

A Novel High-Resolution, Accurate-Mass, Orbitrap-Based GC-MS for Routine Analysis of Short Chained Chlorinated Paraffins

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ABSTRACT

Purpose: Short chained chlorinated paraffins (SCCPs) are emerging contaminants that are intentionally manufactured and can have harmful and irreversible effects for humans and the environment. SCCPs are listed in the Stockholm convention and their production and use in Europe is restricted and regulated. Detection and quantification of SCCPs is challenging because these compounds are present in the environment at low levels and as very complex isomeric mixtures, which are difficult to separate chromatographically.

Methods: The performance of a novel benchtop, high-resolution, accurate-mass, Orbitrap™-based GC-MS was tested for the analysis of SCCPs. The experiments focused on assessing the sensitivity, linear dynamic range, selectivity and analytical precision for the analysis of SCCPs technical mixtures. Both electron ionization and negative chemical ionization were used.

Results: Sub-ppm mass accuracy at low and high concentrations provided enough selectivity to confidently separate SCCP-specific low mass ions from the interfering background ions or higher masses for various SCCP homologue groups.

INTRODUCTION

Short chained chlorinated paraffins are emerging contaminants that, after released, remain in the environment for long periods and therefore can 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.

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

Carbon skeleton analysis by GC, GC-FID or GC-MS	GC-ECD	GC-NCI-MS
<p>Details:</p> <ul style="list-style-type: none"> uses Pd catalyst held in the gas chromatograph injector to simultaneously dechlorinate the CPs and separate the resulting alkanes. <p>Disadvantages:</p> <ul style="list-style-type: none"> lack in sensitivity and selectivity. no information on the degree of chlorination of the SCCPs can be achieved. 	<p>Details:</p> <ul style="list-style-type: none"> GC coupled to electron capture detector, sensitive for halogenated compounds. <p>Disadvantages:</p> <ul style="list-style-type: none"> relatively unselective. interferences from other halogenated compounds. 	<p>Details:</p> <ul style="list-style-type: none"> Uses soft ionisation (negative chemical ionisation) with methane and/or dichloromethane. <p>Disadvantages:</p> <ul style="list-style-type: none"> low resolution GC-MS nominal mass interferences from higher chlorinated PCBs, toxaphenes and dieldrine-related compounds, have similar molecular masses. high resolution GC-MS (ToF, magnetic sector) trade-off sensitivity with speed and/or resolution.

In this study, the performance of a novel benchtop, 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 SCCP technical mixtures. Both electron ionization (EI) and negative chemical ionization (NCI) were used and the results compared and discussed.

MATERIALS AND METHODS

Two SCCP technical mixtures (63% and 55.5% chlorine) were used to test the performance of the Thermo Scientific™ Exactive™ GC system using both EI and NCI with methane as the reagent gas. The sensitivity of the Exactive GC was tested by injecting low level solvent standards (in cyclohexane) prepared by a serial dilution of the two SCCP technical mixtures. 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 10,000 pg/μL (in cyclohexane). Moreover, a SCCPs 55% chlorine standard was spiked with polychlorinated biphenyls to test the effect of various resolving powers on compound selectivity.

An Exactive GC Orbitrap mass spectrometer was coupled to a Thermo Scientific™ TRACE™ 1310 gas chromatograph for gas-phase separation of target compounds. 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 mode using 60k mass resolution (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.

