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A novel high resolution accurate mass Orbitrap-based GC-MS platform for routine analysis of Short Chained Chlorinated Paraffins

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Introduction

Short Chained Chlorinated Paraffins (SCCPs) are emerging contaminants that, once released, will remain in the environment for long periods of time with the potential to bioaccumulate in living organisms. SCCPs are intentionally manufactured and used as lubricants and coolants in the metal processing industry or as plasticizers and flame retardants in plastic products. Chronic exposure to SCCPs is believed to have harmful and irreversible effects for humans and the environment.[1] As a consequence, SCCPs are listed in the Stockholm convention as chemicals with potential adverse effects and their production and use in Europe is restricted and regulated.[2]

Detection and quantification of SCCPs poses analytical challenges due to the fact that these compounds are present in the environment at low levels, as very complex isomeric mixtures and are difficult to separate chromatographically. Although there is no consensus for the use of a validated analytical procedure for the routine monitoring of SCCPs in environmental samples, there are several analytical methods that are used to detect and quantify SCCPs. Details of these methods and their limitations are listed in Table 1.

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Table 1. Current analytical methodology used for the analysis of SCCPs.

Carbon skeleton analysis by GC-FID or GC-MS	GC-ECD	GC-NCI-MS
Details: Uses Pd catalyst held in the gas chromatograph injector to simultaneously dechlorinate the CPs and separate the resulting alkanes	**Details:** GC coupled to electron capture detector, sensitive for halogenated compounds	**Details:** Uses soft ionisation (negative chemical ionisation) with methane and/or dichloromethane
Disadvantages: • Lack in sensitivity and selectivity • No information on the degree of chlorination of the SCCPs can be achieved	**Disadvantages:** • Relatively non-specific • Interferences from other halogenated compounds	**Disadvantages:** • Low resolution GC-MS nominal mass interferences from higher chlorinated PCBs, toxaphenes and chlordane-related compounds, have similar molecular masses

In this study, the performance of a novel bench top, high resolution accurate mass Orbitrap-based GC-MS was tested for the analysis of SCCPs. System performance was tested using full-scan acquisition and simple instrumental setup. The experiments performed focused on assessing the sensitivity, linear dynamic range, selectivity and analytical precision for the analysis of two SCCPs technical mixtures. Both electron ionization (EI) and negative chemical ionization (NCI) were used and the results compared and discussed.

Experimental

In the experiments described here, a Thermo Scientific™ Exactive™ GC Orbitrap™ mass spectrometer was coupled to a Thermo Scientific™ TRACE™ 1310 Gas Chromatograph for gas-phase separation of target compounds, achieved on a Thermo Scientific™ TraceGOLD TG5-SilMS 15m x 0.25 mm x 0.25 µm column (P/N 26096-1300). Injection of liquid samples was performed automatically using a Thermo Scientific™ TriPlus™ RSH™ autosampler. The Exactive GC was tuned and calibrated in under one minute using PFTBA to achieve the best ion transmission and sub-ppm mass accuracy. The mass spectrometer was operated in full-scan using 60k mass resolution (measured as FWHM at *m/z* 200). Lockmass corrected data was processed with Thermo Scientific™ TraceFinder™ software. Additional details regarding the GC and MS conditions are given in Table 2.

Table 2. Gas chromatography and mass spectrometers analytical parameters.

TRACE 1310 GC System Parameters	
Injection Volume (µL):	2.0
Liner:	LinerGOLD™, single taper (P/N:453A0344-UI)
Inlet (°C):	280
Inlet Module and Mode:	Splitless
Carrier Gas, (mL/min):	He, 1.2
Oven Temperature Program	
Temperature 1 (°C):	100
Hold Time (min):	2.0
Temperature 2 (°C):	310
Rate (°C/min):	20
Hold Time (min):	4.0

Electron Ionization MS Parameters	
Transfer line (°C):	280
Ionization type:	EI
Ion source (°C):	230
Electron energy (eV):	70
Acquisition Mode:	Full-scan
Mass range (Da):	50-650
C-Trap voltage (V):	0.0
Mass resolution (FWHM at *m/z* 200):	15k, 30k and 60k
Lockmass (*m/z*):	207.03235 281.05114 355.06993

