thermo scientific

APPLICATION NOTE 10623

Versatility of GC-Orbitrap mass spectrometry for the ultra-trace detection of persistent organic pollutants in penguin blood from Antarctica

Authors

Nicholas A. Warner[1] and Cristian I. Cojocariu[2]

[1]NILU-Norwegian Institute for Air Research, Fram Centre, Tromsø, Norway, [2]Thermo Fisher Scientific, Runcorn, United Kingdom

Keywords

Persistent organic pollutants, POPs, polychlorinatd biphenyls, PCBs, organochlorine pesticides, King penguin blood, liquid-liquid extraction, GC Orbitrap high-resolution mass spectrometry, full-scan, targeted single ion monitoring, accurate mass, simultaneous full-scan/targeted analysis acquisition

Goal

In this study, the performance of the Thermo Scientific™ Q Exactive™ GC Orbitrap™ mass spectrometer was evaluated for routine analysis of POPs within King penguin blood from Antarctica.

Introduction

Persistent organic pollutants (POPs) have been studied extensively over the past five decades due to their toxicity and environmental persistence with spread and exposure throughout the global environment. International treaties, such as the Stockholm Convention, effectively banned or restricted the use of POPs such as polychlorinated biphenyls (PCBs) and organochlorine (OC) pesticides in 2004. However, many of these chemicals are still present in the environment today, accumulating in the food chains and posing risks for biota and humans even when present in trace amounts. PCBs and OC pesticides are the dominant contaminants found in remote polar regions, as these regions act as deposition sinks for contaminants that have undergone long-range transport via atmospheric or oceanic currents.[1] Although Arctic exposure to POPs has been studied extensively, information is scarce within Antarctica. Historically, chemical usage within the Southern Hemisphere has been lower compared to the Northern Hemisphere, making detection of POPs more challenging as concentrations are much lower. However, the Antarctica continent does receive atmospheric inputs of POPs.[2]

ThermoFisher SCIENTIFIC

This together with increasing industrial and agricultural activity within the Southern Hemisphere indicates an exposure risk to this region and highlights the need to address analytical challenges associated with monitoring POPs in Antarctica biological matrices.[3]

As environmental concentrations are approaching the detection capabilities of current analytical instrumentation, sample amount and preparation is key. However, sample material obtained through non-invasive sampling (i.e., blood or plasma) is often limited. Employing extensive sample clean-up strategies will increase sample processing time and costs and potentially result in lower detection frequency, as targeted compounds present at low concentrations will be lost through various clean-up steps. In addition, dilution of samples to reduce effects of co-extracted matrix may dilute targeted analytes below detection limits and reduce detection frequency. High-resolution Orbitrap™ mass spectrometry provides distinct advantages to help address these analytical challenges. With the potential to run routine full-scan and/or targeted single ion monitoring (t-SIM) analysis at 60,000 mass resolution (measured at *m/z* 200 as full width half maximum) greatly enhances the ability to selectively target and separate the analytes of interest away from co-extracted matrix interferences, reducing chemical noise and lowering detection limits.

Experimental conditions

Sample preparation

Blood samples from King penguins (0.5–1.0 g wet weight) were spiked with ^{13}C mass-labeled internal standards (PCBs and OC pesticides) and extracted using a liquid-liquid extraction procedure. Then, 2 mL ethanol, 2 mL of deionized water saturated with ammonium sulfate, and 6 mL of hexane were added to the sample material. Samples were vortexed and hexane supernatant was collected. Sample extracts were evaporated to dryness, then reconstituted in 0.5 mL of hexane and cleaned up further using automated solid phase extraction using 1 gram of activated Florisil® (450 °C) and eluted with 12 mL of 10% DCM/hexane. Sample extracts were evaporated close to dryness and quantitatively transferred to a GC autosampler micro-insert vial. ^{13}C-PCB 159 was added as a syringe standard. Isotope dilution quantification of target compounds in the samples was performed taking into account the corresponding internal standard response and using a single point calibration.

Instrument and method setup

Sample extracts were analyzed using the Q Exactive GC Orbitrap mass spectrometer. Automatic sample injection was performed using a Thermo Scientific™ TriPlus™ RSH™ autosampler, and chromatographic separation was obtained with a Thermo Scientific™ TRACE™ 1310 Gas Chromatograph and a Thermo Scientific™ TraceGOLD™ TG-5SilMS 30 m × 0.25 mm I.D. × 0.25 µm film capillary column with a 5 m integrated guard (P/N 26096-1425). Sample analysis was performed using full-scan and t-SIM acquisition. Additional details of instrument parameters are displayed in Table 1 and Table 2.

