# Determination of Short and Medium Chained Chlorinated Paraffins in Salmon Samples using GC Orbitrap-MS

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## ABSTRACT

**Purpose:** The Thermo Scientific™ Q Exactive™ GC Orbitrap™ GC-MS/MS system was tested for linear dynamic range, sensitivity and selectivity using full-scan acquisition and negative chemical ionisation at 60,000 and 120,000 resolution. Mixtures of different chlorinated paraffins (CPs) and polychlorinated biphenyls (PCBs) standards were examined as well as real food samples which were prepared with and without separation of co-eluting persistent organic pollutants (POPs) during sample clean-up.

**Methods:** Standard solutions resembling technical mixtures of short-chain chlorinated paraffins (SCCPs) (C10-C13 55.5% Cl) and medium-chain chlorinated paraffins (MCCPs) (C14-C17 42% Cl) were used. For calibration, solutions of SCCP and MCCP with the concentration 0.1 ppm, 0.5 ppm, 1 ppm, 5 ppm, 10 ppm, 15 ppm and the addition of 0.1 ppm 1,5,5,6,6,10-13C-Hexachlorodecane and 0.05 ppm ε-HCH in cyclohexane. Salmon samples were extracted using accelerated solvent extraction with and without a clean-up step. Experiments performed using a Q Exactive GC Orbitrap GC-MS/MS system coupled with a Thermo Scientific™ TRACE™ 1310 gas chromatograph equipped with a Thermo Scientific™ TraceGOLD TG5-SilMS GC column 15m x 0.25 mm x 0.25 µm (P/N 26096-1300). Automatic tuning of the Q Exactive GC mass spectrometer was made using FC-43 as tuning reagent and methane as ionization gas.

**Results:** The linearity and dynamic range was assessed for both SCCP and MCCP technical mixtures (55.5% and 42% chlorine, respectively) using a dilution series in cyclohexane which resembles the usual analytical range of CP sample analysis in food. The coefficient of determination of over 0.99 for almost all chosen congeners when assigned the concentration of the technical mixture. Taking their percentage of the technical mixtures in account, a limit of quantification (LOQ) of 1.5 ppb (MCCP) and 0.1 ppb (SCCP) could be achieved for some congeners, with the corresponding limit of detection (LOD) being as low as 0.3 ppb (MCCP) and estimated below 0.05 ppb (SCCP). A mixture of the SCCP and MCCP technical mixtures spiked with a high concentration of PCBs was analyzed data showing no significant influence on peak shapes in comparison with the separate technical mixtures, even in the presence of high levels of PCB congeners. This was further verified by the sum concentrations of SCCP and MCCP which were determined both in the standards and the standard mix. Different samples of salmon were prepared with two different clean-up methods and analysed. The sum concentration of SCCPs and MCCPs was additionally obtained beforehand by GC-EI-LRMS/MS. Samples that were not cleaned-up show many additional, overlapping compounds such as several PCBs, dieldrin, DDT and DDD as well as toxaphenes. The comparison of sum SCCP and MCCP concentration as determined by Q Exactive GC Orbitrap MS system and as determined by GC-EI-LRMS/MS showed good agreement between the differently cleaned samples for the Orbitrap measurements with less than 10% deviation.

## INTRODUCTION

The coupling of gas chromatography (GC) to high resolution mass spectrometry (HRMS) using the Orbitrap technology opens up a broad spectrum of possible applications in environmental and food/feed analysis. Although known for several decades and widely used as plasticisers or flame retardants,[1] SCCPs have only been recently added to Annex A of the Stockholm Convention list of POPs.[2] Caused by previous efforts to ban SCCPs, medium-chain CP (MCCP) production increased, often to replace SCCPs.[3] As SCCPs alone consist of several thousand congeners with only four different carbon chain lengths to choose from, quantification of SCCPs and MCCPs in samples is a highly complex problem. In addition to that, other halogenated POPs like PCBs are known to co-elute and add to the complexity of any analysis. With this in mind, experiments focusing on linear dynamic range, sensitivity and selectivity were performed using full-scan acquisition and negative chemical ionization (NCI) at 60,000 and 120,000 resolution (FWHM, *m/z* 200). In this study, mixtures of different CP and PCB standards were examined as well as food samples which were prepared with and without separation of co-eluting POPs during sample clean-up.

