotope cluster were used. Due to the fact that the CHROMTECH EVOLUTION Triple Quadrupole GC-MS/MS enables very short dwell times, an average of 6 to 8 data points across a chromatographic peak is possible thus allowing for reliable peak integration and quantitation.
The unique selectivity and sensitivity of the CHROMTECH EVOLUTION GC-MS/MS system virtually eliminates complex matrix interference, therefore accurate identification and quantification of the PCB congeners in low picogram (pg) levels is made possible.
The rugged and reliable ion source together with the patented CHROMTECH IonRail collision cell technolgy ensure satisfying analysis results even when running long sequences.

```
Data Path : C:\msdchem\1\DATA\
Data File : Hecht_3.D
Acq On    : 17 Sep 2009  13:42
Operator  :
Sample    : Hecht µL
Misc      : Hecht leber 2 µL
ALS Vial  : 1   Sample Multiplier: 1

Quant Time: Nov 29 08:53:39 2010
Quant Method : C:\msdchem\1\METHODS\CT_PCB_Q10_ext.M
Quant Title  : PCB
QLast Update : Thu Sep 17 08:41:08 2009
Response via : Initial Calibration
```

Compound	R.T.	QIon	Response	Conc	Units	Dev(Min)
Target Compounds						Qvalue
1) 13C-1,2,4-Trichlorbenzol	6.290	150	293650	1		
2) 1,2,4-Trichlorbenzol	0.000		0	N.D.		
3) 13C-Hexachlor-1,3-buta...	6.673	194	1074180	1		
4) Hexachlor-1,3-butadien	0.000		0	N.D.		
5) 13C-Pentachlorbenzol	9.435	221	231479	1		
6) Pentachlorbenzol	0.000		0	N.D.		
7) 13C-HCB	10.955	255	400091	1		
8) HCB	10.959	249	48786	3.33	pg/µL#	80
9) 13C-PCB 28	12.079	198	1061618	1		
10) PCB 28	12.083	186	129374	3.59	pg/µL#	9
11) 13C-PCB 52	12.535	269	289337	1		
12) PCB 52	12.541	257	75340	5.78	pg/µL#	68
13) 13C-PCB 101	13.674	268	296571	1		
14) PCB 101	13.679	291	922252	106.54	pg/µL#	66
15) 13C-PCB 138	14.745	302	241803	1		
16) PCB 138	14.754	290	6057482	513.68	pg/µL#	67
17) 13C-PCB 153	15.098	302	218997	1		
18) PCB 153	15.104	290	4411206	374.84	pg/µL#	73
19) 13C-PCB 180	15.902	371	128957	1		
20) PCB 180	15.906	324	1998423	245.28	pg/µL	95

Fig.9: Quantitation Report, PCBs in pike liver

Fig.10: CHROMTECH EVOLUTION Triple Quadrupole GC-MS/MS
Offering unique selectivity and sensitivity for analysis of target compounds in difficult matrices