Table 1. GC and injector conditions

TRACE 1310 GC System Parameters	
Injection volume:	1 µL
Liner:	Thermo Scientific™ LinerGOLD™ GC Liner (P/N 453A1345-UI)
Inlet:	250 °C
Carrier gas:	He, 1.2 mL/min (constant flow)
Oven temperature program	
Temperature 1:	40 °C
Hold time:	1.5 min
Temperature 2:	180 °C
Rate:	25 °C/min
Hold time:	0 min
Temperature 3:	280 °C
Rate:	5 °C/min
Hold time:	0 min
Temperature 3:	320 °C
Rate:	40 °C/min
Hold time:	5 min

Table 2. Mass spectrometer conditions for data acquisition using simultaneous full-scan and targeted single ion monitoring (t-SIM) mode

Q Exactive GC Mass Spectrometer Parameters	
Transfer line:	280 °C
Ionization type:	Electron Ion (EI)
Ion source:	250 °C
Electron energy:	70 eV
Acquisition mode:	Full-scan and t-SIM
Isolation window:	8 Da
Mass range:	50–600 Da
Resolving power:	30,000 & 60,000 (FWHM at *m/z* 200)
Lock mass, column bleed:	207.03235 *m/z*

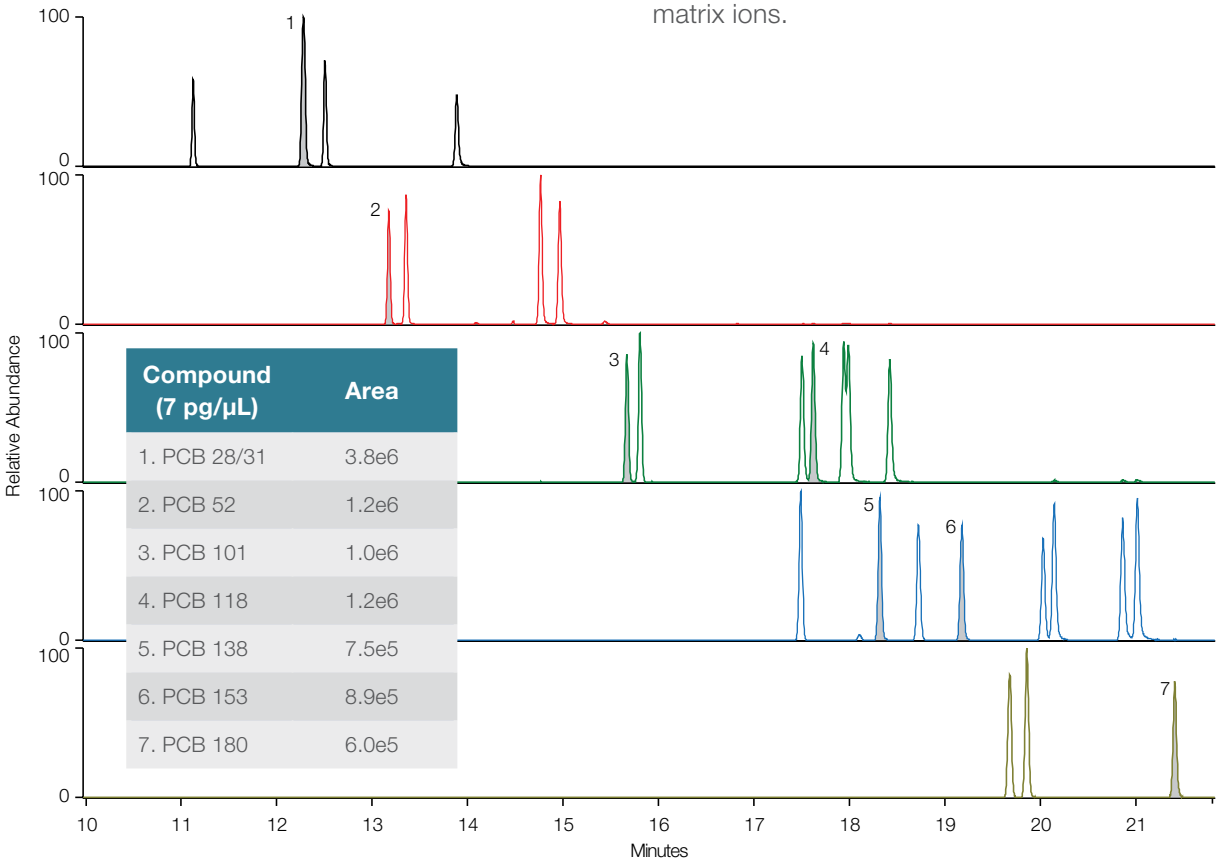
Data processing

Data were acquired using Thermo Scientific™ TraceFinder™ Environmental and Food Safety software version 4.1. Performance of the Q Exactive GC Orbitrap mass spectrometer to measure POPs in King penguin blood was evaluated in both full-scan and t-SIM acquisition modes. For targeted analysis, accurate masses for both target and qualifier ions were automatically acquired from quantification standards using TraceFinder software. Positive detection of compounds required both the target and qualifier ion to be detected within a mass accuracy of ±5 ppm and a target/qualifier ion ratio within 20% of that obtained from the quantification standard. All concentrations were blank corrected (if detected) with 3 and 10 times the blank variation being used to determine limits of detection and quantification, respectively.

