An Executive Summary

# Orbitrap GC-MS: An Opportunity to Help Address the Challenges of Chlorinated Paraffins Analysis

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The flexibility of operation modes and high resolving power of the Thermo Scientific™ Q Exactive™ GC Orbitrap™ GC-MS/MS means that the system is well suited to the analysis of highly complex mixtures of chemicals such as chlorinated paraffins.

## Overview

This summary describes the utilization of the Thermo Scientific™ Q Exactive™ GC Orbitrap™ GC-MS/MS system by the European Union Reference Laboratory for Dioxins and Polychlorinated Biphenyls (PCBs) for the analysis of chlorinated paraffins. This emerging class of contaminants is of increasing concern because of their persistence in the environment and potential effects on human health. The detection and accurate quantification of chlorinated paraffins in food is known to be challenging, but the high sensitivity, high resolving power, and excellent accurate mass accuracy of the GC-Orbitrap system enabled isomer-level quantitation of short- and medium-chain length chlorinated paraffins (CPs). The GC-Orbitrap system also enabled the simultaneous identification and quantitation of potentially interfering species such as PCBs.

## Introduction to CPs

CPs are members of a complex family of man-made compounds in which straight-chain alkanes have been chlorine-substituted at multiple positions. Chlorination is a largely random process, producing many thousands of isomers for each length of carbon chain.

These isomers are generally subdivided into three subcategories: short-chain CPs (SCCPs, $C_{10–13}$), medium-chain CPs (MCCPs, $C_{14–17}$); and long-chain CPs (LCCPs, $C_{>17}$). In their pure form, CPs are liquid until the chain length reaches about $C_{20}$ or more.

CPs are commonly used as flame retardants and in the manufacture of plastics, rubber, paints, adhesives, and sealants, as well as components in the lubricants and coolants used in metal working. The manufacturing of SCCPs in Europe is regulated by Regulation EC 850/2004 as amended by Regulation EU 2015/2030, and limits for surface water are set in Directive 2000/60/EC as amended by Directive 2008/105/EC. The worldwide annual production of CPs exceeds 1.1 million tons per year.

SCCPs have recently come under scrutiny because they are toxic, highly persistent in the environment, and fat soluble, with the potential to bioaccumulate in organisms as they move up the food chain. This led to the addition of SCCPs to Annex A of the Stockholm Convention on POPs earlier this year. CPs undergo long-range environmental transport and

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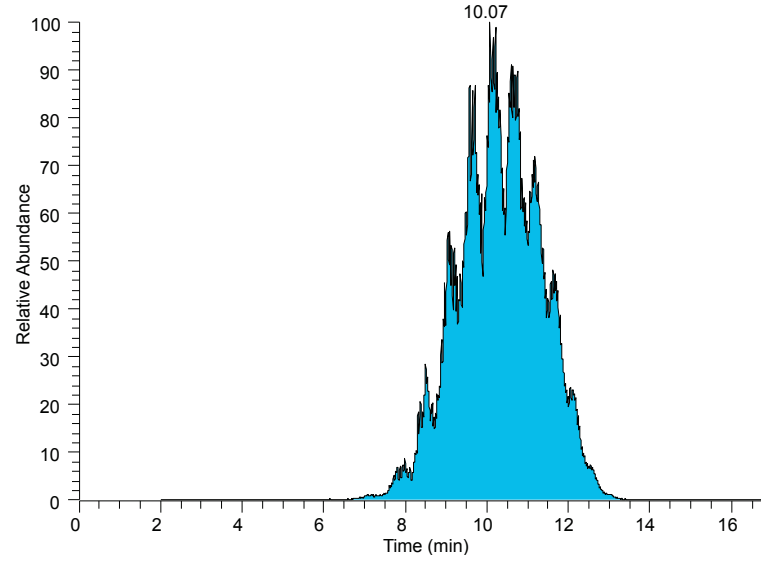
have been measured in arctic biota at similar concentrations to other known persistent organic pollutants (POPs).