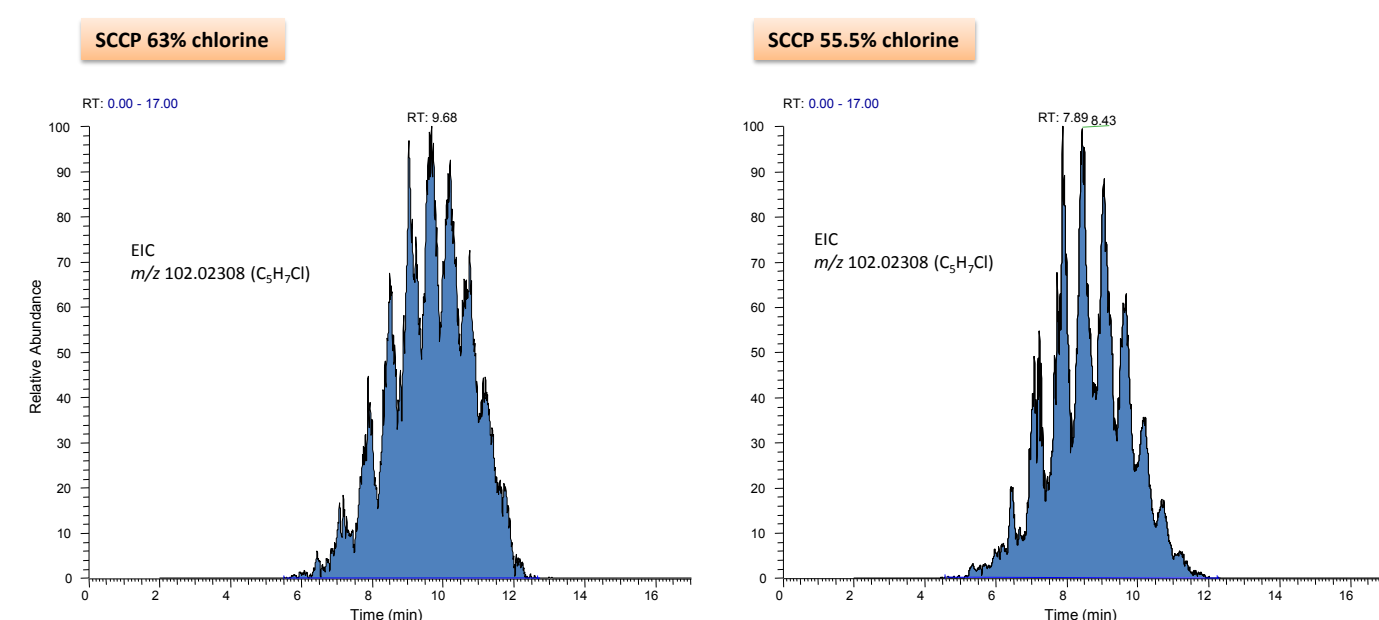
TRACE 1310 GC Parameters		Electron Ionization MS Parameters	
Injection Volume (μL):	2.0	Transfer line (°C):	280
Liner:	LinerGOLD™, single taper (P/N:453A0344-UI)	Ionization type:	EI
Inlet (°C):	280	Ion source (°C):	230
Inlet Module and Mode:	splitless	Electron energy (eV):	70
Carrier Gas, (mL/min):	He, 1.2	Acquisition Mode:	full scan
Oven Temperature Program:		Mass range (Da):	50-650
Temperature 1 (°C):	100	C-Trap voltage (V):	0.0
Hold Time (min):	2.0	Mass resolution (FWHM at m/z 200):	15k, 30k and 60k
Temperature 2 (°C):	310	Lockmass (m/z):	207.03235
Rate (°C/min):	20		281.05114
Hold Time (min):	4.0		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		