Results and discussion

Full-scan acquisition with triple quadrupole sensitivity

Examples of the levels of sensitivity obtained for targeted POPs using the Q Exactive GC Orbitrap mass spectrometer are illustrated in Figures 1 and 2. Excellent response was observed in full-scan mode for both PCBs and OC pesticide quantification in solvent standards. Several compounds typically found within biological matrices within remote regions are highlighted, with area response ranging from 4e5 to 1e7 for injection of 1 to 38 pg on column (compound dependent). Substantial chemical noise can be observed in the full-scan analysis of the penguin blood extract (Figure 3). Total ion count (TIC) greater than 1e10 indicates substantial co-extracted sample matrix remains despite the clean-up strategies employed. However, even in the presence of such high levels of chemical background, detection of several PCBs (28/31, 66, 153) and OC pesticides (HCB, *pp*-DDE, and mirex) (Figure 4) could be achieved in full-scan acquisition. The high resolving power of the Q Exactive GC Orbitrap mass spectrometer provided selective mass separation (using ±5 ppm extraction window) of targeted compounds from co-extracted matrix ions.



Compound (7 pg/µL)	Area
1. PCB 28/31	3.8e6
2. PCB 52	1.2e6
3. PCB 101	1.0e6
4. PCB 118	1.2e6
5. PCB 138	7.5e5
6. PCB 153	8.9e5
7. PCB 180	6.0e5

Figure 1. Extracted ion chromatogram from full-scan acquisition of tri-, tetra-, penta-, hexa-, and heptachlorinated PCBs within a quantification standard (7 pg/µL)

Compound	Area
1. HCB (1.3 pg/µL)	4.8e5
2. α-HCH (38 pg/µL)	1.0e7
3. β–HCH (10 pg/µL)	1.8e6
4. γ-HCH (26 pg/µL)	5.5e6
5. *p,p'*-DDE (13 pg/µL)	4.3e6
6. *p,p'*-DDT (13 pg/µL)	1.7e6
7. *trans*-chlordane (10 pg/µL)	1.3e6
8. *trans*-nonachlor (10 pg/µL)	1.4e6
9. Mirex (26 pg/µL)	7.4e6