Negative Chemical Ionization MS Parameters	
Transfer line (°C):	280
Ionization type:	NCI
Ion source (°C):	200
Reagent gas and flow (mL/min):	Methane, 1.2
Electron energy (eV):	70
Electron Lens Voltage (V):	10
Emission current (µA):	150
C-Trap voltage:	2.0
Acquisition Mode:	Full-scan
Mass range (Da):	200-550
Mass resolution (FWHM at *m/z* 200):	60k
Lockmass (*m/z*):	234.94104

Complete separation of SCCPs is very difficult due to the very high numbers of isomers and homologues with similar physiochemical properties (Figure 1). Due to the fragmentation obtained in EI, it is often difficult to find homologue specific ions with sufficient intensity for use as quantification masses, highly efficient electron ionization can be used to detect and quantify SCCPs.

To provide even higher selectivity for homologue groups, GC-MS with negative chemical ionization is the method preferred by many laboratories for the detection and quantification of SCCPs. NCI allows for sensitive and selective detection of SCCPs by using ions characteristic for various homologue groups in a mixture (in particular [M-Cl]-, [M-HCl]-). Using NCI, the SCCP congeners in the two technical mixtures were easily separated based on the number of chlorine substitutes for a certain carbon chain length and according to the number of carbon atoms and chlorine atoms for various carbon chain lengths. Examples of congener specific extracted ion chromatograms are given in Figure 2.

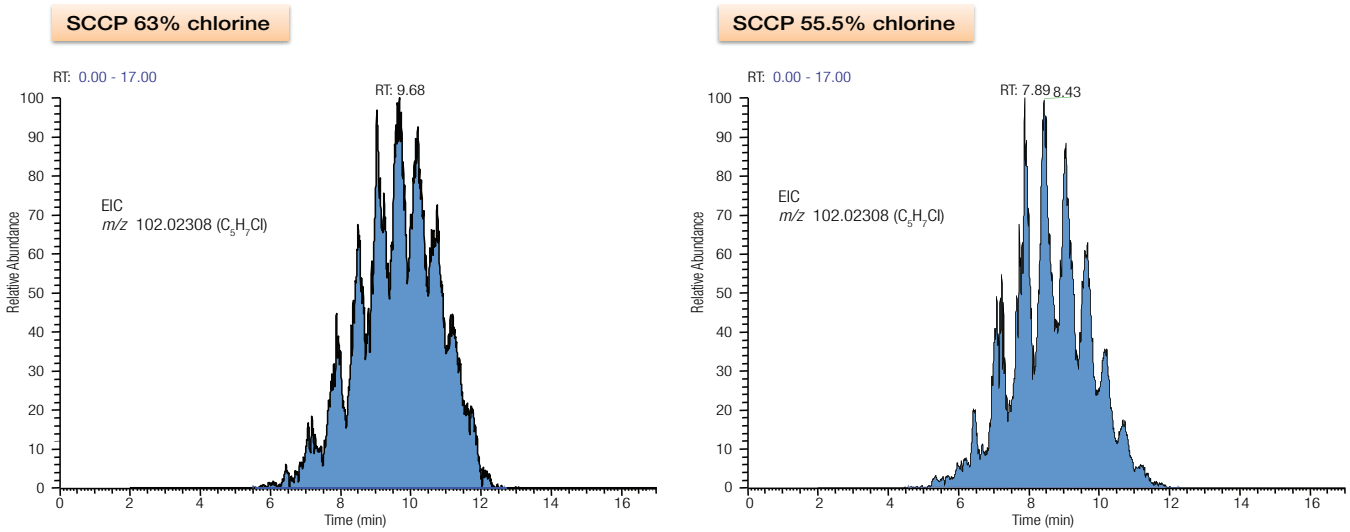

Figure 1. Extracted ion chromatogram of the fragment ion *m/z* 102.02308 corresponding to $C_5H_7Cl_7$, ±5ppm extraction window) showing the chromatographic complexity of two SCCP technical mixtures (63% and 55.5% chlorine). Data acquired in EI, full-scan, using 60k resolution.