RESULTS

Chromatography

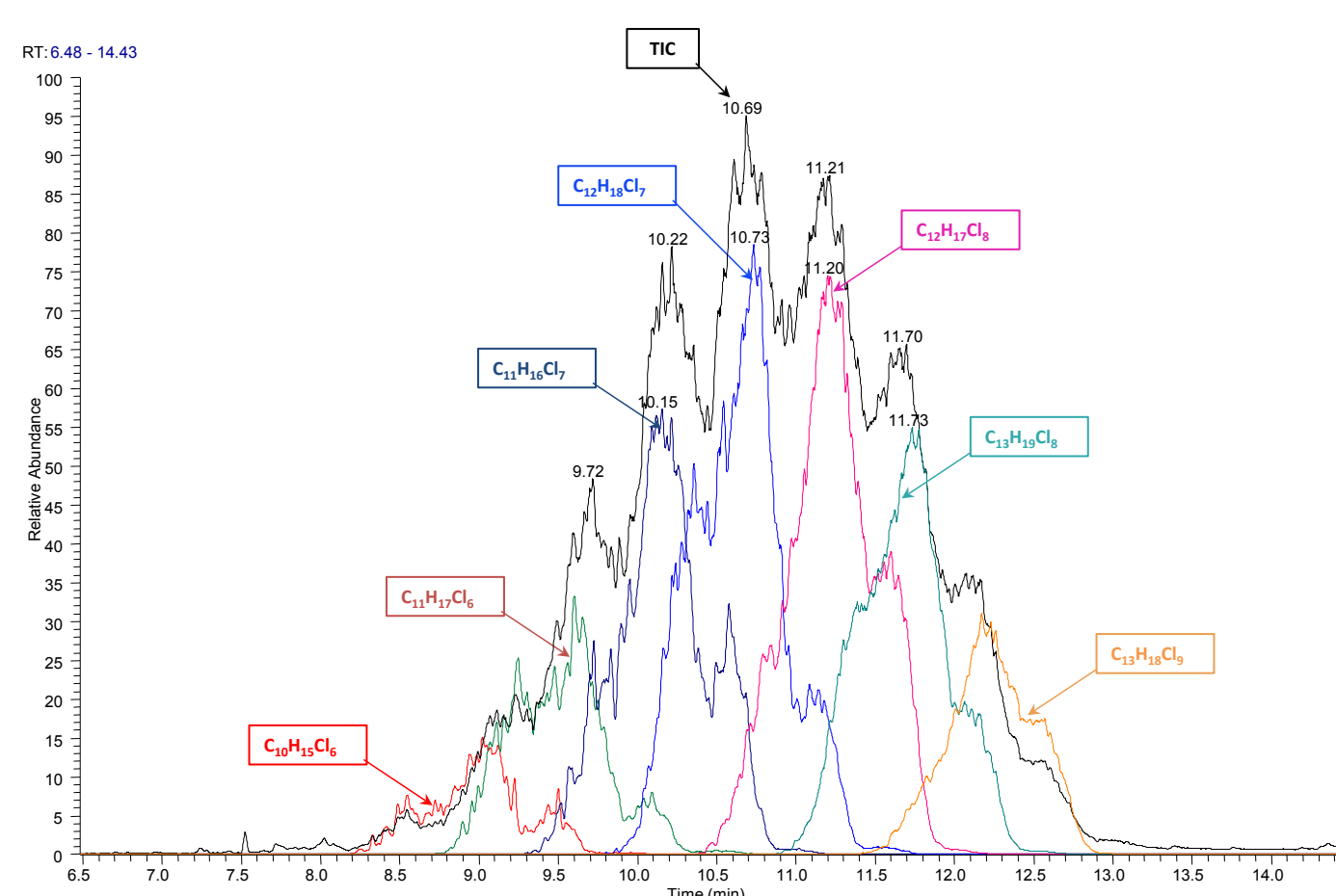
Complete separation of SCCPs is exceptionally difficult due to the very high numbers of isomers and homologues with similar physicochemical properties (Figure 1).

Figure 1. Extracted ion chromatogram of the fragment ion m/z 102.02308 corresponding to $C_8H_7Cl_2$, ± 5 ppm 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.



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 2. High-resolution, accurate-mass selectivity demonstrated for the C10-C13 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).

Figure 3. Extracted ion chromatograms (m/z 102.02308, mass window ± 5 ppm) representing the total SCCP C10-C13 55.5% chlorine homologues. Peak area responses at 20, 50, 100, and 200 pg on column concentrations, are shown.

