Guidance Document on the Analysis of Polychlorinated Alkanes

Analytical Parameters for food and feed analysis

VERSION 2.0

23 NOVEMBER 2026

EURL POPs

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Disclaimer

This document refers to a number of products (analytical standards and equipment) as examples of items that may be used in the described procedures. Alternative sources of these or similar products may be available, and the document does not endorse or recommend any particular product for use in the described procedures.

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Table of Contents

1 General 8
1.1 Scope and Field of Application 8
1.2 Background 10
1.2.1 Classification as POPs 10
1.2.2 Prevalence in Human Tissue 11
1.3 Terms and Definitions 12
1.3.1 Terms for Analytical Standards 12
1.3.2 Definitions of Analytes 12
1.3.3 PCA groups 15
1.4 Quality control and quality assurance 16
1.4.1 Recommendations for Laboratories 16
1.4.2 General aspects regarding sample pre-treatment and storage 16
1.4.3 Identification and quantification criteria 17
1.4.4 Estimation of LOD/LOQ 18
1.4.5 Measurement uncertainty 19
1.4.6 Validation 19
1.4.7 Continuous QC measures 20
1.5 Reporting 21
1.5.1 Reporting of occurrence data for PCA sum parameter (Monitoring) 21
1.5.2 Recommendations for reporting results of PCA characterisation analysis 22
1.5.3 Reporting to EFSA 23
2 General analytical methodologies 24
2.1 Considerations on choosing a suitable method 24
2.2 Choice of Instrumentation 25
2.3 Analytical standards 29
2.3.1 Native standards for quantification 29
2.3.2 Syringe standard 30
2.3.3 Internal Standard 30
2.4 Blank level management 31
2.4.1 General aspects to avoid contamination / high blank levels 31
2.4.2 Pretreatment of consumables and glass ware 31
2.4.3 Precautions to avoid blank contribution from laboratory equipment 32

3 Analytical methods 34
3.1 Module 1: Sample pre-treatment and extraction 34
Module 1A: Cold extraction 35
Module 1B: Extraction procedure using high-performance dispersion extraction 35
Module 1C: Glass column extraction 36
Module 1D: Microwave extraction 36
Module 1E: Pressurised liquid extraction 37
3.2 Module 2: Clean-up 38
Module 2A: Lipid removal - Direct acid treatment 39
Module 2B: Lipid removal / clean-up - Acidic silica column 39
Module 2C: General clean-up - Neutral silica column 40
Module 2D: Removal of co-eluting contaminants - Florisil® column 40
Module 2E: Automated clean-up 41
3.3 Module 3: Measurement 42
Module 3A: GC-EI-MS/MS 43
Module 3B: GC-ECNI-LRMS 44
Module 3C: LC-ESI-MS/MS 45
Module 3D: GC- and LC-HRMS analysis 46
3.4 Module 4: Quantification methods 48
Module 4A: Quantification strategy for GC-EI-MS/MS monitoring method 48
Module 4B: Calibration corrected for chlorination degree 50
Module 4C: Gaussian curve method 51
Module 4D: Deconvolution method 52
Applications and scripts for quantification 54
4 References 55
5 ANNEXES 62

ANNEXES:

ANNEX I Guidance for monitoring of sum parameter ΣPCAs-$C_{10\text{-}17}$ by GC-EI-MS/MS

ANNEX II Additional information for GC-ECNI-LRMS analysis (Module 3B)

ANNEX III Additional information for LC-MS/MS analysis (Module 3C)

ANNEX IV Additional information for GC-ECNI-HRMS and LC-ESI-HRMS analysis (Module 3D)

Abbreviation	Definition
ACN	Acetonitrile
ASE	Accelerated solvent extraction
CE	Collision energy
CWG	Core working group
CPs	Chlorinated paraffins (refers to commercial products)
SCCPs	Short-chain chlorinated paraffins
MCCPs	Medium-chain chlorinated paraffins
LCCPs	Long-chain chlorinated paraffins
CRM	Certified reference material
DCM	Dichloromethane
ECNI	Electron capture negative ionisation
EFSA	European Food Safety Authority
EI	Electron ionisation
(H)ESI	(Heated) electrospray ionisation
EURL	European Union Reference Laboratory
FWHM	Full width at half maximum height
GC	Gas chromatograph(y)
HRMS	High resolution mass spectrometry
iLOD	Instrument limit of detection
ILS	Interlaboratory study
IS	Internal standard
ISO	International Organisation for Standardisation
IUPAC	International Union of Pure and Applied Chemistry
LC	Liquid chromatograph(y)
(U)HPLC	(Ultra) High performance liquid chromatograph(y)
LOD	Limit of detection
LOQ	Limit of quantification
LRMS	Low resolution mass spectrometry
MAE	Microwave assisted extraction
m/z	Mass-to-charge ratio
MS/MS	Tandem mass spectrometry
MOSH/MOAH	Mineral oil saturated hydrocarbons / mineral oil aliphatic hydrocarbons
MU	Measurement uncertainty
NCI	Negative chemical ionisation
NRL	National Reference Laboratory
OFL	Official Laboratory
PCAs	Polychlorinated alkanes
SC-PCAs	Short-chain chlorinated alkanes (C_{10}-C_{13})
MC-PCAs	Medium-chain chlorinated alkanes (C_{14}-C_{17})
LC-PCAs	Long-chain chlorinated alkanes (≥C_{18})
PBDEs	Polybrominated diphenyl ethers

PCNs	Polychlorinated naphthalenes
PCBs	Polychlorinated biphenyls
PCDDs	Polychlorinated dibenzo-*p*-dioxins
PCDFs	Polychlorinated dibenzofurans
PFE	Pressurised fluid extraction
POP(s)	Persistent organic pollutant(s)
PT	Proficiency test
PTFE	Polytetrafluoroethylene
RRF	Relative response factor
S/N	Signal-to-noise ratio
U	Expanded measurement uncertainty
UNEP	United Nations Environment Programme
TIC	Total ion chromatogram
TOF	Time-of-flight (mass analyser)
w.w.	Wet weight

1 General

1.1 Scope and Field of Application

This guidance document describing the determination of polychlorinated alkanes (PCAs) as main components of the technical products chlorinated paraffins (CPs) is intended for laboratories involved in the official control of contaminants in food and feed.

Note: In this document the term chlorinated paraffin (CP) is used for the technical/industrial product whereas the term polychlorinated alkane (PCA) refers to the actual determined analyte class representing the main component of technical CP products.

This guidance document was developed by a working group within the network of the European Union Reference Laboratory (EURL) for halogenated persistent organic pollutants (POPs) in Feed and Food, the respective National Reference Laboratories (NRLs) of EU member states and international experts in the analysis of POPs and PCAs. The document provides general guidance on analytical methodologies for laboratories and particularly for those that do not have an existing method for PCAs. The methodologies described cover both, sum parameter/monitoring and characterisation approaches (**Figure 1**). The practical determination is described in a modular format composed of different techniques for each stage of analysis. This allows choice of individual modules based on the overall analytical aim (e.g. monitoring/screening *vs* characterisation) and allows laboratories the flexibility of utilising existing equipment and expertise. This document aims to contribute to further harmonization and the assurance of reliable data as part of the EURL's official mandate and scope of work.

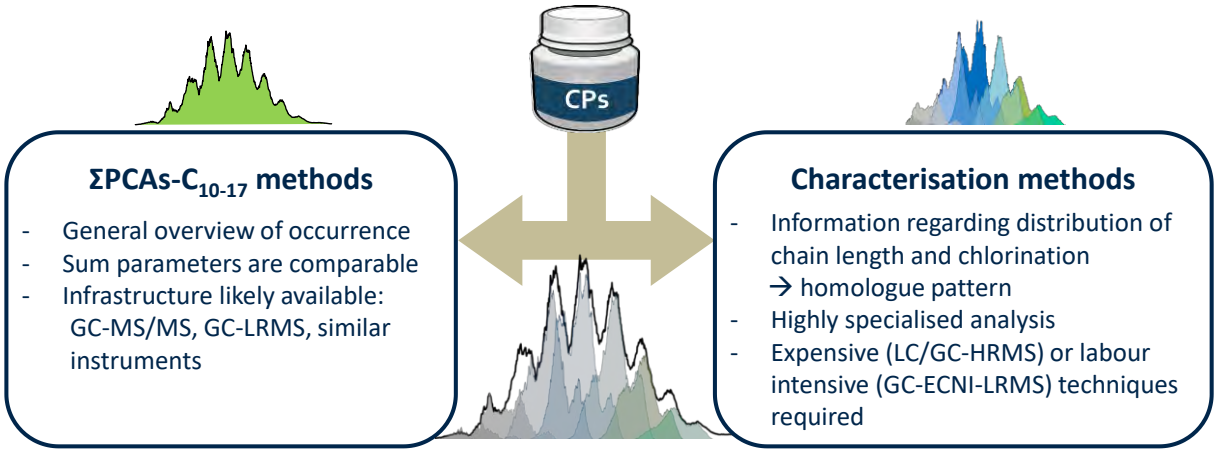


Figure 1 Overview of different methodological approaches for the analysis of PCAs.

The main purpose of this document is to provide guidance to allow PCA analysis in food and feed to be established throughout the European Union as part of the EURL's official mandate and scope of work.

The recommendations in this guidance document are designed to allow the monitoring of PCA concentrations in food and feed as part of studies that will enable the determination of current occurrence levels for

- the establishment/maintenance of databases,
- the assessment of human exposure through dietary intake and assessment of risk.

The guidance could also be used for studies on time trends and patterns of PCA occurrence in order to identify possible source(s) of contamination, particularly during incidents involving such contamination.