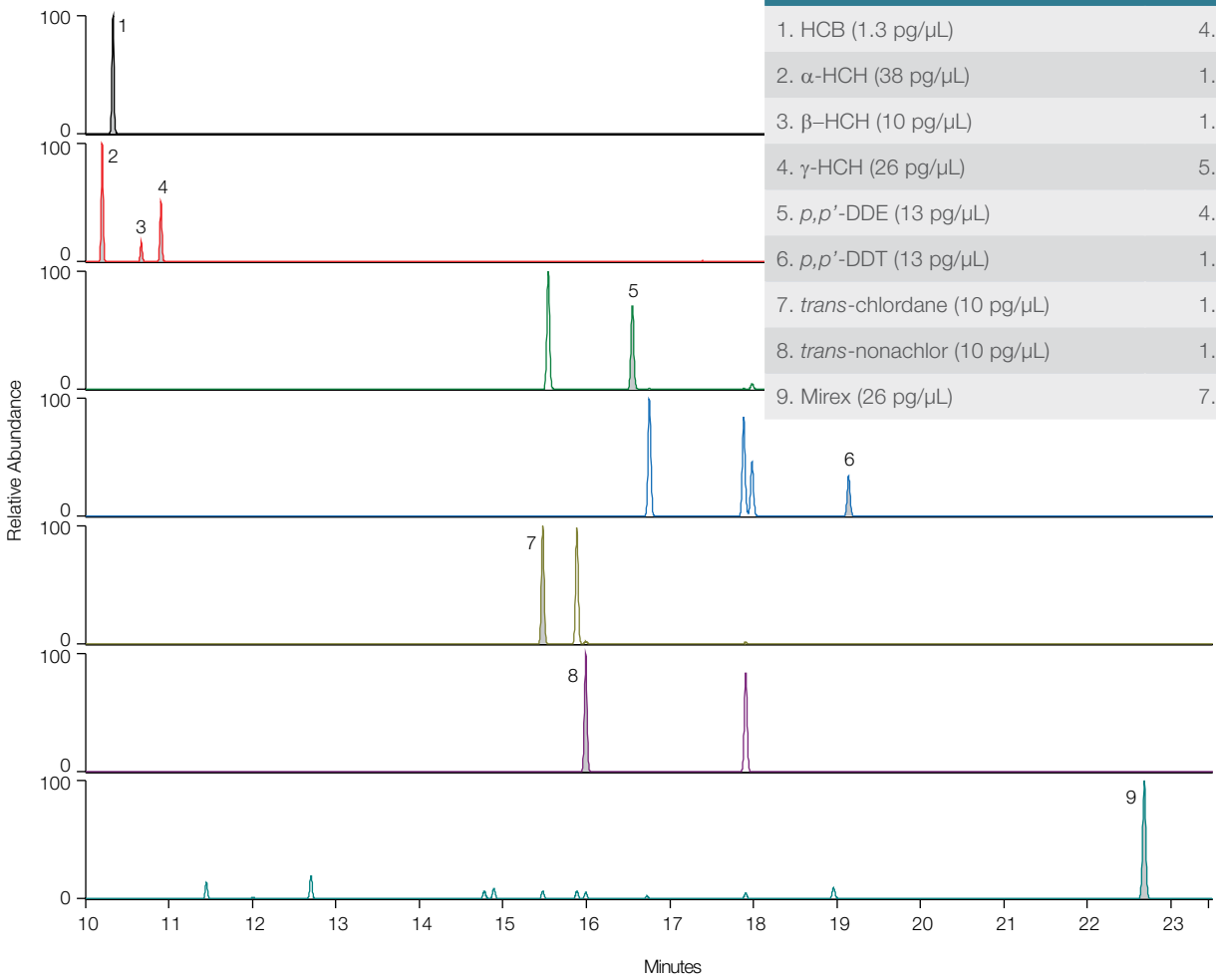


Figure 2. Extracted ion chromatogram from full-scan acquisition of OC pesticides quantification standard. Concentrations of individual pesticides range from 1 to 38 pg/µL.