## MATERIALS AND METHODS

### Sample Preparation

Two standard solutions resembling technical mixtures of SCCP (100mg/L in cyclohexane, C10-C13 55.5% Cl) and MCCP (100mg/L in cyclohexane, C14-C17 42% Cl) were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). As internal standards 1,5,5,6,6,10-13C-Hexachloro-decane (100mg/L in nonane) was purchased from Cambridge Isotope Laboratories, Inc. (Andover, USA) and ε-HCH (100mg/L in cyclohexane) was purchased from Dr. Ehrenstorfer GmbH. For calibration, solutions of SCCP and MCCP with the concentration 0.1 ppm, 0.5 ppm, 1 ppm, 5 ppm, 10 ppm, 15 ppm and the addition of 0.1 ppm 1,5,5,6,6,10-13C-Hexachlorodecane and 0.05 ppm ε-HCH were prepared in cyclohexane. The samples were acquired from supermarkets and vendors in Baden-Württemberg as part of the food control. Part of the homogenized sample was extracted using accelerated solvent extraction (ASE) at 150° C with acetone/n-hexane 1:3 (v/v) followed by elution through a sulphuric acid primed silica gel column with further clean up using a Florisil® column and eluting the PCB fraction with n-hexane and the CP fraction with dichloromethane. Some of the samples were additionally prepared without the clean-up on a Florisil column.

### GC-MS Analysis

Experiments were performed using a Q Exactive GC Orbitrap GC-MS/MS system coupled with a TRACE 1310 gas chromatograph equipped with a TraceGOLD TG5-SilMS GC column 15m x 0.25 mm x 0.25 µm (P/N 26096-1300). Automatic tuning of the Q Exactive GC mass spectrometer was made using FC-43 as tuning reagent and methane as ionisation gas. Full scans of the standards and samples were obtained using a mass range of m/z 50-650. Further details regarding the analytical system are given in Table 1.

**Table 1. Parameters of the Q Exactive GC Orbitrap GC-MS/MS system used in this project.**

| TRACE 1310 GC System Parameters | |
|---|---|
| Injection Volume: | 1.5 µL |
| Liner: | Single gooseneck (P/N:4530924-UI) |
| Inlet: | 280 °C |
| Inlet Module and Mode: | Splitless/Surge (9 psi for 1 min) |
| Splitless time: | 1.2 min. |
| Split flow: | 50 mL/min. |
| Column flow: | 1.4 mL/min. |
| **Oven Temperature Program:** | |
| Temperature 1: | 60 °C |
| Hold Time: | 2 min. |
| Temperature 2: | 300 °C |
| Rate: | 50 °C/min. |
| Hold Time: | 5 min. |

| Q Exactive GC Orbitrap GC-MS/MS parameters | |
|---|---|
| Transfer Line: | 280 °C |
| Ionization Type: | NCI (methane) |
| Ion Source: | 180 °C |
| Electron Energy: | 70 eV |
| Acquisition Mode: | Full-scan |
| C-Trap Energy: | 2 V |
| Mass Range: | 50-650 *m/z* |
| Mass Resolution: | 60k and 120k FWHM at *m/z* 200 |

## RESULTS

### Selectivity

One of the biggest challenges of CP analysis is the high complexity of the compound mixtures found in both samples and standards (Figure 1). One of the biggest challenges of CP analysis is the high complexity of the compound mixtures found in both samples and standards (Figure 1). In addition to a high degree of overlapping of the different CP homologues, other persistent organic pollutants such as PCBs are known to co-elute, thus further complicating the analysis. To investigate possible influences, a mixture of the SCCP and MCCP technical mixtures spiked with a high concentration of PCBs was analysed. The mixture showed no significant influence on peak shapes in comparison with the separate technical mixtures (Figure 2), even with the PCB congeners clearly dominating the total ion chromatogram (TIC) and degrading the CP chromatographic hump to mere baseline disturbance. Therefore the high resolution of the Q Exactive GC Orbitrap GC-MS/MS system allows for the quantification of both SCCP and MCCP even in the presence of significant amounts of PCBs in samples without congener groups being overestimated due to mass overlaps.