A 2015 draft risk profile by the working group on SCCPs under the POPs Review Committee of the Stockholm Convention stated that SCCPs could lead to "significant adverse environmental and human health effects" and that "global action is warranted." Specifically, SCCPs have been found to be toxic to aquatic organisms and carcinogenic in rodents, as well as negatively affecting the function of human cells *in vitro*.

## Analysis of CPs

CPs analysis is analytically challenging because CPs rarely exist as pure compounds, but instead as highly complex mixtures of isomers and homologues that cannot be separated chromatographically. This, together with the low concentrations, makes their detection and quantification in food doubly difficult. The difficulty is highlighted by the separation of a SCCP containing 63% chlorine, shown in **Figure 1**. This figure shows an ion extracted chromatogram for *m/z* 122, a common fragment observed using electron impact (EI) ionization.

**Figure 1:** Extracted ion chromatogram of a short-chain paraffin containing 63% chlorine.




Because of these challenges, there is no consensus on a validated method for the routine monitoring of CPs in food and feed. The different methods typically used are shown in **Figure 2**.

In general, gas chromatography is the preferred separation technique, coupled to either analogue detectors or

**Figure 2:** Analytical methodology.

| Method | Advantages | Disadvantages |
|---|---|---|
| GC-ECNI-MS | • equipment available in most CP laboratories<br>• most commonly used in publications → comparable results<br>• information about homologue pattern attainable | • interferences with other co-eluting POPs (e.g. PCBs)<br>• interferences within CP homologues (overlapping fragment/molecule ions; not with expensive HRMS)<br>• dependent on chlorination degree |
| GC-EI-MS | • response is influenced neither by chlorination degree nor by the chain length | • high degree of fragmentation<br>• no information about homologue pattern attainable<br>• standards with similar chlorination degree necessary |
| GC-NCI-MS | • only little dependency on the chlorine content of the CPs contained in the quantification standards<br>• low chlorinated CPs detectable | • High instrumental demands<br>• Only rarely used → results not comparable |
| GC-ECD | • lower chlorinated CPs detectable<br>• generally sensitive for halogenated compounds | • GCxGC needed for qualitative identification of CPs, otherwise relatively unspecific<br>• interferences with other organo halogenated compounds with same retention time (e.g. PCBs) |
| (LC)-APCI-qTOF-MS | • simultaneous quantification of CP homologues possible<br>• less false positive results (compared to ECNI-MS)<br>• low interferences due to DCM addition to eluent | • cost<br>• complex quantification<br>• authentic quantification standards necessary |
| GC-FID | • low chlorinated CPs detectable (carbon-skeleton analysis) | • no information about congener pattern attainable<br>• no information on chlorination degree attainable |

mass spectrometers with each of these approaches having advantages and disadvantages.

Systems such as GC-flame ionization detectors or GC-electron capture detectors suffer from very poor selectivity, which makes it impossible to distinguish between CPs and other halogenated chemicals that may co-elute with the targeted CPs of interest. GC-MS (nominal mass) with EI or negative chemical ionization are preferred over analogue detectors, but these techniques also suffer from chemical interferences from other persistent organic pollutant (POP) compounds such as PCBs and polybrominated diphenyl ethers when analyzing real-world samples.

For in-depth studies of the chlorinated parts in congener patterns, analytical laboratories are increasingly turning to GC systems coupled to high-resolution accurate mass (HRAM) mass spectrometry such as the Thermo Scientific™ Q Exactive™ GC Orbitrap™ GC-MS/MS system. The high resolving power, and consistent sub-ppm mass accuracy can provide selective detection and excellent quantification of CPs, even in complex matrices, and also enable the simultaneous detection, identification, and quantification of POPs providing additional valuable results at minimal extra cost.