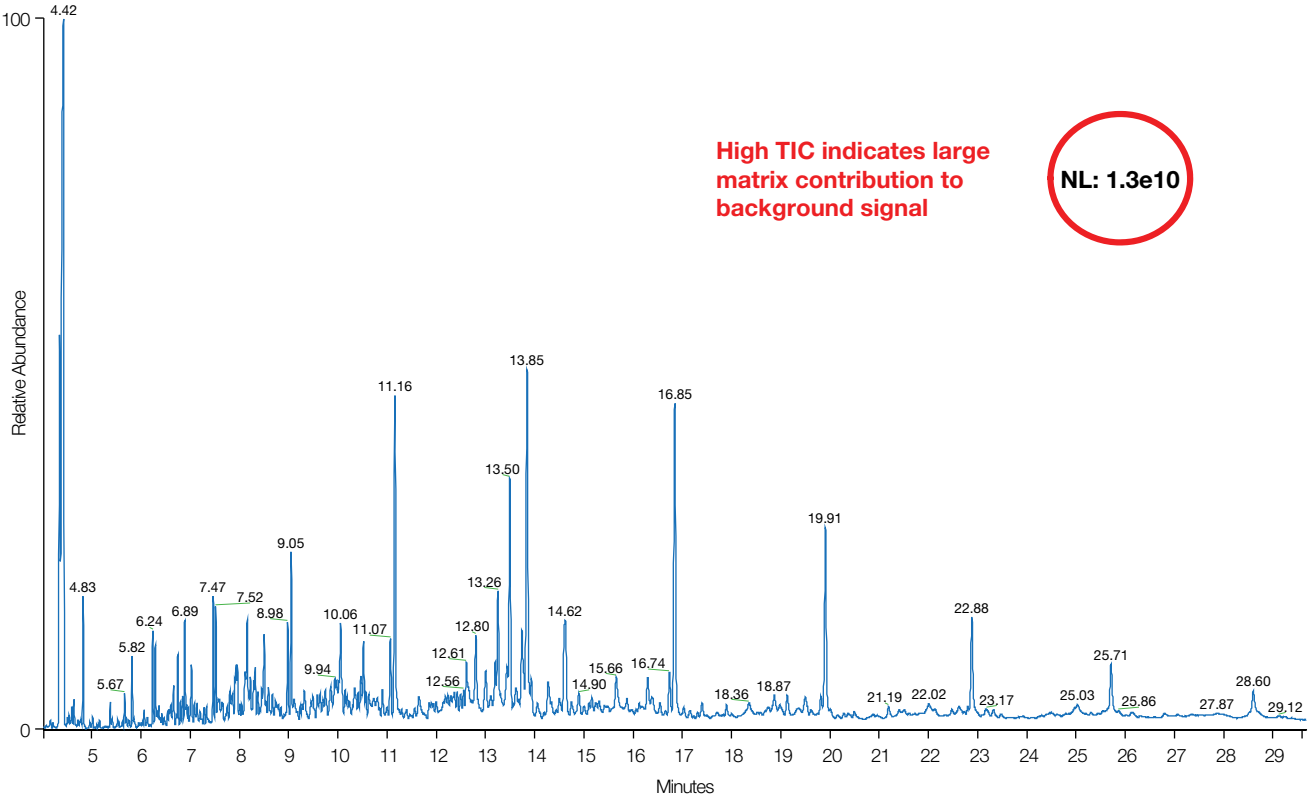


Figure 3. Full-scan total ion chromatogram (TIC) of King penguin blood extract highlighting substantial chemical noise from co-extracted sample matrix remains in final extract

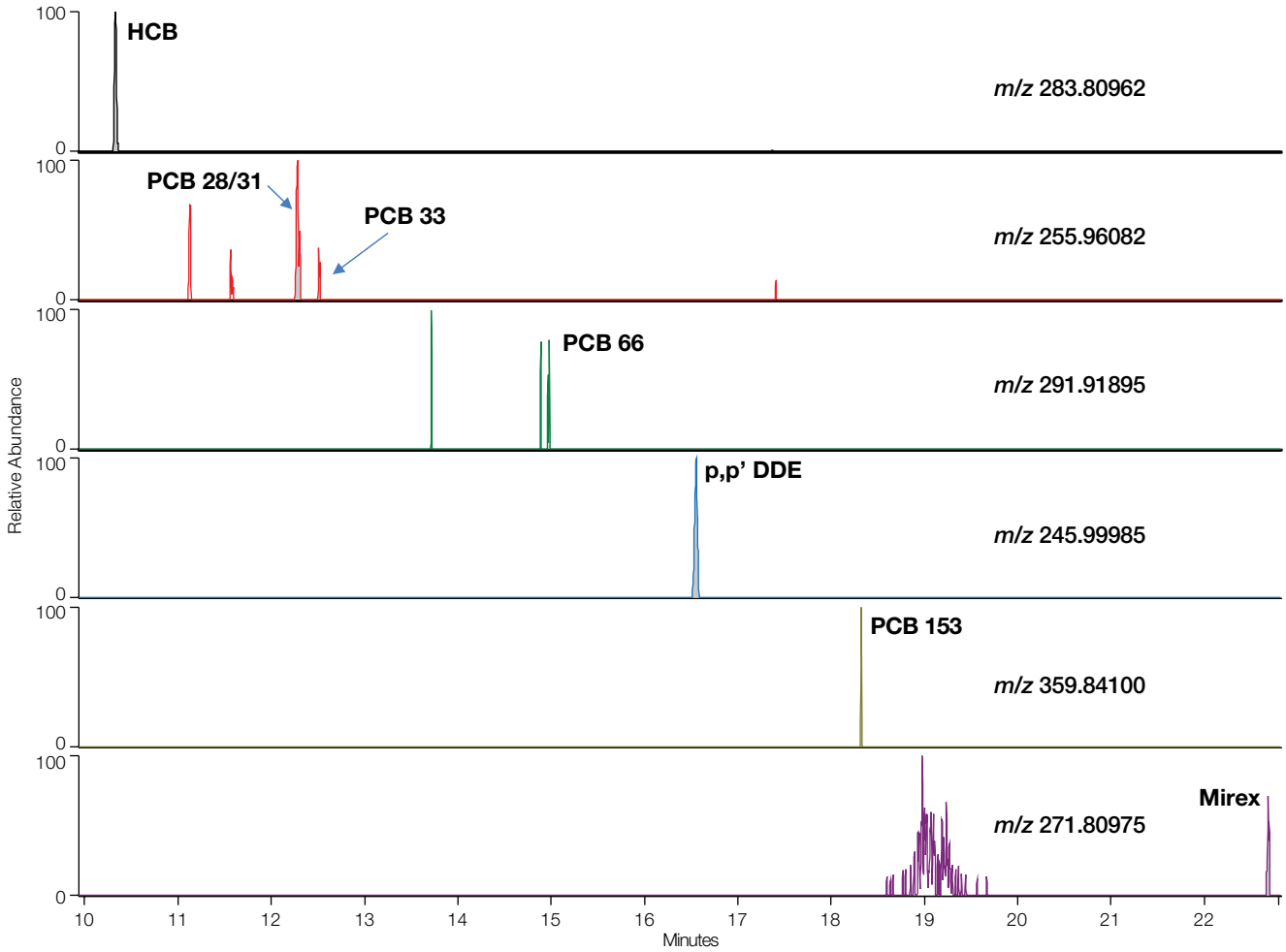


Figure 4. Extracted ion chromatogram of detected PCBs and OC pesticides in King penguin blood acquired in full-scan acquisition