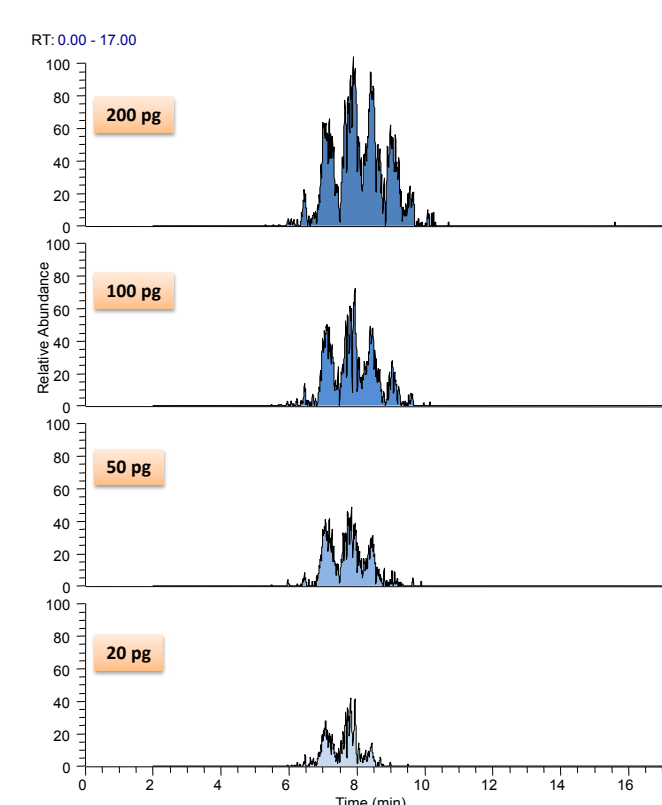


Table 3. Peak area repeatability calculated as %RSD from n=10 repeat injections at 25 pg/μL level for two SCCP congeners acquired using NCI. IDL calculated taking into account the Student's distribution critical values for the corresponding degrees of freedom (99% confidence).

inj. no	m/z 492.8546 ($C_{10}H_{17}Cl_2$)	m/z 458.8936 ($C_{11}H_{17}Cl_2$)
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

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 10,000 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. Moreover, selected individual homologue masses showed excellent linearity when acquired in NCI with R^2 values >0.999 (Figure 5).

Figure 4. Example of linearity obtained for the C10-C13 55.5% chlorine homologues across 1–10,000 pg/μL in EI and NCI at 60k resolution.