To improve comparability and to support the exchange of information within the EURL/NRL/OFL network and beyond, the EURL for halogenated POPs in Feed and Food established a Core Working Group (CWG) on Chlorinated Paraffins (CPs) in 2016. As one standardised analytical approach for food and feed is not feasible given the diversity of methodologies and instrumentation, harmonisation and simplification in this analytical field is difficult to achieve.

A current aim of the CWG is to encourage more laboratories to analyse PCAs as predominant component of CPs in feed and food samples. By focusing on sum parameters and applying analytical methods for which equipment is available in most laboratories, the efforts for measurement and data analysis can be reduced and simplified. The *Commission Recommendation (EU) (2026/2335) on monitoring the presence of chlorinated paraffins in food* [1] focuses on the use of these methods for the determination of the PCA sum parameter ΣPCAs-$C_{10\text{-}17}$ to collect occurrence data for different food matrices.
Therefore, this guidance document provides information on a simplified quantitative screening method for quantification of the sum parameter ΣPCAs-$C_{10\text{-}17}$ (this method is referred to as "monitoring method" in this document), including example methodologies and guidance on a set of analytical parameters that lead to satisfactory method performance, together with advice on analytical standards and quantification (compiled in **Annex I**).

Information on PCA homologue patterns, i.e. distribution of chain length and chlorination degree are essential, particularly when assessment of exposure and impact on human health requires speciation of the occurrence and relative proportions of short-, medium- and long-chain PCA homologue groups. This information can also help to identify the source of contamination. Thus, this document also compiles guidance on methodologies for characterisation analysis.

Examples of methods for the different types of analysis, i.e. screening/monitoring and characterisation, are provided in modular format for the different steps of the analytical procedure in **chapter 3**. The example methods given are applied in the EURL/NRL network and were developed for different food types but may generally also be applicable to animal feed after appropriate modification and validation for the respective matrices.

1.2 Background

Chlorinated paraffins (CPs) are high-production volume chemicals used in various industries as plasticizers, high-pressure lubricants or flame retardants. Annual CP production was predicted as surpassing 1 million metric tons already for the early 2010s, with further increase observed until 2020s, especially on the Chinese market [2,3]. Depending on the desired properties, different alkane feedstocks are chlorinated to a specific degree. Technical CP products are commonly categorized by alkyl chain lengths (of the paraffin feedstocks) in three main groups: short-chain chlorinated paraffins (SCCPs, C_{10}- to C_{13}-alkanes, 40-70% chlorine), medium-chain chlorinated paraffins (MCCPs, C_{14}- to C_{17}-alkanes, 30-70% chlorine) and long-chain chlorinated paraffins (LCCPs, $C_{\geq 18}$-alkanes, chlorination degree varies widely). In addition to PCAs (the main constituents), technical CP products may also contain various other substances, e.g. unreacted alkanes, chlorinated alkenes, polychlorinated branched chain/*iso*-alkanes, stabilizers, chlorinated fatty acid methyl esters, as well as polychlorinated biphenyls (PCBs), polychlorinated naphthalenes (PCNs) and polychlorinated dibenzo-*p*-dioxins and furans (PCDD/F) as by-products [4].

All technical CP products are composed of several thousand PCA congeners with varying degrees of chlorination [5]. Analytically, these mixtures cannot be individually characterised at present, so there is little knowledge about individual chemical structures or whether any configurations predominate.

1.2.1 Classification as POPs

The technical product defined as SCCPs (C_{10}-C_{13}, >30%Cl) has proven to be persistent [6], bioaccumulative [7–9] and toxic [10–12], just as MCCPs (C_{14}-C_{17}, >48%Cl), though to a slightly lesser degree [7–10,13–15]. Some of these properties have also been reported for LCCPs [8,9,16], and the amount of data continues to grow. Currently, both SCCPs and MCCPs have been regulated as POPs under the Stockholm Convention [17,18].

The risk assessment of CPs published by the European Food Safety Authority (EFSA) in 2020 noted limitations in the toxicokinetic and toxicological database and limited data on the occurrence of SCCPs and MCCPs in food. A robust exposure assessment, a risk characterisation and consequently a complete risk assessment could not be performed [19].

Note: At the time of publication of the EFSA scientific opinion in 2020, the recommendation to use PCA nomenclature had not been published [3].

A broad monitoring of PCAs in food and the environment is needed to collect occurrence data for robust exposure estimates to identify environmental concerns and in order to support regulatory efforts. However, the complexity of CP products engenders an analytical challenge and no general standardised analytical method for food and feed has been established to date. In recent years, standardised methods applying GC-ECNI-MS for determination of SC-PCAs (ΣPCAs-$C_{10\text{-}13}$) in drinking water [20,21] and sediments [22,23] have been developed and adapted to leather [24,25] and textiles [26] to additionally cover MC-PCAs (ΣPCAs-$C_{14\text{-}17}$).

For food and feed samples, a large variety of methods based on different principles and using various instruments has been reported in the literature [27–29]. Although interlaboratory studies showed improvement in comparability, there are still difficulties when different quantification methods or instrument types are used [20,29,30]. These factors make laboratories often very hesitant to start establishing PCA analysis.

1.2.2 Prevalence in Human Tissue

One strong indicator of a compound (group) of concern is bioaccumulation in the human body. In the case of most halogenated POPs, this accumulation can be observed best in fatty tissue and human milk. The available data from the United Nations Environmental Programme (UNEP) human milk study of 2015-2019 allowed insights into PCA levels in human milk together with other POPs on a global scale, demonstrating the presence of PCAs even in samples from remote Pacific Island States (**Figure 2**). This unique dataset also allowed a direct comparison to other, better established POPs, showing PCAs (here: ΣPCAs-C_{10-17}) to be among the most dominant contaminants detected in the human milk samples across all countries [31]. The modelled time trends also indicate an alarming conclusion: while most other POPs seem to have decreased in human milk over time, PCAs seem to have increased in samples especially from Central- and Eastern Europe and Asia UN regional groups, but also globally [32]. These findings emphasise the need for reliable methods to gain insight in dietary sources of PCAs as a step towards lowering human exposure.

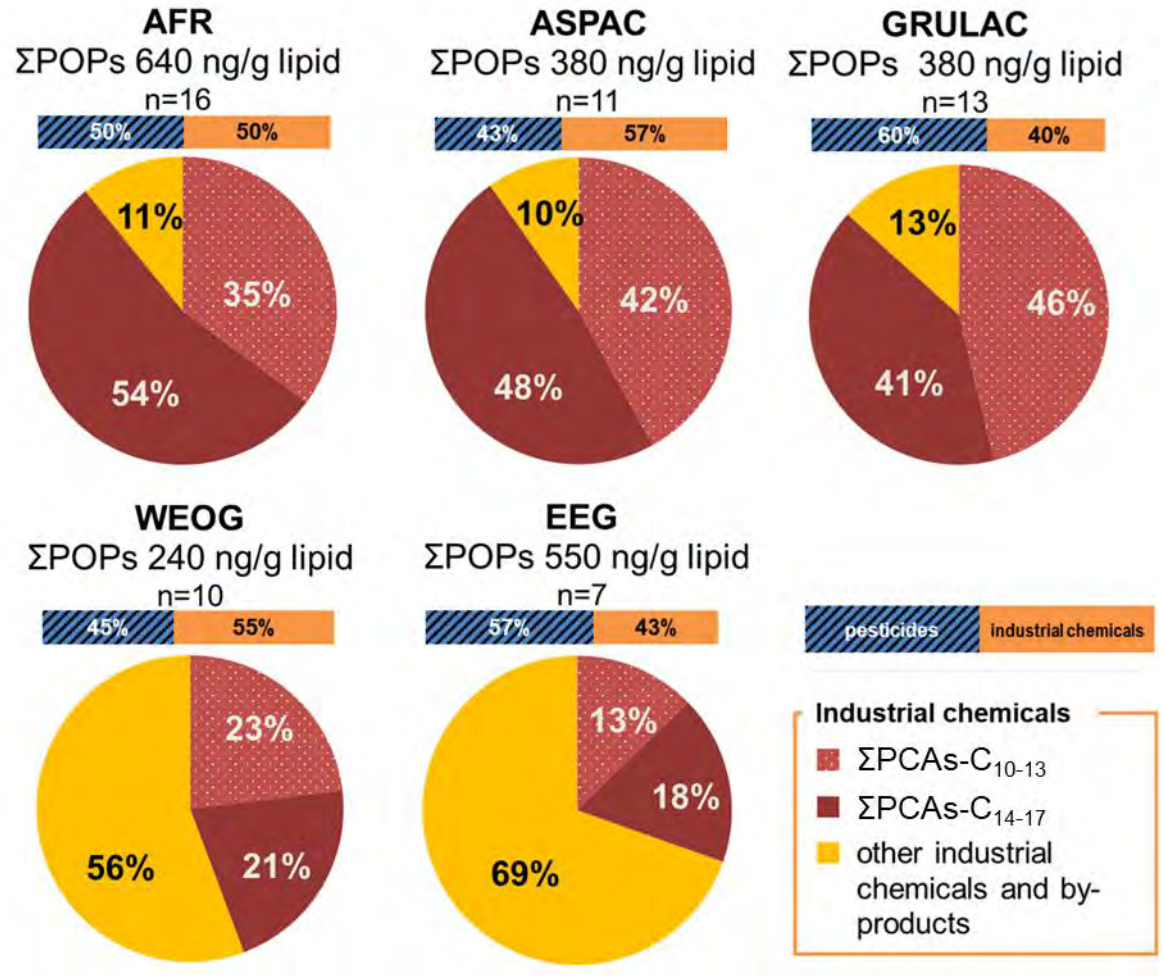


Figure 2 Median sum of all POPs except PFAS and distribution of different POPs groups in pooled human milk samples from 2015-2019 for the UN regions (AFR Africa; ASPAC Asia-Pacific; GRULAC Group of Latin American and Caribbean Countries; WEOG Western Europe and Others Group; CEE Central and Eastern Europe). *Pesticides: aldrin, chlordane, chlordecone, DDT, dicofol; dieldrin, endosulfan, endrin, heptachlor, α-HCH, β-HCH; γ-HCH; mirex, pentachlorobenzene, pentachlorophenol (including pentachloroanisole) and toxaphene;*
(Other) Industrial chemicals and by-products: hexabromobiphenyl (HBB), HBCDD, HCB, hexachlorobutadiene; PBDE; PCB, PCDD, PCDF and PCN, as well as ΣPCAs-C_{10-13} and ΣPCAs-C_{14-17}.