**Figure 1. Overlaid chromatograms of SCCP, MCCP, and PCB standards with added extracted ion chromatograms of selected CP homologues measured with the Q Exactive GC Orbitrap GC-MS/MS system.**

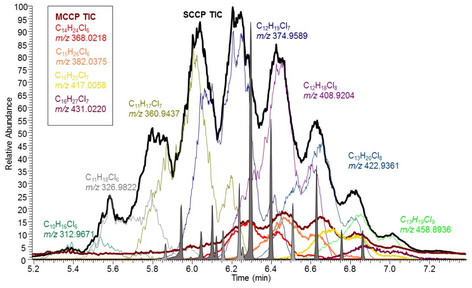
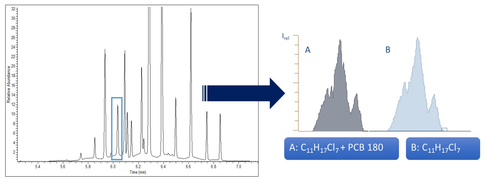



**Figure 2. TIC of a mixture of SCCP, MCCP, and PCBs with extracted ion chromatograms of a CP homologue that is isobar with PCB 180 in its nominal mass and therefore eluting simultaneously. No significant influence on the peak shape and peak area could be observed.**




This was further verified by the sum concentrations of SCCP and MCCP which were determined both in the standards and the standard mix. The twelve homologues chosen to serve as examples gave in ten cases relative standard deviations of <10% from the sum concentration determined in the single standards. The slightly elevated concentrations of both groups of CPs shown in Table 2 most likely stem from the known impurities of both standards; a small amount of SCCPs could be found in the MCCP standard and it has been commented on in literature that SCCP standards seem to contain MCCPs.[4]

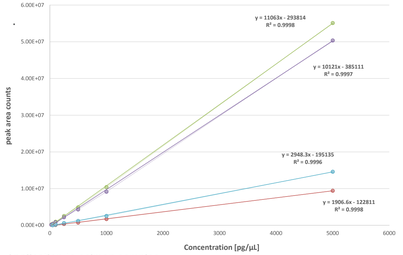
**Table 2. Concentrations of the sum of SCCPs and MCCPs in the mix of SCCPs, MCCPs, and PCBs as well as the single standards determined using different homologues (1-12).**

| | ΣSCCP concentration [ppm] | | | | ΣMCCP concentration [ppm] | | |
|---|---|---|---|---|---|---|---|
| | SCCP standard | Standard mix | $s_{x,rel}$ | | MCCP standard | Standard mix | $s_{x,rel}$ |
| **1** | 4,04 | 4,49 | 8% | **7** | 4,87 | 5,28 | 6% |
| **2** | 3,99 | 4,49 | 9% | **8** | 4,86 | 6,38 | 22% |
| **3** | 4,22 | 4,45 | 4% | **9** | 4,83 | 4,95 | 2% |
| **4** | 4,29 | 4,64 | 6% | **10** | 4,81 | 5,50 | 10% |
| **5** | 4,22 | 4,45 | 4% | **11** | 4,82 | 5,03 | 3% |
| **6** | 4,25 | 4,56 | 5% | **12** | 4,85 | 10,82 | 87% |

### Linerity and dynamic range

The linearity and dynamic range was assessed for both SCCP and MCCP technical mixtures (55.5% and 42% chlorine, respectively) using a dilution series in cyclohexane that resembles the usual analytical range of CP sample analysis in food. The coefficient of determination (R2) of over 0.99 indicates good linearity beyond this concentration range for almost all chosen congeners when assigned the concentration of the technical mixture. Taking their percentage of the technical mixtures in account, an LOQ of 1.5 ppb (MCCP) and 0.1 ppb (SCCP) could be achieved for some congeners, with the corresponding LOD being as low as 0.3 ppb (MCCP) and estimated below 0.05 ppb (SCCP).