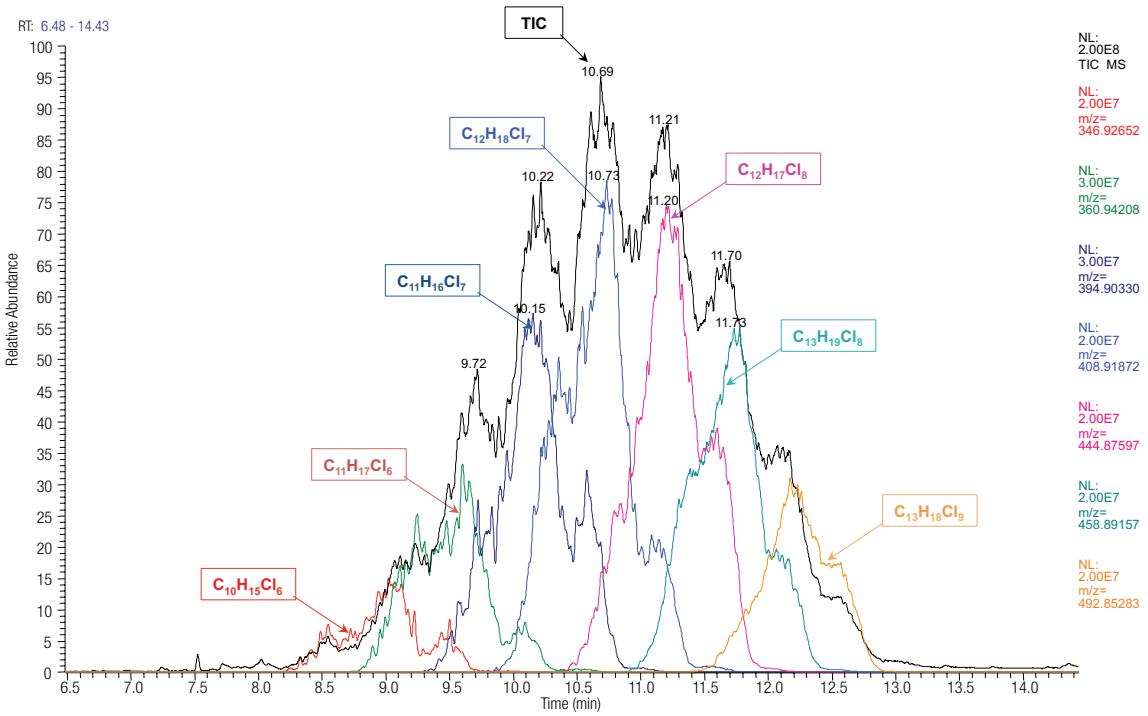

Figure 2. High resolution accurate mass selectivity demonstrated for the C_{10}-C_{13} 63% technical mix acquired in NCI at 60k resolution. Examples of extracted ion chromatograms for individual homologues with various chlorination degrees are shown.

Sensitivity

The sensitivity of the Exactive GC was tested by injecting low concentration solvent standards (in cyclohexane) prepared by a serial dilution of the two SCCP technical mixtures (Figure 3). The limit of detection varies depending on the relative concentration of a particular congener in a technical mixture. From the EI data, the instrumental detection limits (IDL) were ~10 pg/µL (calculated as total homologues response for each of the two SCCPs technical mixtures). In addition, the NCI data demonstrates that IDLs as low as 3 pg/µL can be obtained for individual homologue groups (Table 3).