The analysis of a real-world sample (salmon) is shown in **Figure 3**. The data was acquired in full scan, using negative chemical ionization and 60,000 resolving power. Extracting the exact mass of ions corresponding to $C_{10}$ and $C_{11}$ and using a narrow extraction window (3 ppm) enables the generation of highly selected extracted ion chromatograms (**Figure 3,** left side). This approach reduces chemical interferences from matrix ions, other CPs, and any other halogenated compounds, such as PCBs, that may be present in the sample.

By contrast, the analysis of the same sample using a low-resolution instrument and a nominal mass extraction window (**Figure 3**, right side) does not provide enough selectivity to detect the two CP ions ($C_{10}$ and $C_{11}$) and thus, will lead to an erroneous quantification.

Meanwhile, the Q Exactive GC mass spectrometer system provides a unique view for CP analysis by bringing together several important technologies to provide enhanced capabilities (see **Figure 4**). These include:

- The Thermo Scientific™ ExtractaBrite™ Ion Source, which can be used for either electron impact or chemical ionization. This source is proven in routine applications and is removable without breaking vacuum, for maintenance, or switching between chemical and electron ionization. Also, a source plug enables GC columns to be exchanged, without venting the MS system. All features designed to maximize uptime and productivity.
- From there, ions travel to a bent flatapole for the lowest possible noise and maximum robustness.
- The advanced quadrupole technology (AQT) allows for high transmission of selected masses for low-level detection and quantitation of low-abundance compounds in highly complex matrices.
- The ions are then trapped in a curved linear ion trap (C Trap) ready for precise ion injection into the Orbitrap analyser. The number of ions in the trap is controlled by the automatic gain control. The result is excellent in spectrum dynamic range and outstanding HRAM performance across a wide range of concentrations.
- Higher-energy collisional dissociation for MS/MS characterization of ions (in combination with chemical ionization) helps elucidate chemical structures for "unknown" compounds.
- The Orbitrap ion trap mass analyzer separates ions with resolving powers up to 120,000 FWHM at *m/z* 200, which ensures very high selectivity. Also, mass accuracy of below 1 ppm is achieved in every scan, for every compound, every day, for at all concentrations.