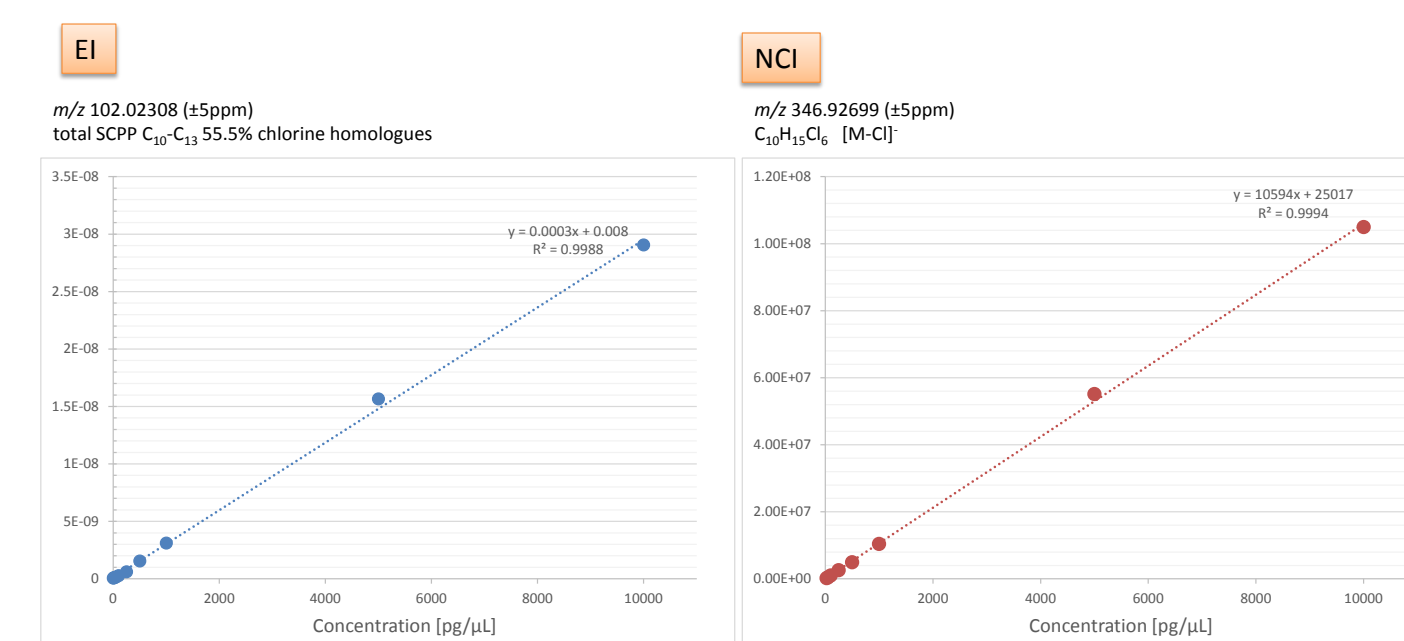
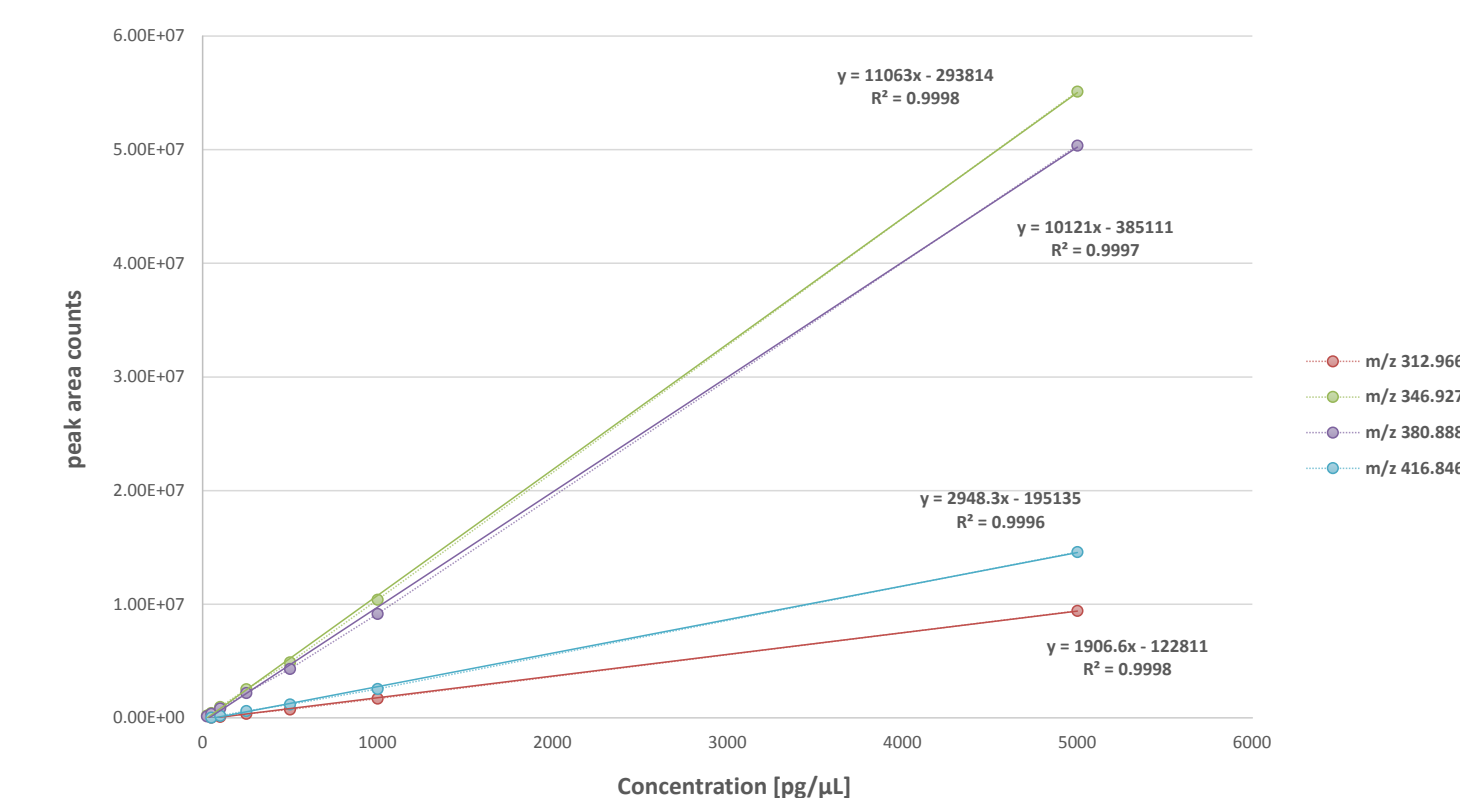