Table 3. Peak area repeatability calculated as %RSD from n=10 repeat injections at 25 pg/µL level for two SCCP congeners acquired using NCI. IDL calculated taking into account the Student's-t critical values for the corresponding degrees of freedom (99% confidence).

inj. no	*m/z* 492.8546 ($C_{13}H_{18}Cl_9$)	*m/z* 458.8936 ($C_{13}H_{19}Cl_8$)
1	765881	1308232
2	822551	1428540
3	795041	1361253
4	781911	1363928
5	776597	1321808
6	731874	1250508
7	761201	1305483
8	749797	1284342
9	737987	1257718
10	757772	1286412
mean	768061	1316822
StDev	27217	54540
%RSD	**3.5**	**4.1**
IDL	**2.5**	**2.9**

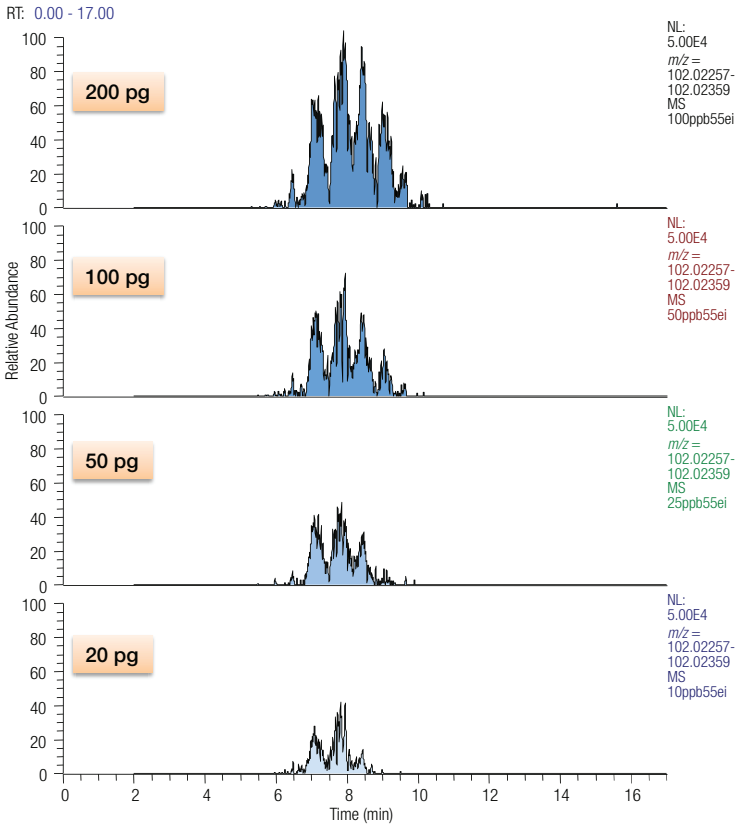

Figure 3. Extracted ion chromatograms (*m/z* 102.02308, mass window ±5ppm) representing the total SCCP C_{10}-C_{13} 55.5% chlorine homologues. Peak area response at 20, 50, 100 and 200 pg on column concentrations) are shown.

Linearity and dynamic range

SCCPs linearity and dynamic range was assessed for each SCCP technical mix (63% and 55.5%) using the following dilution series: 1, 10, 25, 50, 100, 250, 500, 1000, 5000 and 10000 pg/µL (in cyclohexane). This test was performed using both EI and NCI and examples are given in Figure 4. The coefficient of determination was >0.99 indicating excellent linearity across this concentration range.