Enhanced sensitivity/detection capabilities

Data acquisition using t-SIM mode can help improve detection limits/capabilities even further in complex samples in which significant co-extracted sample matrix remains. By utilizing the advanced quadrupole technology (AQT), mass regions of interest (i.e., isolation windows) can be targeted while minimizing introduction of co-extracted sample matrix into the C-trap of the Q Exactive GC Orbitrap system. Analysis using t-SIM acquisition of the same King penguin blood extract run previously in full-scan mode showed an improvement in the number of PCBs (Figure 5) and OC pesticides (Figure 6) detected. In addition to PCB 28/31, 33, 66, and 153 detected in full-scan (Figure 4), several other PCB congeners were detected using t-SIM acquisition (Figure 5). Similar findings were also observed for OC pesticides with the HCH isomers, *p,p'*-DDD, *p,p'*-DDT, chlordane, and nonachlor isomers being detected along with HCB, *p,p'*-DDE, and mirex (Figure 6).

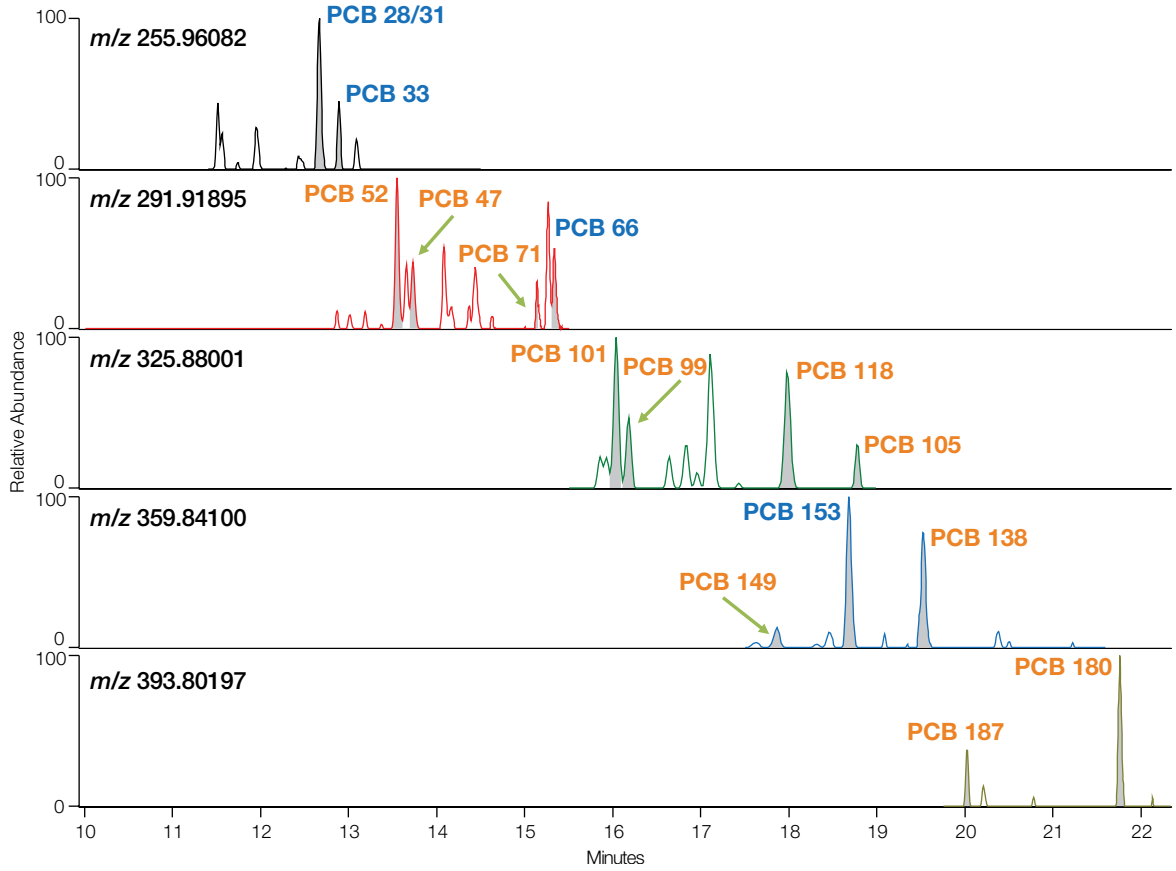


Figure 5. Extracted ion chromatogram of detected PCBs in King penguin blood extract with t-SIM acquisition. Compounds marked in green were not detected in full-scan acquisition.