**Figure 3:** Selectivity through high resolving power.

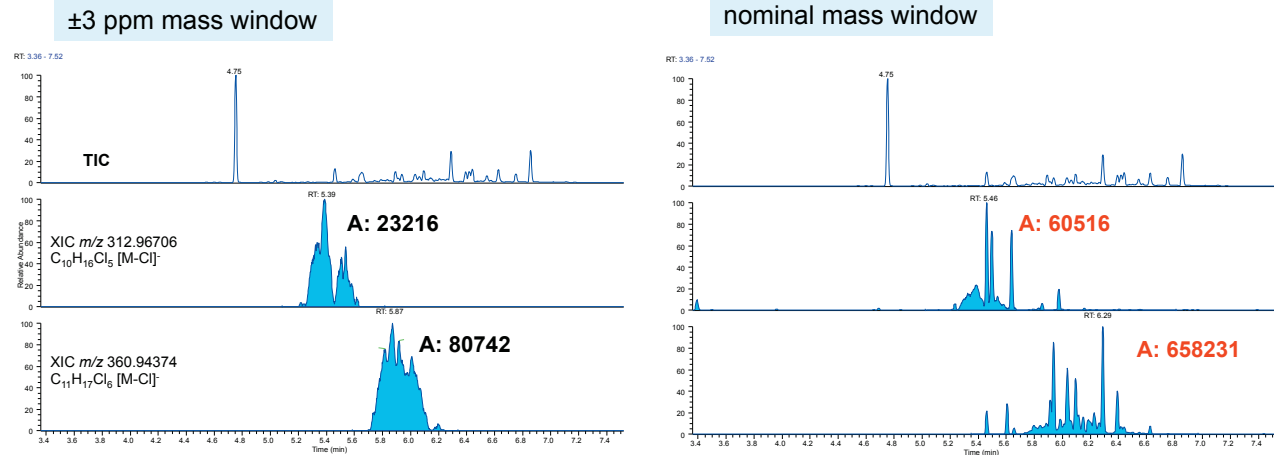
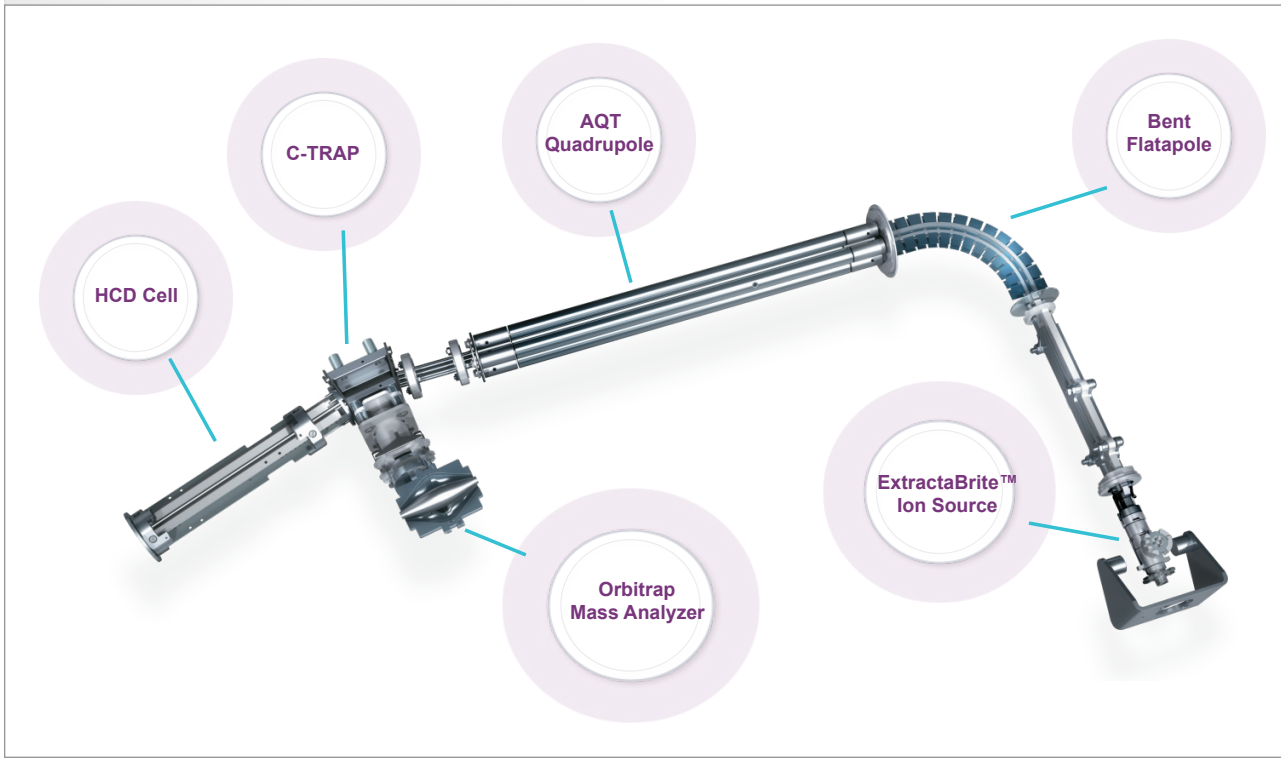


**Figure 4:** Q Exactive GC Orbitrap GC-MS/MS system: The technology inside.




## CP Analysis Example

In a recent study, the European Union Reference Laboratory for Dioxins and PCBs in Feed and Food found that using the Q Exactive GC Orbitrap GC-MS/MS system provided more reliable SCCP results.

As mentioned, the complexity of CPs makes full isolation of individual isomers impossible without an unreasonable amount of effort, even with multidimensional techniques. However, by fractionating the sample into more subunits for analysis can provide more information on environmental disposition, regional fingerprinting, and assessment of the relative toxicities of different fractions.