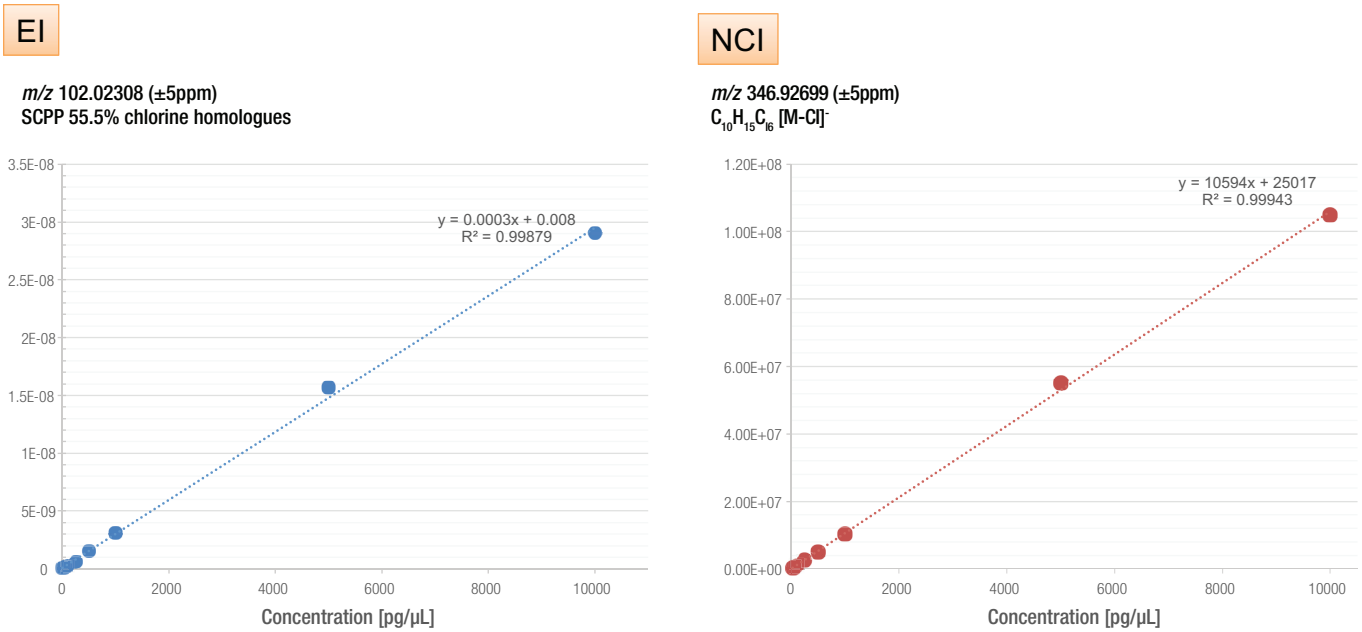

Figure 4. Example of linearity obtained for the C_{10}-C_{13} 55.5% chlorine homologues across 10 – 10000 pg/µL in EI and NCI at 60k resolution.

Moreover, selected individual homologue masses showed excellent linearity when acquired in NCI with R^2 values >0.999 (Figure 5).