1.3 Terms and Definitions

For the purpose of this guidance document, the following terms and definitions apply.

1.3.1 Terms for Analytical Standards

***Internal standard**:* A standard that is added to the sample during the first step of preparation to monitor or account for losses during sample preparation and the whole analytical procedure. Often an isotope-labelled compound is used as it should not be naturally present in the sample but possess similar physicochemical properties as the analyte.

***Syringe standard** also referred to as injection or volumetric standard:* A standard that is added at the very end of sample processing, prior to instrumental analysis which does not have to be structurally similar to the analyte, as it accounts only for losses caused by problems with injection or other steps of instrumental analysis/measurement.

1.3.2 Definitions of Analytes

Polychlorinated alkanes (general sum formula $C_nH_{2n+2-m}Cl_m$) are complex mixtures of several thousand compounds. As such, there is no official definition or definite CAS numbers available (currently, more than a dozen CAS numbers are active for “chlorinated paraffins”, often describing mixtures of PCAs and other CP constituents) to characterize the analytes or analyte groups.

EFSA categorized PCAs by carbon chain length (SCCPs: C_{10}-C_{13}, MCCPs: C_{14}-C_{17}, LCCPs: $C_{\geq 18}$, [19]), while the chemical description in the Stockholm Convention listing for SCCPs and MCCPs is more specific, i.e. for SCCPs C_{10}-C_{13}, min. 48% Cl [17], for MCCP C_{14}-C_{17}, min. 45% Cl or/and presence of PCA-C_{14}/C_{15} with ≥Cl_5 or PCA-C_{16}/C_{17} with ≥ Cl_6 [17].

Increasing the complexity even further, currently produced CPs often do not adhere to the rough labels of SCCPs, MCCPs and LCCPs. Instead, they are characterized by their overall average chlorination degree [5,33].

In order to harmonise the analytical efforts and clarify reporting, the following analyte / analyte group definitions are proposed in accordance with Fernandes *et al.* 2023 (further details on PCA nomenclature are described in this publication) [4].

General terms for PCAs

Beside the actual analytes or analyte groups, there are also some other frequently used terms for the characterisation of PCAs. This guidance document follows the IUPAC Gold Book as much as possible, with restrictions relevant to the scope. Stereochemistry is not taken into consideration in this document.

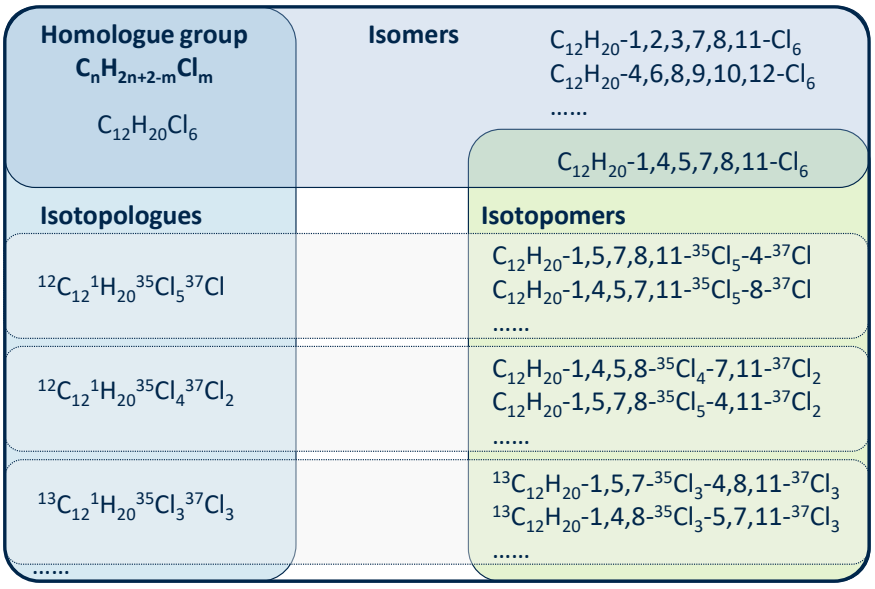


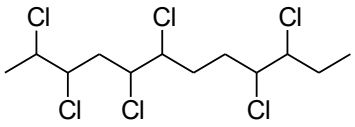
Figure 3 Overview of some general terms regarding PCA nomenclature with examples for the homologue group $C_{12}H_{20}Cl_6$.

Isomer: One of several molecules with the same molecular formula but different structural formulae, e.g. CH_3OCH_3 and CH_3CH_2OH, both with molecular formula C_2H_6O.

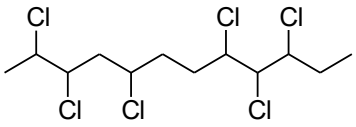
Congener: One of several molecules related to each other by origin, structure, or function.

Polychlorinated alkane (PCA) congener is a molecule with the chemical formula $C_nH_{2n+2-m}Cl_m$, with a defined positioning of Cl atom substitutions on the carbon backbone, e.g. 2,3,5,6,9,10-hexachlorododecane. Congeners can differ in position of Cl substitution, number of Cl atoms and/or carbon chain length.

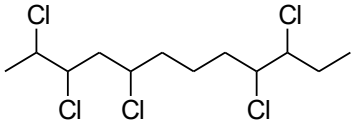
Example



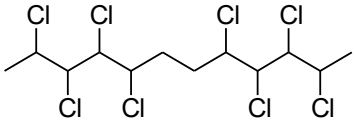
(1): 2,3,5,6,9,10-hexachlorododecane



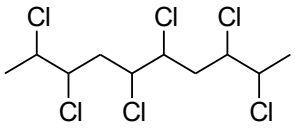
(2): 2,3,5,8,9,10-hexachlorododecane



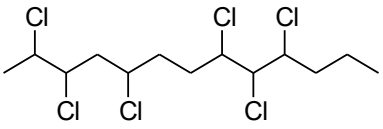
(3): 2,3,5,9,10-pentachlorododecane



(4): 2,3,4,5,8,9,10,11-octachlorododecane



(5): 2,3,5,6,8,9-hexachlorodecane



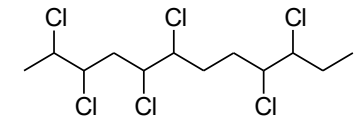
(6): 2,3,5,8,9,10-hexachlorotridecane

Two PCA congeners can belong to the same or to different PCA homologue groups:

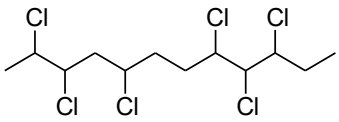
- Congeners (1) and (2) belong to the same $C_{12}Cl_6$ homologue group (they are isomers).
- Congeners (1), (3), (4), (5) and (6) belong to different homologue groups (they are not isomers).

PCA homologue group is a group of PCA configurational isomers with the same carbon chain length and the same number of chlorine atoms, e.g. the $C_{12}Cl_6$ homologue group comprises all PCA congeners with the molecular formula $C_{12}H_{20}Cl_6$; sometimes shortened to "homologue" or called "congener group" in the literature.

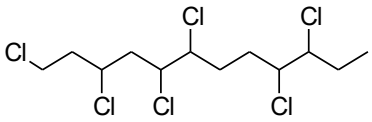
Example



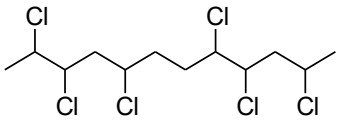
(1): 2,3,5,6,9,10-hexachlorododecane



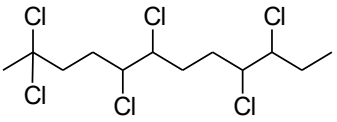
(2): 2,3,5,8,9,10-hexachlorododecane



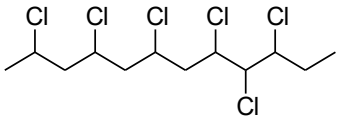
(7): 1,3,5,9,10-hexachlorododecane



(8): 2,3,5,8,9,11-hexachlorododecane



(9): 2,2',5,6,9,10-hexachlorododecane

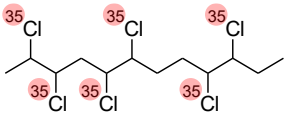


(10): 2,4,6,8,9,10-hexachlorododecane

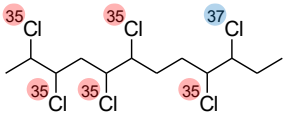
- The $C_{12}Cl_6$ homologue group comprises all PCA congeners with the molecular formula $C_{12}H_{20}Cl_6$. They are not discriminated by mass spectrometry. Their chromatographic separation results in a hump.
- These PCA congeners are isomers.

PCA isotopologues are PCA congeners belonging to the same homologue group (i.e., same molecular formula) that differ only in isotopic composition (number of isotopic substitution), e.g. $^{12}C_{12}{}^{1}H_{20}{}^{35}Cl_6$, $^{12}C_{12}{}^{1}H_{20}{}^{35}Cl_5{}^{37}Cl$ and $^{12}C_{12}{}^{1}H_{20}{}^{35}Cl_4{}^{37}Cl_2$ are different isotopologue groups. They manifest as different peaks in the mass spectrum.