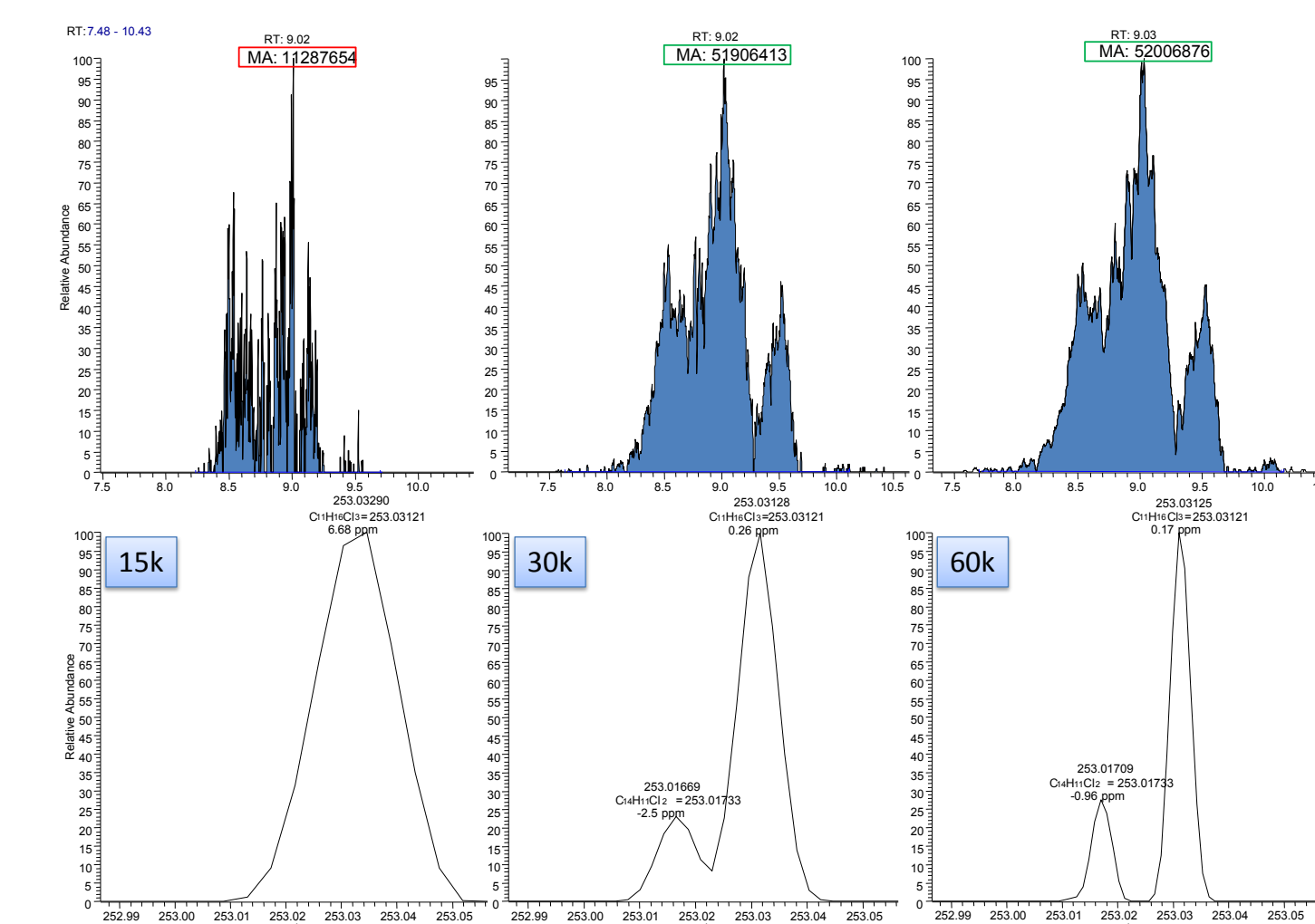
Figure 5. Example of linearity obtained for individual SCCP congeners in the C10-C13 63% technical mixture across 1–5000 pg/μL concentration range. Data acquired in full scan, NCI at 60k resolution.



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 groups, 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 a 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 253.03121 is demonstrated for a 55% SCCPs sample spiked with polychlorinated biphenyls.



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 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 alkane chains with various chlorination degrees, making quantification of homologues with similar Cl content possible.
- 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

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TRADEMARKS/LICENSES

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