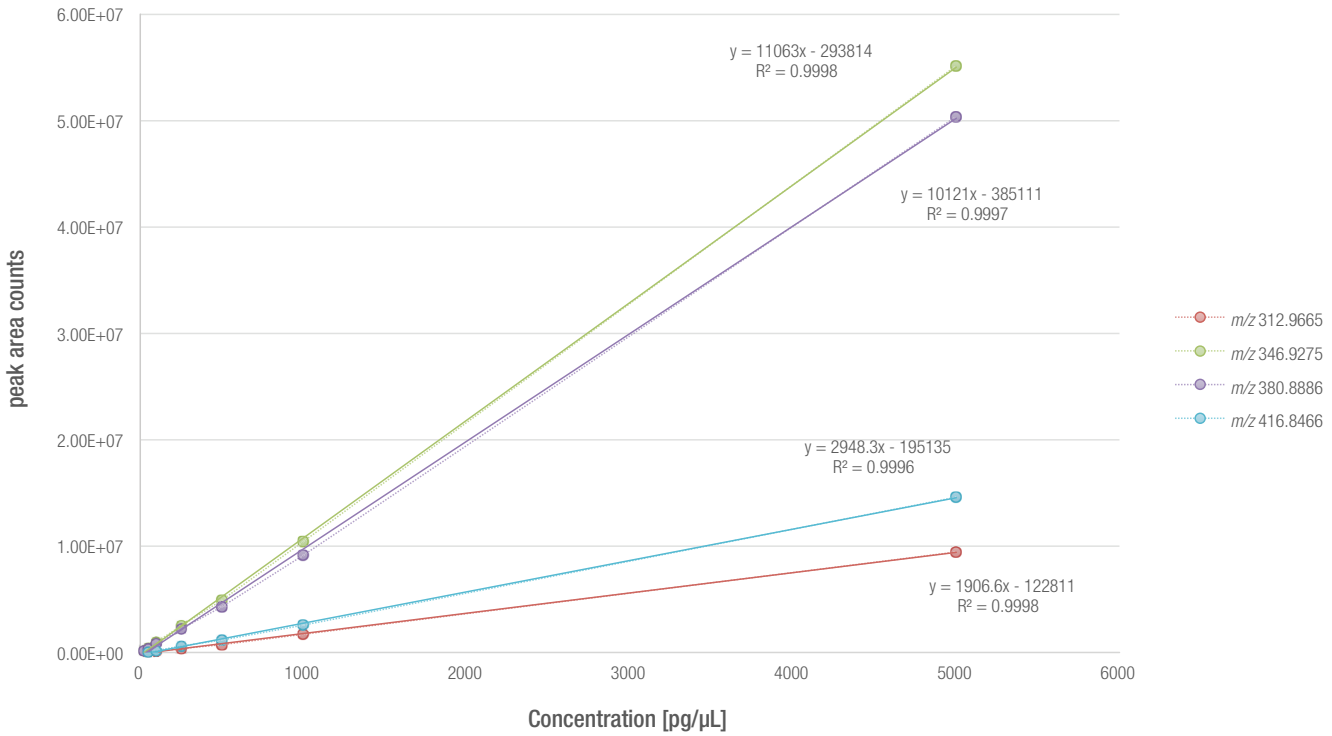

Figure 5. Example of linearity obtained individual SCCP congeners in the C_{10}-C_{13} 63% technical mixture across 1 – 5000 pg/µL concentration range. Data acquired in full-scan, NCI at 60k resolution.

Selectivity through high mass resolution

High resolution and high mass accuracy enable excellent selectivity and specificity. This is particularly important for full-scan data when background masses such as matrix ions, other organochloro contaminants or ions from other SCCP homologous group interfere with the target masses, leading to erroneous quantification.

As demonstrated in Figure 6 for a SCCPs 55% chlorine standard spiked with polychlorinated biphenyls, a resolving power of 15k is not sufficient to differentiate between an SCCP ion (*m/z* 253.03121, $C_{11}H_{16}Cl_3$) and a PCB interference (*m/z* 253.01733, $C_{14}H_{11}Cl_2$). Instead, at 15k resolution a single ion is detected, which in turn will significantly affect the peak area determination and precise estimation of SCCP concentration. At low resolving power, the extracted peak area of the target SCCP ion *m/z* 253.03121 is significantly lower than those obtained at 30k or 60k resolution due to higher errors in mass measurements (ppm). To achieve the sub-ppm mass accuracy and the selectivity required for consistent separation and quantification of target compounds, resolving powers of >30k are needed.