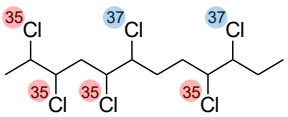
Example of congeners of the $C_{12}Cl_6$ homologue group that belong to the same or different isotopologue group



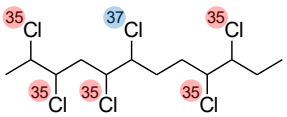
(1.1): $^{12}C_{12}{}^{1}H_{20}{}^{35}Cl_6$
2,3,5,6,9,10-$^{35}Cl_6$-dodecane



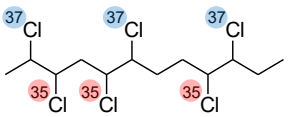
(1.2): $^{12}C_{12}{}^{1}H_{20}{}^{35}Cl_5{}^{37}Cl$
2,3,5,6,9-$^{35}Cl_5$,10-^{37}Cl-dodecane



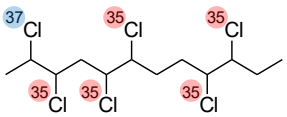
(1.3): $^{12}C_{12}{}^{1}H_{20}{}^{35}Cl_4{}^{37}Cl_2$
2,3,5,9-$^{35}Cl_4$,6,10-$^{37}Cl_2$-dodecane



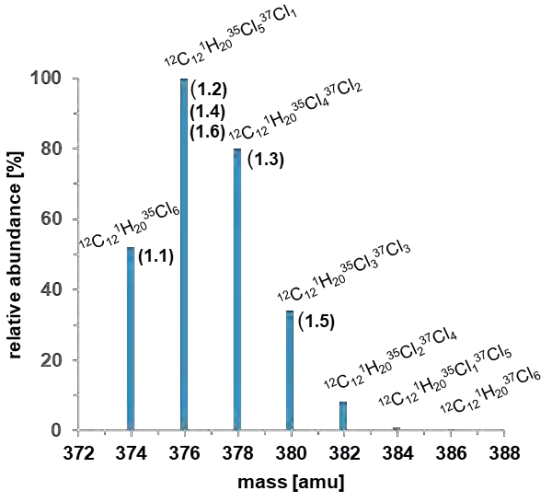
(1.4): $^{12}C_{12}{}^{1}H_{20}{}^{35}Cl_5{}^{37}Cl$
2,3,5,9,10-$^{35}Cl_5$,6-^{37}Cl-dodecane



(1.5): $^{12}C_{12}{}^{1}H_{20}{}^{35}Cl_3{}^{37}Cl_3$
3,5,9-$^{35}Cl_3$,2,6,10-$^{37}Cl_3$-dodecane



(1.6): $^{12}C_{12}{}^{1}H_{20}{}^{35}Cl_5{}^{37}Cl$
3,5,6,9,10-$^{35}Cl_5$,2-^{37}Cl-dodecane



- When considering the ^{12}C isotope for C and the ^{1}H isotope for H only, the PCA configurational isomer/congener ***1,3,5,6,9,10-hexachlorododecane*** (1) is composed of ***7 isotopologues*** arising from ^{35}Cl and ^{37}Cl isotope substitutions. Their

isotopic compositions are $^{12}C_{12}{}^{1}H_{20}{}^{35}Cl_6$, $^{12}C_{12}{}^{1}H_{20}{}^{35}Cl_5{}^{37}Cl$, $^{12}C_{12}{}^{1}H_{20}{}^{35}Cl_4{}^{37}Cl_2$, $^{12}C_{12}{}^{1}H_{20}{}^{35}Cl_3{}^{37}Cl_3$, $^{12}C_{12}{}^{1}H_{20}{}^{35}Cl_2{}^{37}Cl_4$, $^{12}C_{12}{}^{1}H_{20}{}^{35}Cl{}^{37}Cl_5$, $^{12}C_{12}{}^{1}H_{20}{}^{37}Cl_6$.

- The simulation of the isotope cluster of this homologue ($^{12}C_{12}{}^{1}H_{20}Cl_6$) shows 7 peaks (corresponding to the 7 isotopologues) with differing relative abundance.
  - Congeners (1.1), (1.2), (1.3) and (1.5) belong to different isotopologues of the PCA congener (1).
  - Congeners (1.2), (1.4) and (1.6) have the same isotopic formula $^{12}C_{12}{}^{1}H_{20}{}^{35}Cl_5{}^{37}Cl$ and thus belong to the same isotopologue of the PCA congener (1).

PCA isotopomers are configurational isomers with the same number of each isotopic atom (i.e., same isotopic composition) differing only in the position of isotopes (e.g. chlorine substitution), e.g. 2,3,5,6,9-$^{35}Cl_5$,10-^{37}Cl-dodecane and 2,3,5,9,10-$^{35}Cl_5$,-6-^{37}Cl-dodecane. They are not discriminated by mass spectrometry.

1.3.3 PCA groups

Sum parameters should always be reported with the range of carbon chain length covered. Conventionally used groups include:

- ***Sum of SC-PCAs (ΣPCAs-C_{10-13})***
  Calculated as sum of PCAs with carbon chain length C_{10}-C_{13} based on at least the sum of all corresponding Cl_5-Cl_9 congeners (five most abundant homologue groups for GC and LC instruments, more if possible).
- ***Sum of MC-PCAs (ΣPCAs-C_{14-17})***
  Calculated as sum of PCAs with carbon chain length C_{14}-C_{17} based on at least the sum of all corresponding Cl_6-Cl_{10} congeners (five most abundant homologue groups for GC and LC instruments, more if possible).
- ***Sum of LC-PCAs (ΣPCAs-C_{18-n})***
  Calculated as sum of PCAs with carbon chain length C_{18}-C_n (n>18), upper chain length included should be specified as index. Due to the wide range of compounds included in this parameter and different availability of standards, no further characterisation is given at this time.
- ***Chain length specific results (ΣPCA-C_n)***
  Sum parameter for PCAs of a certain carbon chain length. The same minimum homologue groups and criteria for positive compound identification as for the corresponding group parameters apply. Example: ΣPCA-C_{14} – sum of all PCAs with 14 carbon atoms, including at least congeners with six to ten chlorine atoms.
- ***Sum of all measured PCAs (ΣPCAs-C_{a-z})***
  Sum concentration of all PCAs detected in a sample, specified with the covered carbon chain length. Depending on the instrument used, this parameter may include LC-PCAs. For GC-based methods, this is commonly defined as sum of ΣPCAs-C_{10}-C_{17}. For LC-based methods, also PCAs with longer carbon chain lengths can be determined. Thus, when reporting a sum parameter for PCAs the range of chain length covered by the method should be specified as index.

1.4 Quality control and quality assurance

1.4.1 Recommendations for Laboratories

- Laboratories should be accredited by a recognised body operating in accordance with EN ISO/IEC 17011 [34] to ensure that they are applying analytical quality assurance. The methods and scope of the laboratory in general should be accredited according to the EN ISO/IEC 17025 standard [35].
- Laboratories should demonstrate proficiency in the analysis of PCAs at the concentration range of interest (range of thresholds of Monitoring Recommendation) by validation, ongoing internal quality control and continuous successful participation in inter-laboratory studies conducted by accredited bodies, e.g. the EURL POPs.

1.4.2 General aspects regarding sample pre-treatment and storage

- Where required, laboratory samples should be thoroughly mixed by fine grinding, blending, etc. using a process that has been demonstrated to achieve complete homogenisation (e.g. ground to pass a 1 mm sieve for animal feed); samples may have to be dried before grinding if the moisture content is too high (the moisture content may optionally be determined for animal feed).
- The sample quantity used for the extraction should be sufficient to fulfil the requirements with respect to a sufficiently low working range including the concentrations at the suggested LOQs.
- The specific sample pre-treatment procedures used for the products under consideration shall follow European Regulations [36,37].
- Appropriate measures should be taken to ensure that the sample composition is representative of the consumed food or animal feed; e.g., removal of bones. In the case of fish, the skin has to be removed, as the contaminant content will relate to muscle meat without skin. However, it is necessary that all remaining muscle meat and fat tissue on the inner side of the skin is carefully and completely scraped off from the skin and added to the sample to be analysed.
- Storage of samples in appropriate containers to avoid contamination

Note: The equipment and material used for sample pre-treatment, homogenisation and analysis, and storage should be verified regarding their contribution to blank levels. More detailed information regarding blank management is described in chapter 2.4.

1.4.3 Identification and quantification criteria

Monitoring methods (using GC-EI-MS/MS) should identify the PCA hump by comparison to the calibration standards with regard to retention time range, visual match (check for visible interfering peaks within the hump), and the use of the three required mass transitions for GC-EI-MS/MS analysis (*m/z* 102 → 67, *m/z* 102 → 65 and *m/z* 91 → 53; see **Annex I** for more information). Note: Due to different PCA patterns between calibration standard and samples the appearance and substructures of the hump may differ. For positive identification of PCAs the substructures of the hump should be clearly visible on all three transitions.

Quantification criteria have been derived from the outcome of an ILS within the CWG CP primarily for the monitoring method.