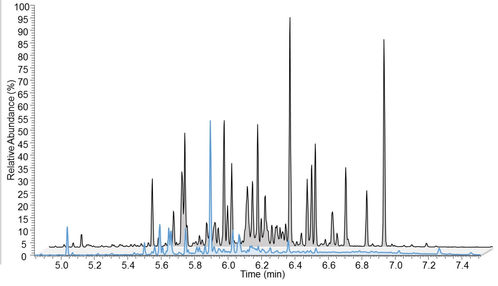
**Figure 3 Example of linearity obtained individual SCCP congeners in the C10-C13 63% technical mixture across 1 – 5000 pg/µL concentration range. Data acquired in full-scan, NCI at 60k resolution.**




### Application to salmon samples

To explore the performance of the Orbitrap-MS system with real-life matrices, different samples of salmon prepared with two different clean-up methods were analyzed. The sum concentration of SCCPs and MCCPs respectively was additionally obtained beforehand by GC-EI-LRMS/MS. As seen in Figure 4, samples that were not subjected to a Florisil clean-up step show many additional, overlapping compounds (gray chromatogram) in comparison to the regularly prepared sample (blue chromatogram). The identified additional compounds in the gray chromatogram included several PCBs, dieldrin, DDT, and DDD as well as several toxaphenes. In the present experiment, a deviation of the CP pattern in comparison to cleansed samples could be observed. Although even between the two cleaned samples a slight deviation is visible, the differences to the uncleaned sample are more pronounced, in particular looking at the relation between selected homologues. Especially, indication of certain SCCPs being held back during Florisil clean-up should be investigated further. The comparison of sum SCCP and MCCP concentration, as determined by the Q Exactive GC Orbitrap GC-MS system and as determined by GC-EI-LRMS/MS, showed good agreement between the differently cleaned samples for the Orbitrap measurements with less than 10% deviation. On the other hand, a reliable determination of CP concentrations using low-resolution mass spectrometry was almost impossible in the uncleaned sample, leading to more than 50% deviation from the results obtained using the clean sample due to significant overestimation. The comparison of regular samples with samples from the same fish that were not cleaned using a Florisil column is therefore only possible because of the high selectivity of the Orbitrap-MS system, as other methods are too affected by the sheer number of different, overlaying compounds in the chromatographic window.

**Figure 4 Q Exactive GC Orbitrap-MS full-scan TIC chromatograms of a regularly prepared salmon sample (blue) and of the same sample prepared without further clean-up (gray).**




## CONCLUSIONS

The results of this study demonstrate very good linearity at concentrations of < 2 ppb. Determination of both CPs and PCBs in the same sample in one run is possible, suggesting the same for other halogenated compounds.

- TraceFinder software is an intuitive tool for processing data from full-scan analyses, allowing fast quantification and unprecedented insights into the pattern and content of CPs.
- A shortened sample preparation without separation of co-eluting compounds showed no influence on analysis results, while other instrumental setups struggled with the high number of compounds.
- Furthermore, the high selectivity of the Q Exactive GC Orbitrap GC-MS/MS system showed that possibly some CPs are held back during clean-up procedures, therefore influencing quantitative and qualitative results.
- Taken together, the Q Exactive GC mass spectrometer is a powerful analytical tool with simple setup and fullscan high-resolution experiments at a high selectivity, representing a potential for shorter sample preparation and quicker analyses of several types of POPs in one run, which is crucial considering the ever-growing list of compounds to be monitored in food and feed.

## REFERENCES

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