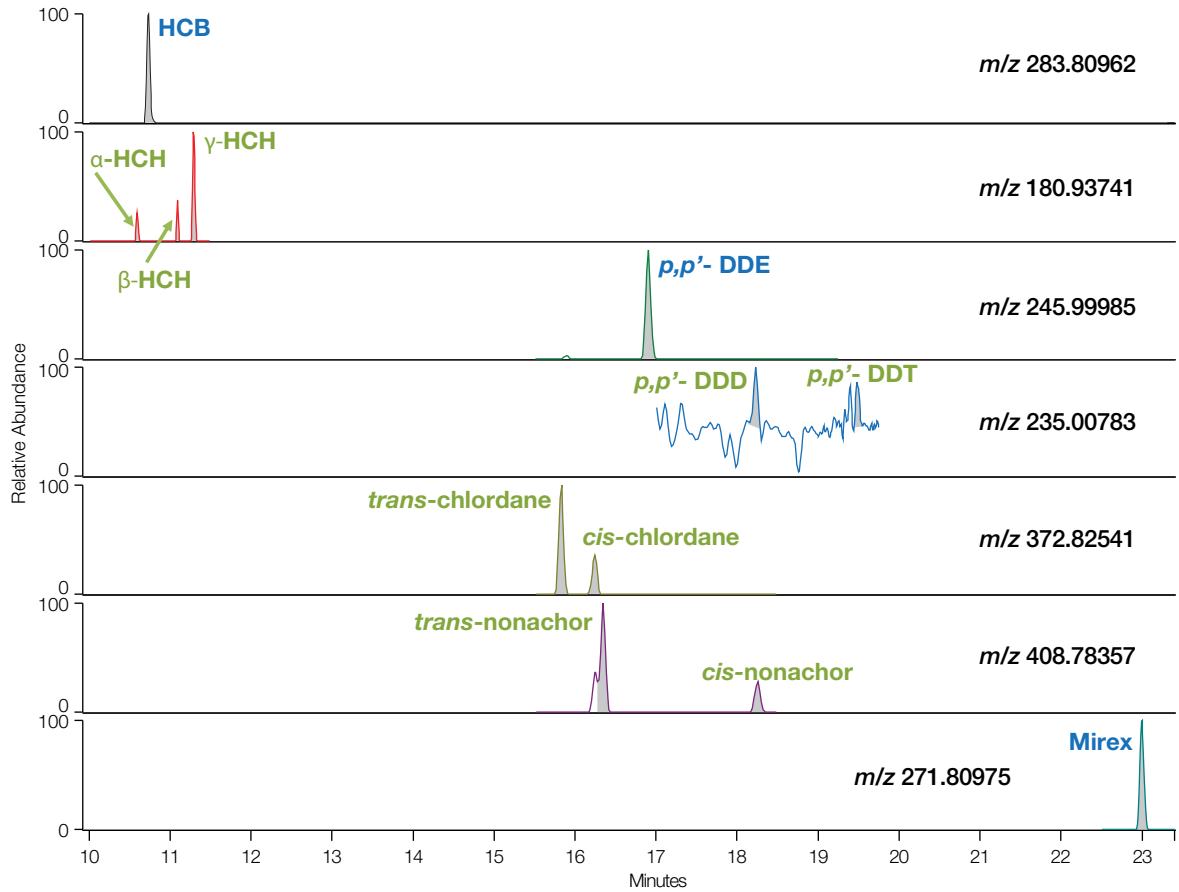


Figure 6. Extracted ion chromatogram of detected OC pesticides in King penguin blood from t-SIM acquisition. Compounds marked in green not detected in full-scan only acquisition.

Quantification of POPs in penguin samples

Detected compounds above blank levels were present at part per trillion (ppt) concentrations within King penguin blood (Tables 3 and 4). Highest concentrations were observed for HCB (290 pg/g ww), while *cis*-nonachlor was detected at 0.9 pg/g ww, demonstrating the sensitivity of the Q Exactive GC Orbitrap system. Limits of quantification (LOQ) were compound-dependent based on blank levels and ranged from 0.1 to 14.2 pg/g ww. Using t-SIM acquisition mode, the Q Exactive GC Orbitrap system was able to remove mass regions containing co-extracted sample matrix entering the C-trap, improving detection frequency at the low part per trillion concentrations.