Internal standard (IS) recovery

The internal standard recovery, determined relative to the syringe standard, represents the primary decision tool for the reliability and usability of a sample analysis. **Do not correct monitoring results for internal standard recovery.**

The following criteria describe the recovery range and recommended actions:

- IS recovery < 30%: repeat sample preparation
- 30% ≤ IS recovery < 50%: consider repeat of sample preparation
- The upper end of the acceptable recovery range is still to be defined

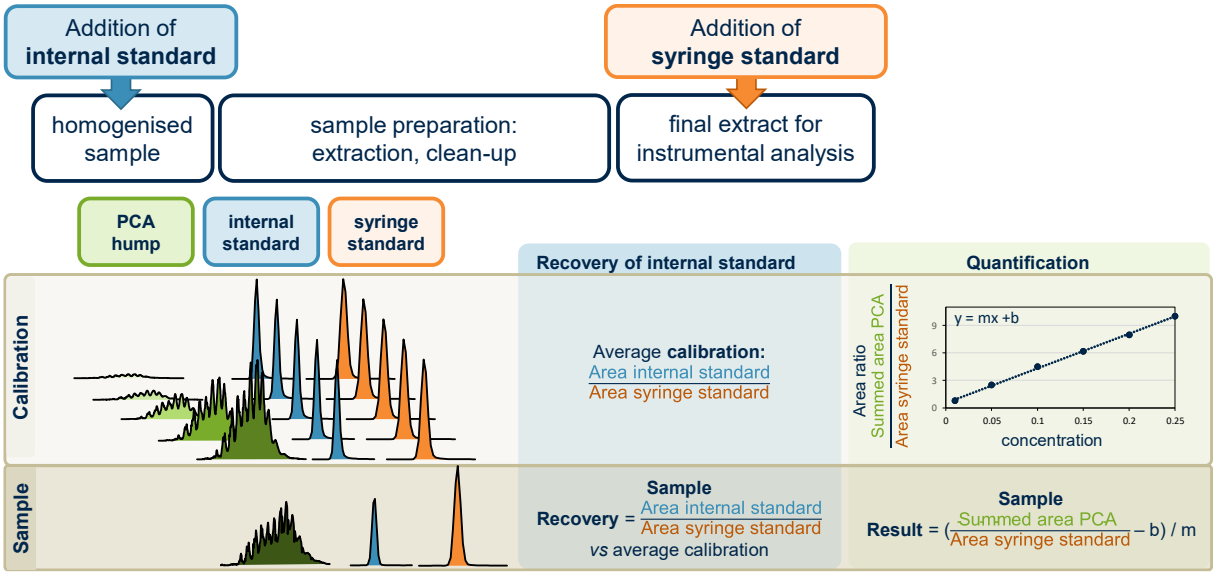


Figure 4 Overview on addition and use of internal and syringe standard for PCA analysis using the quantitative GC-EI-MS/MS screening method for monitoring of ΣPCAs-$C_{10\text{-}17}$.

Note: Currently, results acquired using the GC-EI-MS/MS monitoring method should not be corrected for internal standard recovery. Instead, for quantification, the area of the PCA hump should be normalized on the syringe standard added prior to instrumental analysis. The recovery of the internal standard added at the beginning of sample preparation relative to the syringe standard is used as quality control criterion. Low recoveries might be caused by divergent elution from the Florisil® column of different batches during clean-up which is an essential part of sample preparation for the monitoring method.

LOQ (lowest validated level) should be

- ≤ ½ threshold level per matrix(group) on [wet weight] basis
- ≤ ⅓ threshold level per matrix(group) on [fat] basis

For the purpose of monitoring, laboratories should strive to establish LOQs as low as possible.

For characterisation methods peak identification criteria similar to other contaminants shall be applied to the extracted mass humps.

The identification of a PCA homologue group peak can be achieved by the following parameters (as shown in **Figure 5** from [38]):

- For HRMS accurate mass of the quantifier and two qualifier ions (the three most abundant isotopologues or adducts of the homologue group) should be matched within ± 5 ppm
- Ratio between quantifier and qualifier ion should be within ± 30% of the theoretical value
- Retention time (range) of quantifier and qualifier should match the calibration

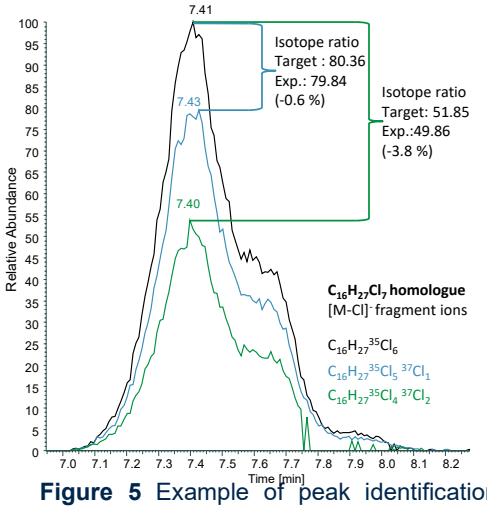


Figure 5 Example of peak identification criteria demonstrated on XIC (quantifier and qualifier) for $C_{16}H_{27}Cl_7$ (i.e., PCA-$C_{16}Cl_7$) (GC-HRMS). Figure from [36].

While the above-mentioned criteria for IS recovery can also be applied to characterisation methods, the criteria for LOQs can only be applied to monitoring methods where a threshold has been assigned to the matrix group.

Please refer to **chapter 1.4.4** for details on how to determine LOQs. Other performance criteria such as repeatability/reproducibility and trueness remain under discussion pending further data from comparative studies and QC samples.

1.4.4 Estimation of LOD/LOQ

For the different methods of estimation of LOD or LOQ, please refer to the Joint Guidance Documents of the four Contaminant EURLs *"Guidance Document on the Estimation of LOD and LOQ for Measurements in the Field of Contaminants in Feed and Food"* [39] and *"Guidance Document on the Estimation of LOQ for Measurements in the Field of Contaminants in Feed and Food"* [40]. The application of the lowest validated level approach is recommended.

For *monitoring methods*, LOQs should be established in relation to the thresholds defined in the Monitoring Recommendation (**Table 2**) to ensure reliable monitoring results (see **chapter 1.4.3**). If no threshold is defined for the matrix category, then fortification experiments in matrix should be carried out to determine a target working range before validating the LOQ. For the purpose of monitoring, laboratories should strive to establish LOQs as low as possible.

This also applies to all characterisation methods.

1.4.5 Measurement uncertainty

Measurement Uncertainty (MU) for PCAs can be estimated using the empirical or semi-empirical approach described in the *EURL Guidance Document on measurement uncertainty for Laboratories performing PCDD/F and PCB analysis using isotope dilution mass spectrometry* [41].

Although improvements in comparability of results should always be the goal, a target measurement uncertainty of ±50% seems prudent at this time. However, this value will be reviewed based on analytical improvements and cumulative data from interlaboratory exercises.

1.4.6 Validation

Prior to routine analysis of PCAs in food and feed samples, the performance of the method should be demonstrated during a validation procedure. Below are some points of attention for designing an appropriate validation study (**Table 1**).

Table 1 Recommendations and points of attention for validation of PCA analysis.

Grouping of matrices	▪ Use of a single food or feed matrix to represent a matrix group if the matrices in the group share similar physicochemical properties ▪ Examples of a matrix group: meat, offal, fish, etc. ▪ Further sub-grouping might be necessary for analytes and/or methods if significant matrix effects are observed.
Blanks	▪ Sufficient blank management should be demonstrated by including procedural blank analysis ▪ Stable procedural blank levels should be taken into account when determining the method LOQ, i.e. LOQs should not be lower than the average procedural blank levels.
Selectivity of the analytical procedure	▪ Analytical methods should demonstrate the ability to reliably and consistently separate the analytes of interest from other co-extracted and possibly interfering compounds that may be present. ▪ **Monitoring method:** Due to the lack of chromatographic separation of the analytes itself, the method should demonstrate that the presence of interferants co-eluting with the PCA hump are not significantly influencing the result (e.g., effect on result is within measurement uncertainty)
Analytical working range	▪ **Monitoring method:** Analytical methods should demonstrate the ability to reliably and consistently determine PCAs in the range of the thresholds and above (see **Table 2**).
Trueness	▪ The analytical method used must provide a valid estimate of the true concentration in a sample. ▪ Trueness can be estimated from fortification experiments, regular analysis of certified reference materials, or successful participation in inter-laboratory studies.

Precision	▪ Precision can be calculated from results generated under repeatability or within-laboratory reproducibility conditions (e.g. regular analysis of quality control samples).
Limit of quantification	▪ **Monitoring Method:** LOQs for PCA sum parameters should be established in relation to the thresholds using the lowest validated level approach (see **chapter 1.4.3 and 1.4.4**)

Target values and ranges for performance criteria such as repeatability/reproducibility and trueness, including measurement uncertainty, remain under discussion pending further data from comparative studies and QC samples.

1.4.7 Continuous QC measures

To ensure consistent performance and quality of analytical results, certain continuous quality control or quality assurance measures should be followed:

- Regular participation in interlaboratory study or proficiency test schemes (EURL, Quasimeme, …) or co-analysis of samples with at least one other laboratory if no such test is available
- Regular check of blank levels (ideally with each sample batch)
- Use of PT/ILS material as QC samples, ideally with each batch
- Use of certified reference material (CRM), where applicable
- Especially in the case of LC-based methods, new matrices should be checked for ion suppression or enhancement effects, e.g. by using standard addition.

1.5 Reporting

Results should be reported in ng/g wet weight or ng/g fat for food, with additional report of the sample fat content and, if available, average PCA chlorine content. For feed samples, results should be reported in µg/kg product or µg/kg (12% moisture content) with additional report of the moisture content, where applicable. In all cases, results should be given with two significant digits.
For sum parameters, the covered chain length should be specified, e.g. ΣPCAs-C_{10-17}. If possible, results defined by PCA carbon chain length are preferred. Analytes should only be reported as specific as they have been validated for the quantification method in use.