For this study, chromatographic separation was performed using a short column (Thermo Scientific TraceGOLD™ TG5-SilMS 15m x 0.25 mm x 0.25 $\mu$m). The ramp rate was 50 °C/min, resulting in short (<10 min) run times. Such a short, wide GC column and fast ramp rate serves to stack the samples, rather than to provide an optimal separation of isomers, as the analysis was primarily intended to evaluate the relative abundance of isomer groups.

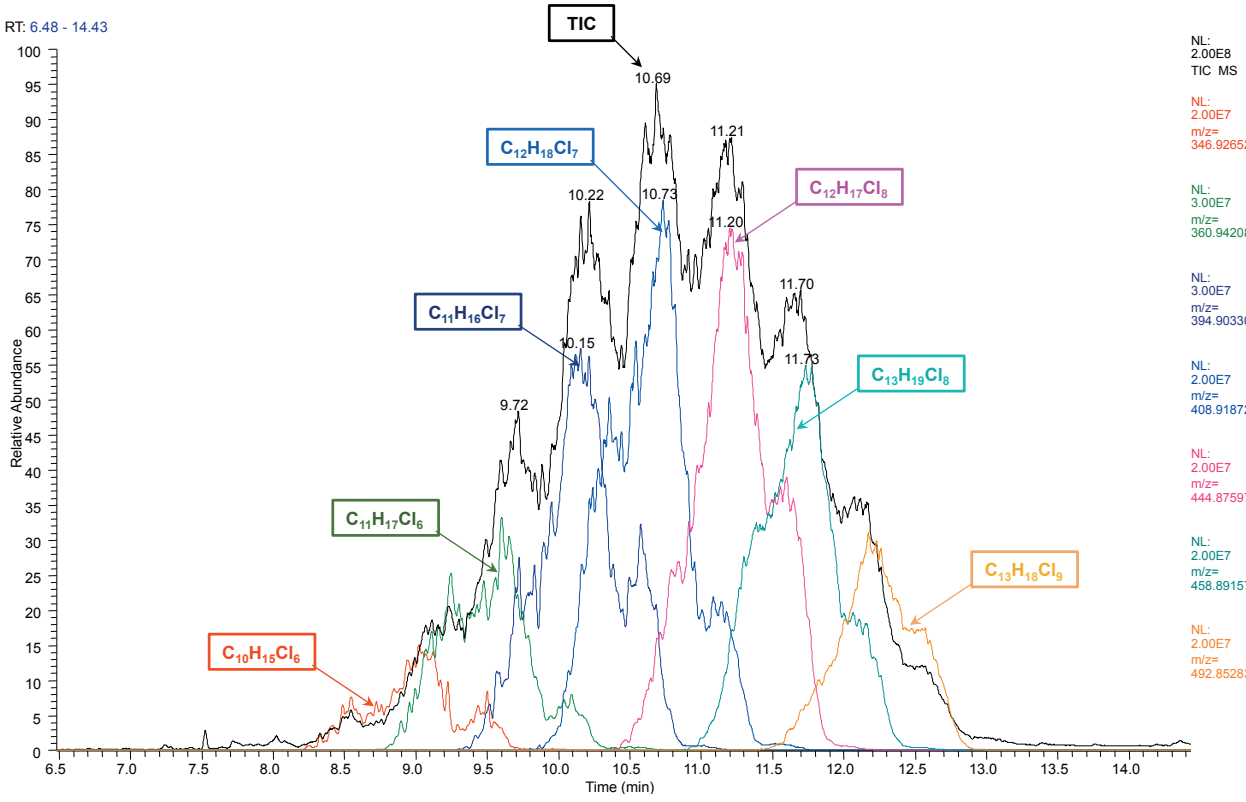
For the mass spectrometric analysis, both EI and negative chemical ionization (NCI) were evaluated with different resolving powers. EI resulted in unwanted fragmentation, which made it impossible to distinguish between homologues and between SCCPs and MCCPs. Thus, NCI was used for the remainder of the study. The resolving power as set at 60 K because it allowed homologue groups, and even homologues, to be distinguished. Data was acquired in full-scan over the mass range of 50–630 *m/z*. The first-stage flatapole proved an excellent way of excluding the peptides early in the beam path and preventing contamination of the quadrupole.