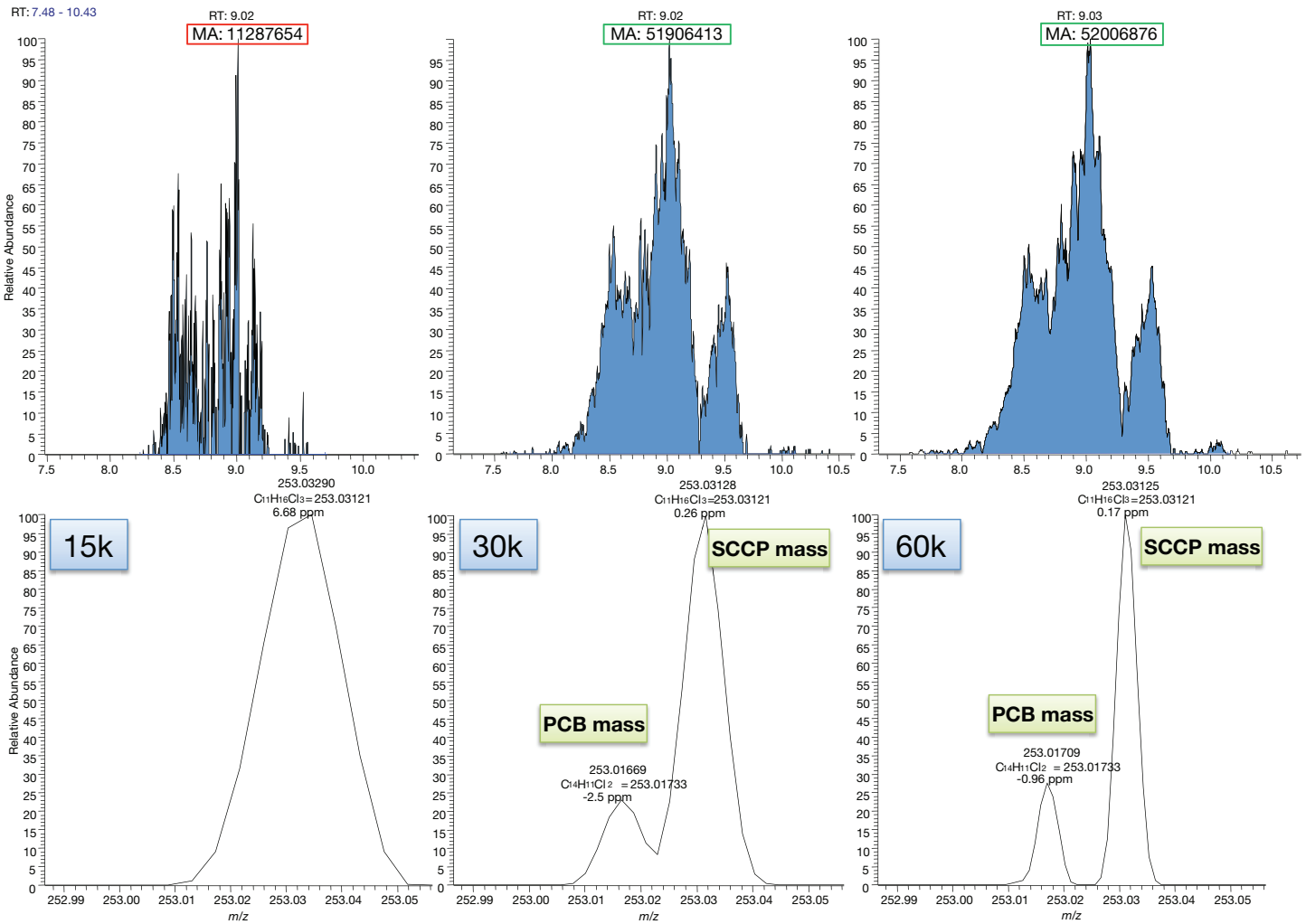

Figure 6. Selectivity enhancement by using narrow mass tolerance windows is possible at high resolving power. The effect of increasing resolving powers of the mass accuracy and peak area of a SCCP ion *m/z* 250.03121 is demonstrated for a 55% SCCPs sample spiked with polychlorinated biphenyls. Data acquired in full-scan, EI.