1.5.1 Reporting of occurrence data for PCA sum parameter (Monitoring)

The *Commission Recommendation (EU) 2026/2335 on monitoring the presence of chlorinated paraffins in food* requests the analysis of a unified sum parameter: the sum of short and medium-chain PCAs, i.e. ΣPCAs-C_{10-17}, in comparison to a threshold value (without consideration of MU) (**Figure 6**). The specified thresholds (see also **Table 2**) serve as trigger for a more thorough characterisation of a monitoring sample: within the EU official food control network, such samples should be communicated to EURL POPs, who then advises on an expert laboratory that will do the characterisation analysis. The EURL POPs should also be informed if the determined level is very high (i.e. > 50 ng/g wet weight or 300 ng/g fat) and no threshold is defined for the analysed sample matrix. The full procedure is described in detail in the sample distribution scheme document.



Figure 6 Workflow of analysis, reporting and further sample distribution for characterisation analysis in the context of the *Commission Recommendation (EU) 2026/2335 on monitoring the presence of chlorinated paraffins in food.*

Reporting of occurrence data (i.e. the sum parameter ΣPCAs-C_{10-17}) acquired in the context of the *Commission Recommendation (EU) 2026/2335 on monitoring the presence of chlorinated paraffins in food* and beyond should include:

- A brief description of the used methodology
- Fat content of the sample (For feed samples moisture content)
- Concentration of sum parameter ΣPCAs-C_{10-17} in the same unit as the threshold value. If no threshold value is defined for the category, report in ng/g wet weight.
  Note: Feed is not specifically included in this recommendation, but reporting units as stated at the beginning of **chapter 1.5** should be followed.
- LOQ (in particular in case of concentrations below LOQ)
- Recovery and identity of internal standard
- (Expanded) measurement uncertainty

Table 2 Threshold values for the sum parameter ΣPCAs-C_{10-17} as trigger for further characterisation analysis in accordance with the *Commission Recommendation (EU) 2026/2335 on monitoring the presence of chlorinated paraffins in food*.

Food category	Threshold for ΣPCAs-C_{10-17}
Fish	100 ng/g wet weight
Milk and dairy products	20 ng/g wet weight
Oils and fats	1000 ng/g fat
Other products of animal origin (e.g. meat, eggs, …)	300 ng/g fat
Processed foods (whole meals)	50 ng/g wet weight
Babyfood*	20 ng/g wet weight

* In the context of the *Commission Recommendation (EU) on monitoring the presence of chlorinated paraffins in food* the threshold for the category "babyfood" is recommended to be applied to all kinds of food intended for infants and young children including infant formula.

1.5.2 Recommendations for reporting results of PCA characterisation analysis

Reports should include:

- A brief description of the used methodology
  - Specification of the analytical instrument: type of chromatography, ionisation, mass analyser
  - For LC methods: mobile phase and modifier
  - Type of ions/adducts used for quantification
  - For HRMS methods: mass resolution and mass tolerance
  - Calibration standards used
  - Data treatment and quantification strategy
  - Further method-specific parameters if applicable such as limitation of quantification range (e.g., GC method or standard availability), goodness of fit R^2 (deconvolution method)
- Fat content of the sample (For feed samples moisture content)
- Concentration of sum parameters (e.g., ΣPCAs-C_{10-13}; ΣPCAs-C_{14-17}; ΣPCAs-C_{10-17})
- Chain length-specific PCA concentrations (ΣPCAs-C_i)
- Chain length specific PCA responses (relative PCA homologue group pattern)
- Occurrence/quantification of PCAs-$C_{>17}$ in the sample
- (Method) limit of quantification and how it was determined
- Recovery of internal standard and information on internal and syringe standard
- *Optionally, expanded measurement uncertainty and how it was determined*
- *Optionally, a comment regarding matrix effects for the matrices reported*

When PCA homologue group patterns are reported, a brief description how response patterns were obtained and standardised should be given as part of the data treatment strategy specifications. Additionally, laboratories are encouraged to report the homologue group patterns of the LGC / Dr. Ehrenstorfer mixed standards given in **Table 3** alongside their samples for harmonisation purposes.

Table 3 Mixed PCA standards recommended for PCA homologue group pattern harmonisation with article number from Dr. Ehrenstorfer / LGC (Augsburg, Germany).

Mixed PCA standards: "Chloroparaffin …"		
C_{10}-C_{13} 51.5%Cl *DRE-X23105100CY*	C_{14}-C_{17} 42%Cl *DRE-X23144200CY*	C_{18}-C_{20} 36%Cl *DRE-X23183600CY*
C_{10}-C_{13} 55.5%Cl *DRE-X23105500CY*	C_{14}-C_{17} 52%Cl *DRE-X23145200CY*	C_{18}-C_{20} 49%Cl *DRE-X23184900CY*
C_{10}-C_{13} 63%Cl *DRE-X23106300CY*	C_{14}-C_{17} 57%Cl *DRE-X23145700CY*	

1.5.3 Reporting to EFSA

Reporting of all monitoring results (below or above the threshold), as well as of results of characterisation analysis to EFSA should be done in accordance with the requirements of EFSA using the respective parameter codes (**Table 4**) and considering information from the annual calls for continuous collection of chemical contaminants occurrence data in food and feed of EFSA [42].

Table 4 Compound descriptions and parameter codes for reporting PCA results to EFSA, as found in the 2026 EFSA parameter catalogue. Please check the current parameter code for updates or additional compounds; https://www.efsa.europa.eu/en/calls/data.

PARAM Codes for PCA sum parameters			
Compound description	PARAMcode	Compound description	PARAMcode
ΣPCAs-C_{10-17}	RF-00015701-PAR	**ΣPCAs-C_{10-13}**	RF-00015724-PAR
Alkanes, C_{18-28}, chloro	RF-00004800-PAR	**ΣPCAs-C_{14-17}**	RF-00015725-PAR
PARAM Codes for chain length specific sums			
Compound description	PARAMcode	Compound description	PARAMcode
ΣPCAs-C_9	RF-00015702-PAR	**ΣPCAs-C_{20}**	RF-00015713-PAR
ΣPCAs-C_{10}	RF-00015703-PAR	**ΣPCAs-C_{21}**	RF-00015714-PAR
ΣPCAs-C_{11}	RF-00015704-PAR	**ΣPCAs-C_{22}**	RF-00015715-PAR
ΣPCAs-C_{12}	RF-00015705-PAR	**ΣPCAs-C_{23}**	RF-00015716-PAR
ΣPCAs-C_{13}	RF-00015706-PAR	**ΣPCAs-C_{24}**	RF-00015717-PAR
ΣPCAs-C_{14}	RF-00015707-PAR	**ΣPCAs-C_{25}**	RF-00015718-PAR
ΣPCAs-C_{15}	RF-00015708-PAR	**ΣPCAs-C_{26}**	RF-00015719-PAR
ΣPCAs-C_{16}	RF-00015709-PAR	**ΣPCAs-C_{27}**	RF-00015720-PAR
ΣPCAs-C_{17}	RF-00015710-PAR	**ΣPCAs-C_{28}**	RF-00015721-PAR
ΣPCAs-C_{18}	RF-00015711-PAR	**ΣPCAs-C_{29}**	RF-00015722-PAR
ΣPCAs-C_{19}	RF-00015712-PAR	**ΣPCAs-C_{30}**	RF-00015723-PAR

2 General analytical methodologies

2.1 Considerations on choosing a suitable method

The choice of instrumentation should take into account the overall aim of the methodology, i.e. characterisation analysis or screening/monitoring of a sum parameter. Determination of homologue group patterns requires the use of soft ionisation for mass spectrometric detection. Additionally, the use of high-resolution mass spectrometry is beneficial with regard to isobaric overlaps and interferences. Coverage of LC-PCAs with chain length ≥ C_{18} requires the use of LC-based methods (**Figure 7**).