Table 3. Concentration of PCBs (pg/g wet weight) present in King penguin blood sample

PCB Congener	Concentration (pg/g ww)
52	11.5
101	16.3
118	6.4
138	13.2
149	3.8
153	11.6
180	5.3
183	1.4
187	2.0

Table 4. Concentration of OC pesticides (pg/g wet weight) present in King penguin blood sample

Pesticide	Concentration (pg/g ww)
HCB	290
aHCH	3.9
p,p'-DDT	1.4
p,p'-DDD	4.7
p,p'-DDE	101
trans-chlordane	11.7
cis-chlordane	3.5
trans-nonachlor	11.7
cis-nonachlor	0.9
Mirex	38

Increased compound coverage using simultaneous full-scan/t-SIM acquisition

Comparison of results obtained between the full-scan and t-SIM acquisition show that co-extracted sample matrix entering the C-trap can hinder detection of targeted compounds at the low part per trillion-concentration range in full-scan acquisition. However, full-scan mass spectral data can still be obtained without sacrificing trace level sensitivity of compounds for targeted analysis. With the Q Exactive GC Orbitrap system, full-scan and t-SIM data acquisition can be obtained simultaneously within a single GC run. As t-SIM selectively isolates mass windows around compounds of interest, lower mass resolution settings can be utilized to insure an optimal number of data scans is obtained across chromatographic peaks while maintaining sufficient mass resolution for compound identification/detection. Assessment of sensitivity/detection frequency within King penguin blood using simultaneous full-scan (at 60,000 mass resolution)/t-SIM acquisition (30,000 mass resolution) was carried out. The number of PCB congeners detected in simultaneous full-scan/t-SIM acquisition (14) was slightly less that that obtained from t-SIM alone (16), but much greater when obtained in full-scan only acquisition (4) (Figure 7). Detection frequency decreased for OC pesticides as HCH isomers, *p,p'*-DDD, *p,p'*-DDT, and *cis*-chlordane were not detected in the simultaneous full-scan/t-SIM acquisition. However, detection frequency was still greater compared to that obtained from full-scan acquisition alone. This demonstrates that the Q Exactive GC Orbitrap system can still maintain trace level sensitivity while acquiring full-scan data simultaneously for identification of unknown compounds that may pose potential environmental/health risks.

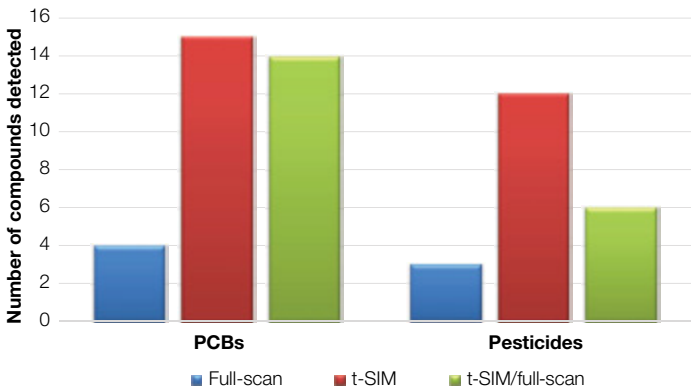


Figure 7. Number of PCBs and OC pesticides detected in King penguin blood in full-scan (blue), t-SIM (red), and simultaneous full-scan/t-SIM acquisition (green). Simultaneous full-scan/t-SIM acquisition utilized 30,000 resolution in for t-SIM acquisition and 60,000 resolution in full-scan acquisition.

Conclusion

The results of this study demonstrate that the Q Exactive GC Orbitrap high-resolution mass spectrometer provides excellent sensitivity, selectivity, and versatility in meeting the analytical challenges associated with trace level quantification of environmental samples from remote regions.

- Despite considerable co-extracted matrix interference, detection of several POP substances at part per trillion levels was achieved using full-scan acquisition at 60,000 mass resolution.
- Improvements in detection limits/capabilities could be obtained using t-SIM acquisition. The number of compounds detected in King penguin blood increased up to a factor of 4 using t-SIM.
- The processing power of the Q Exactive GC Orbitrap GC-MS/MS system provided the means of collecting full-scan mass spectra data without significantly compromising sensitivity of targeted analysis. Simultaneous full-scan and t-SIM data acquisition provided quantification of targeted analytes at part per trillion levels while collecting full-scan mass spectral data for identification of potential unknown compounds impacting remote environments.

Acknowledgments

The authors would like to thank the POLAR-ECOTOX project (project number 243763, Research Council of Norway) for access to sample material and the Strategic Institute Funding by the Research Council of Norway.

References

1. AMAP, AMAP Assessment 2009 - Persistent Organic Pollutants (POPs) in the Arctic. *Science of the Total Environment Special Issue*, *408*, 2851–3051. Elsevier, 2010.
2. Kallenborn, R.; Breivik, K.; Eckhardt, S.; Lunder, C. R.; Manø, S.; Schlabach, M.; Stohl, A., Long-term monitoring of persistent organic pollutants (POPs) at the Norwegian Troll station in Dronning Maud Land, Antarctica. *Atmos. Chem. Phys.* **2013**, *13*, (14), 6983–6992.
3. Bengtson Nash, S., Persistent organic pollutants in Antarctica: current and future research priorities. *Journal of Environmental Monitoring* **2011**, *13*, (3), 497–504.

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