The relative abundance of molecular ions was evaluated, including $[M]^-$, $[M\text{-}HCl]^-$, and $[M\text{-}Cl]^-$. It was found that the $[M\text{-}Cl]^-$ ions were most abundant in all but the molecules with a low degree of chlorination (six or less chlorine atoms). For those, the $[M\text{-}HCl]^-$ ion gave the best signal. Relative standard deviations for repeat injections (n=10) were 4% or lower at 25 ppb.

As seen in **Figure 5**, the complex mixture results in a chromatogram with a characteristic hump with some groups only beginning to become evident. Use of the Orbitrap mass analyzer allows one to distinguish the identity of those subgroups into unresolved groups of isobaric compounds, ranging from $C_{10}H_{15}Cl_6$, to $C_{13}H_{19}Cl_5$. The method was tested for linearity using both 55.5% Cl and 63% Cl standards. The system gave a linear response over the concentration range tested—25 ppb to 10,000 ppb—even without use of internal standards. The presence of high concentration of PCBs was tested and found to have no effect on the peak shapes of compounds of the same nominal mass.

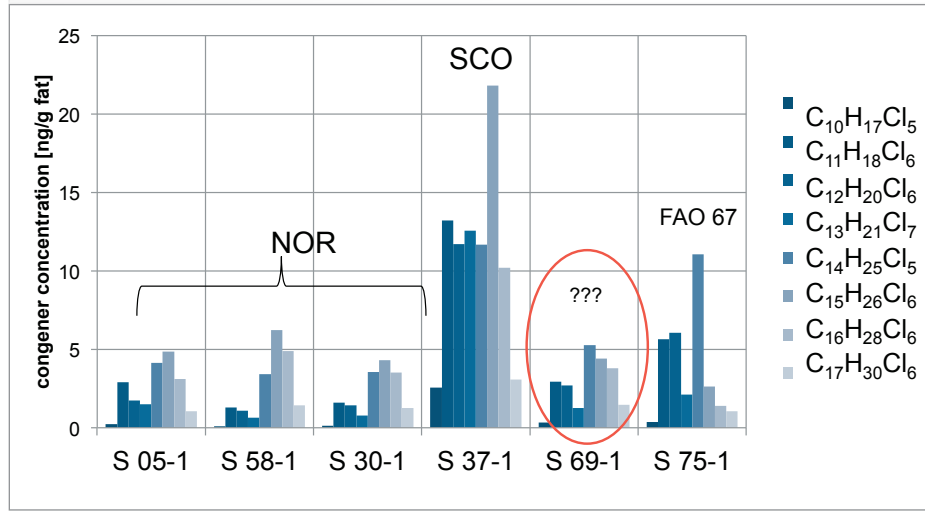
The method was applied to the analysis of samples of wild-caught salmon from Alaska and farm-raised salmon from the Northeast Pacific. The effect of potential interfering contaminants such as PCBs, dieldrin, DDT, and DDD was evaluated, and it was found that they could easily be identified and their interference avoided. While the estimated sum

**Figure 5:** High resolution accurate mass selectivity 63% $C_{10}$-$C_{13}$ technical mix, NCI, 60k resolution.




concentration of SCCP and MCCP only deviates 10–20% compared to the results gained by GC-EI-LRMS/MS, quantification of homologues opens up a new level of insight into the samples as seen in **Figure 6**.