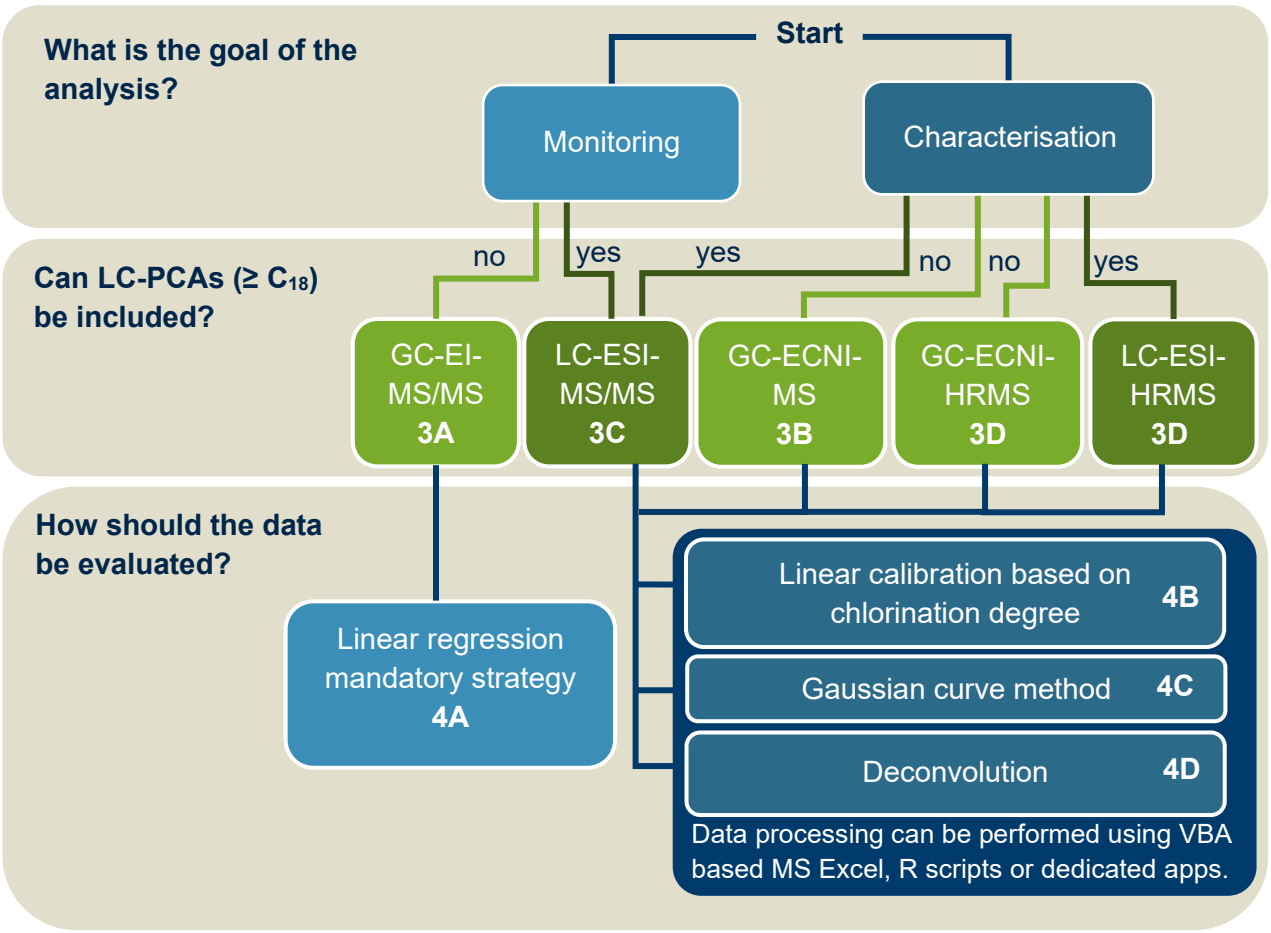


Figure 7 Decision tree on choosing a suitable method for PCA analysis. Depending on the overall aim of the methodology, i.e. characterisation *vs.* monitoring (sum parameter) soft ionisation is needed. Use of high-resolution mass spectrometric systems allows more accurate determination of the pattern. If LC-PCAs should be covered by the method LC-based instrumental analysis is needed. For PCAs up to a chain length of C_{17}, GC-based analysis is sufficient.

2.2 Choice of Instrumentation

The different instrumental methodologies applied for the measurement of PCAs present different characteristics in terms of advantages and limitations that should be considered (summarized in **Table 5**).

Table 5 Overview of different instrumental methods for PCA analysis applied in the EURL/NRL network and further explained in the corresponding modules, with advantages and limitations.

Method	Quan level	Advantages	Limitations	Module
GC-EI-MS/MS	ΣPCAs-C_{10-17}	• Response is influenced neither by chlorination degree nor by the chain length • Equipment available in most laboratories	• High degree of fragmentation • No information on homologue group pattern attainable • Interferences with other organo-halogenated compounds with same retention time range (e.g. PCBs) requires Florisil® clean-up	3A
GC-ECNI-LRMS	ΣPCAs-C_{10-17}, ΣPCAs-C_{10-13}, ΣPCAs- C_{14-17}, ΣPCA-C_n	• Equipment available in most laboratories	• Interferences with other organo-halogenated compounds with same retention time (e.g. PCBs) requires Florisil® clean-up • Response dependent on chlorination degree, low response of low chlorinated PCAs • Interferences between PCA homologue groups (overlapping fragment ions and molecule ions) • LC-PCAs not covered • Rapid contamination of ion source due to reactant gas • Characterisation is time consuming and labour-intensive	3B
GC-ECNI-HRMS	ΣPCAs-C_{10-17}, ΣPCAs-C_{10-13}, ΣPCAs- C_{14-17}, ΣPCA-C_n	• Information on homologue group pattern • NCI / ECNI frequently used in publications → comparable results	• Response dependent on chlorination degree, low response of low chlorinated PCAs • LC-PCAs not covered • Rapid contamination of ion source due to reactant gas	3D
LC-ESI(-)-MS/MS	ΣPCAs-C_{10-17}, ΣPCAs-C_{10-13}, ΣPCAs- C_{14-17}, ΣPCA-C_n	• LC-PCAs detectable • Equipment available in most laboratories • Information on homologue group pattern	• Dopant for ionisation needed • ESI less sensitive towards high chlorinated PCAs • Isobaric interferences: mostly between $C_nH_{2n+2-x}Cl_x$ and $C_{n+5}H_{2(n+5)-x}Cl_x$	3C
LC-ESI(-)-HRMS	ΣPCAs-C_{10-17}, ΣPCAs-C_{10-13}, ΣPCAs- C_{14-17}, ΣPCA-C_n	• LC-PCAs detectable • Low chlorinated PCAs ($n_{Cl} < 5$) detectable	• Dopant for ionisation needed • Possible contamination in Orbitrap analyser • ESI less sensitive towards high chlorinated PCAs	3D

Instrumental methods using APCI are also described in the literature. However, such methods are not applied in the EURL/NRL network and therefore not described in this guidance document.

The analytical methods described in this document either separate analytes in a gaseous or liquid phase. Depending on the choice of separation method, the resulting PCA peak humps show very different characteristics in chromatography:

Independent of the GC instrument set-up used, chromatographic separation of PCAs by **gas chromatography** leads to a hump signal which is further characterised by multiple shoulders, as shown in **Figure 8 A** and **B**. This is in contrast to humps observed in mineral oil analysis (MOSH/MOAH) which are smoother in their appearance (**Figure 8 C**). Although the presence of other analytes or matrix signals might conceal the PCA hump in a TIC, the extracted mass traces should reveal this or a similar shape (**Figure 8 A** and **B**). Even chain-length specific mixture standards or extracted homologue group mass traces will show at least two, more often three of these substructures (**Figure 8 B**). Depending on contamination source or sample matrix, the shape and/or intensity of the sub-structures may vary; nevertheless, they will always be present and clearly defined. The peak shoulders do not represent a homologue or chain length group. Thus, separation by segmenting the TIC based on retention time is not applicable.

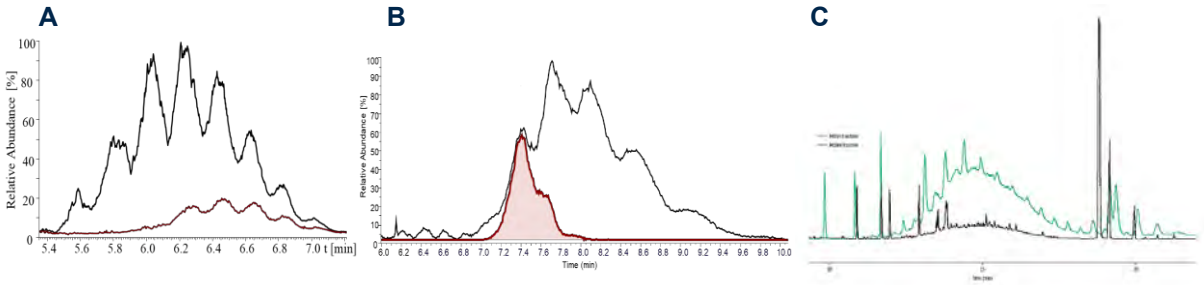


Figure 8 Chromatography of PCAs (hump) in comparison to mineral oil analysis by GC. **(A)** Total ion current chromatogram of a SCCP (black) and MCCP (red) standard analysed by GC-ECNI-HRMS. **(B)** GC-ECNI-HRMS chromatogram of a mixture of SCCPs and MCCPs. Overlaid and filled in red is a corresponding extracted homologue peak hump (here: $C_{16}H_{27}Cl_7$). **(C)** MOSH (green) and MOAH (black) compounds on Rxi-5Sil MS and Hydroguard-treated MXT with Allure Silica (Online LC/GC Coupling). Source: https://ez.restek.com/images/cgram/gc_fs0500.pdf

GC-ECNI-(HR)MS coupling is often used to analyse and quantify PCAs with predominant formation of $[M\text{-}Cl]^-$ and $[M\text{-}HCl]^-$ adducts depending on the overall chlorination degree and ion source temperature [43,44]. Certain GC and ECNI parameters can influence the response of PCAs. Detailed information on the influence and optimization of GC parameters (including column characteristics, oven, GC inlet) as well as the MS source temperature for simultaneous analysis of PCAs with chain-lengths ranging from 10 up to 20 carbon atoms (PCAs-$C_{10\text{-}20}$) are described by Amoura et al [45]. In general instrument response using ECNI is dependent on the overall chlorination degree and blind towards low chlorinated compounds ($Cl_{<5}$).

Liquid chromatography does not yield hump shapes similar to those observed in GC, but rather comparatively smoother humps like those obtained in MOSH/MOAH analysis (**Figure 9 A**, **Figure 8 C**). However, similarly to analysis by GC, the sub-structures of the humps do not translate into single chain length PCA standards or extracted mass traces of homologue groups which may appear, depending on signal smoothing, similar to GC chromatograms (**Figure 9 B**).

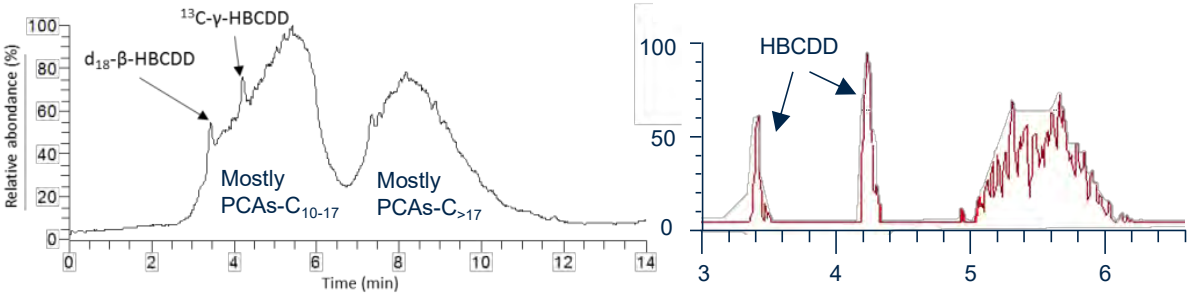


Figure 9 (A) TIC of LC-analysis of a mixture of SCCPs, MCCPs and LCCPs. The latter appears as a second, separate hump with the gradient used in this method. No substructures are observed here. **(B)** The same shape is found in extracted homologue group chromatograms (here: $C_{11}H_{11}Cl_{13}$). Figures kindly provided by M. Mézière, LABERCA, France.