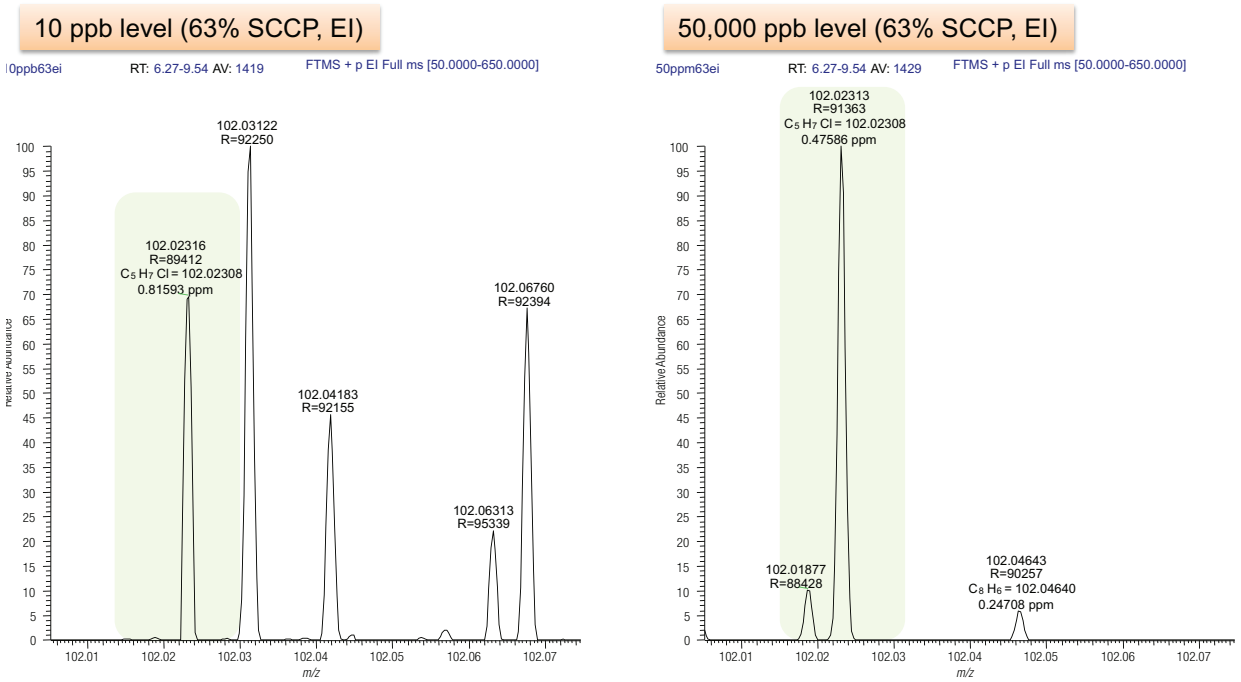

Figure 7. Mass accuracy remained at <1 ppm level irrespective of the by compound concentration as demonstrated for SCCPs fragment ion *m/z* 102.02308 (C_5H_7Cl) acquired in full-scan, EI at 60,000 mass resolution. The exact mass resolution, as well as the mass precision (ppm), used are annotated to each measured ion.

Maintaining sub-ppm mass accuracy at low and high concentrations

Outstanding mass accuracy (<1 ppm) was maintained across all compound concentrations (Figure 7). This is essential, as any compromise in accuracy of mass measurements can result in false identification, erroneous quantification and interferences from matrix ions.

Conclusions

These preliminary results demonstrate that the Exactive GC is a potential solution to address the difficult challenges related to the detection and quantification of SCCPs due to the excellent sensitivity, linearity and selectivity and in combination with an uncomplicated instrumental setup.

From the EI data, low instrumental LODs of ~10 pg/µL, calculated as total homologues response for each of the two SCCPs technical mixtures, can be easily obtained.

Using NCI it is possible to selectively separate C10 alkanes chains with various chlorination degrees making quantification of homologues with similar Cl content achievable.

In addition, with increased selectivity, the NCI data demonstrates that LOQs as low as 3 pg/µL can be reliably obtained for individual homologue groups.

Excellent linearity was obtained across a total SCCP mixture concentration range of 1 – 10,000 pg/µL, making the Exactive GC an ideal quantification tool.

The high resolving power of the Exactive GC facilitates sub-ppm mass accuracy at low and high concentrations, essential for achieving enough selectivity to confidently separate SCCP specific low mass ions from the interfering background ions (in EI), or higher masses (in NCI), for various SCCP homologue groups.

References

1. Geng N, Zhang H, Zhang B, Wu P, Wang F, Yu Z, Chen J. Effects of short-chain chlorinated paraffins exposure on the viability and metabolism of human hepatoma HepG2 cells. *Environ Sci Technol*, **2015** Mar 3;49(5):3076-83. doi: 10.1021/es505802x.
2. European Commission, Commission Regulation (EU) No 519, Off. J. Eur. Union, L 159 1-4, 2012.

Find out more at **thermofisher.com/ExactiveGC**

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