**Figure 6:** Determination of SCCPs and MCCPs in salmon samples.

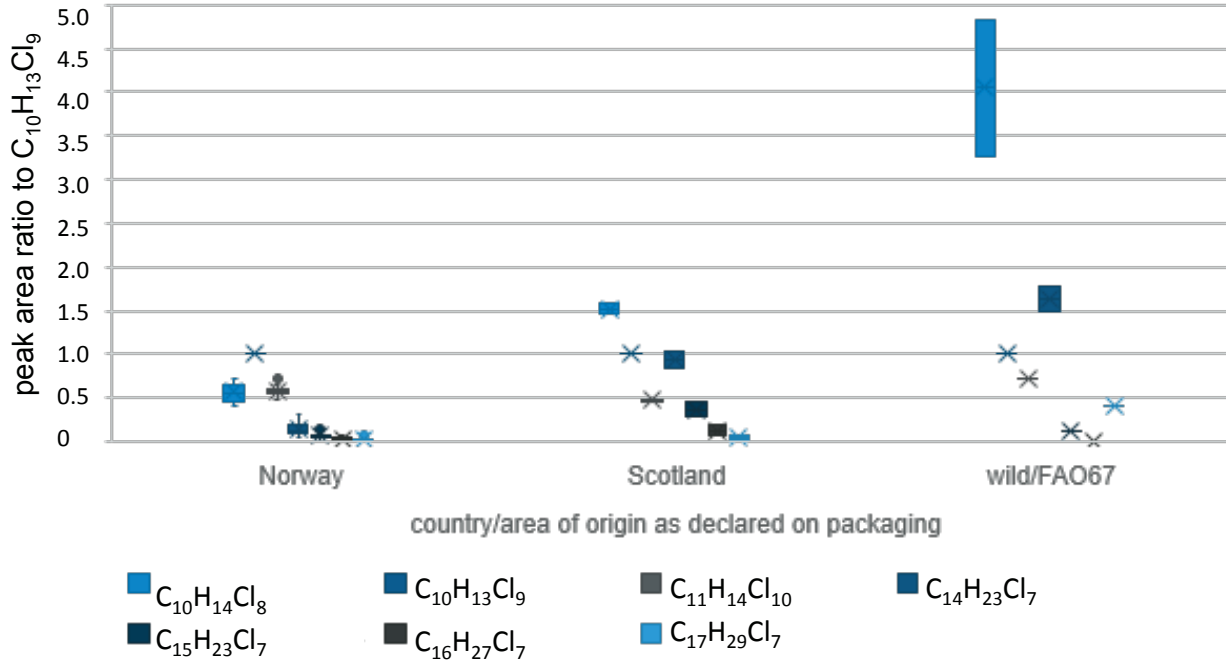



With the use of the Orbitrap mass analyzer, the characteristic hump can be deconvoluted into individual groups of isomers. The pattern of relative abundance appears to provide a possible fingerprint of the regional origin, with the Norwegian samples all showing similar patterns. The patterns were more variable for wild-caught salmon, which could be due to the greater mobility of the animals before capture, or simply the lower fat content. Level of $C_{10}H_{14}C_{18}$ and $C_{14}H_{23}Cl_7$ are significantly different in Norwegian and Scottish salmon as well as significantly higher in the analysed wild salmon from FAO 67 (Bering Sea/Eastern Pacific). (**Figure 7**).

## Conclusion

CPs present a substantial analytical challenge to environmental research and regulation of food safety. The European Union Reference Laboratory for Dioxins and PCBs in Feed and Food researchers found that Q Exactive GC Orbitrap GC-MS/MS system provides sufficient resolution, high

**Figure 7:** CP patterns in salmon samples.




sensitivity, and very low limits of detection and limits of quantitation. The NCI method showed excellent linearity from 25 ppb to 10,000 ppb, a detection limit of ~10 pg/uL and excellent RSDs. Experiments with mixtures of CPs and PCBs demonstrated that quantification of halogenated contaminants in one injection is possible.

The GC-Orbitrap system is an excellent quantitation platform and more than capable of compensating for the limited chromatographic separation. The ability to distinguish homologue patterns opens up new possibilities to assess the impact of CPs on environmental health and to assess the impact of CPs in food on consumer health.