Only LC-based methods are able to detect and quantify LC-PCAs with chain lengths > C_{20} (and higher chlorinated PCA homologue groups with chain length ≥ C_{18}), which are less volatile and therefore not suitable for analysis with GC-based systems.

Efficient ionisation of PCAs using ESI requires adduct formation and several types of ions can be formed. Deprotonated $[M-H]^-$ species provide poor sensitivity for low-chlorinated PCAs, thus additives added to the mobile phase or post-column are commonly applied to promote the formation of $[M+Cl]^-$, $[M+Br]^-$ or $[M+Ac]^-$ adducts thereby enhancing sensitivity [46]. The resulting mass spectra can be very complex due to overlaps between homologue groups from isotopic clusters (distribution of ^{35}Cl and ^{37}Cl) and also from parallel formation of different adduct ion species (**Figure 10**) [47]. While with the unit resolution of LRMS or MS/MS analysis, such overlaps cannot be distinguished, HRMS with resolution ≥ 60,000 allows separation of these complex patterns [27,43].

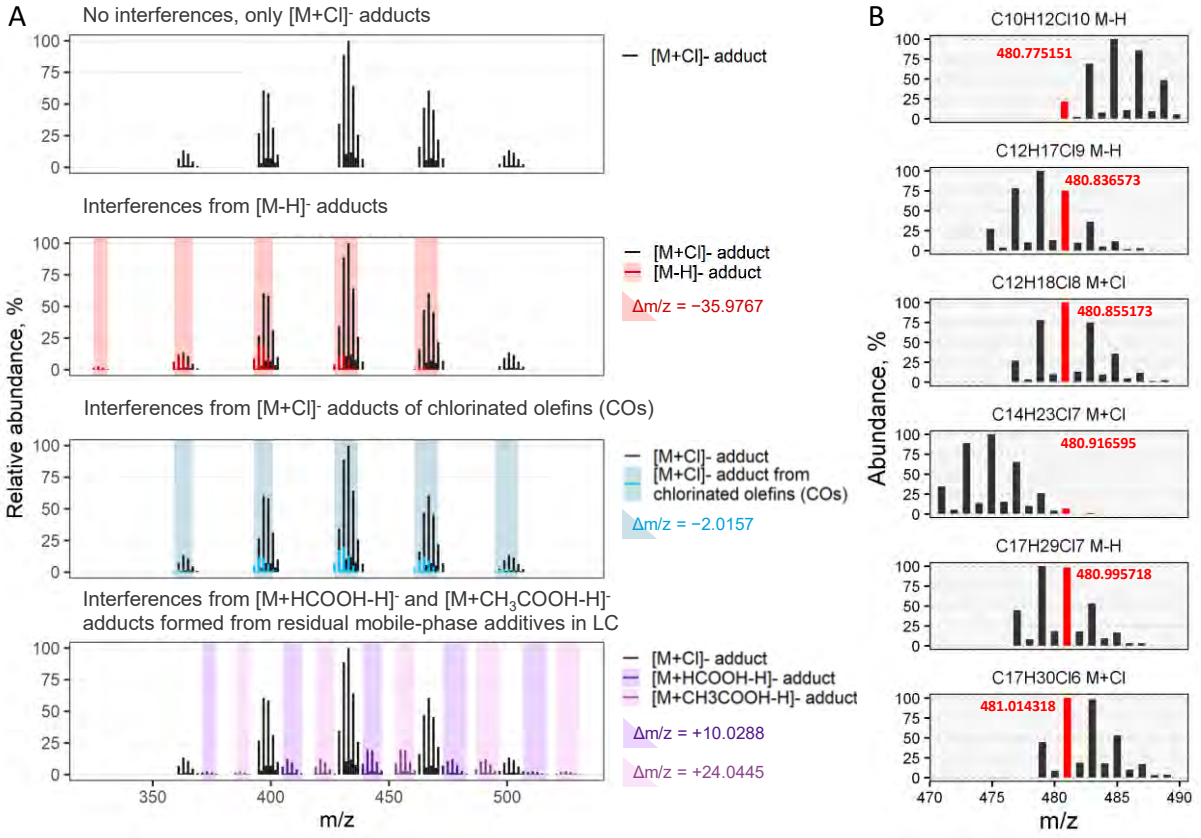


Figure 10 (A) Simulated mass spectra of $[M+Cl]^-$-adduct ions of PCAs-$C_{11}Cl_{5\text{-}9}$ and possible interferences by different adducts and chlorinated olefins. **(B)** Mass spectra of isotopic clusters of different PCA homologues and adduct ions with overlap between *m/z* 480.7-481.1 [47].
Figure adapted based on the original version from Lazdiņa and Pērkons 2026, Environ Sci and Pollut Res https://doi.org/10.1007/s11356-026-38341-3 *licensed under CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/).*

The choice of the mobile phase (i.e. organic modifier methanol *vs.* acetonitrile) and the selection of the stationary phase can impact homologue group pattern by LC-ESI-HRMS analysis. It has been shown, that with post-column addition of dichloromethane, use of a MeOH/H_2O mobile phase is more appropriate for the higher chlorinated PCAs. However, using ACN/H_2O led to fewer ion species, with almost exclusively $[M+Cl]^-$-adducts being formed. Regarding the choice of the stationary phase, separation on a C30 column better highlighted the short-chain PCAs compared to the C18 column conventionally used [48].

Note: The ion adduct used for quantification should be chosen based on the system, dopant, and mobile phase to obtain optimum sensitivity and selectivity. Depending on the different ion species (e.g., $[M-H]^-$, $[M+Cl]^-$, $[M+Ac]^-$) ionisation efficiency is different between different homologue groups and is also affected by the instrument (e.g., ion-source geometry) and method setup. Thus, PCA homologue group patterns obtained using different adduct ions and method setups will not match each other [29,47–50].

2.3 Analytical standards

2.3.1 Native standards for quantification

For the choice of native standards for quantification the overall aim of the methodology, i.e. screening/monitoring of a sum parameter or characterisation analysis should be considered.

For **monitoring** of the sum parameter ΣPCAs-C_{10-17} by GC-EI-MS/MS the use of the mixture standards Chloroparaffin C_{10}-C_{13} 55.5% Cl and Chloroparaffin C_{14}-C_{17} 57% Cl (Dr. Ehrenstorfer, Augsburg, Germany) is recommended. More detailed information on quantification is described in **Annex I**.

In general, single chain PCA standards have proven suitable for more accurate quantification of PCAs using different quantification strategies when information beyond screening level is to be achieved, i.e. **characterisation analysis**. However, due to the currently fast changing market on PCA standards and the different requirements of each quantification method, no definite recommendation for quantification standards for characterisation methods can be given here. Instead, some general remarks are provided to help in selecting an appropriate set of standards:

- **(Technical) mixture standards** can be used for sum parameters ΣPCAs-C_{10-13}, ΣPCAs-C_{14-17}, ΣPCAs-C_{18-20} or monitoring analysis. Additional information on chain length specific concentrations of such mixed standards are not always available, but would be helpful as some commercially available LC-PCA standards also contain other PCAs with shorter chain length, making ΣPCAs-C_{18-20} results calculated based on their total response unreliable [51]. The required quantification standards for the recommended GC-EI-MS/MS monitoring method (Chloroparaffin C_{10}-C_{13} 55.5% Cl, Chloroparaffin C_{14}-C_{17} 57% Cl, Dr. Ehrenstorfer, Augsburg, Germany) fall under this category. These standards are not recommended for characterisation analysis.
- Use of **a wide range of chlorination degrees and chain length standards** appears to provide better results. Quantification based only on one single chain standard cannot objectively reflect PCA composition in the sample and is thus not recommended. Generally, more accurate quantification results are obtained if the PCA homologue patterns of sample and quantification standard(s) are similar.
- **Multiple single-chain PCA mixture standards** can be used for all quantification methods, allowing a metrological traceable quantification on chain length level (lowest comparable quantification level to date). Currently, single chain PCA standards spanning C_{10}-C_{24} in several chlorination degrees are commercially available from LGC/Dr. Ehrenstorfer and Chiron (Trondheim, Norway).
- A number of **configurationally defined individual PCA congener standards** are available from Chiron but the evidence suggests that at least the standards from the first generation (with multiple terminal chlorines) are unsuitable without major adjustments of the methods due to poor instrument response in both LC and GC methods [52]. Apart from individual research requirements, these are currently not recommended. More suitable standards were developed as part of the Eurostars "Chloffin" project and are available from Chiron.

2.3.2 Syringe standard

The choice of syringe standards in publications varies greatly [27,29], including isotope-labelled and unlabelled pesticides (e.g., ε-hexachlorocyclohexane (ε-HCH) [53,54]), *trans*-chlordane, PCB congeners, PBDE congeners/derivatives [54], d_{18}-β-HBCDD [50] and $^{13}C_{10}$-1,5,5,6,6,10-hexachlorodecane.

For the GC-EI-MS/MS monitoring method, the use of ε-HCH as syringe standard is required and should be used for normalization of the PCA and internal standard areas (**chapter 1.4.3**).

2.3.3 Internal Standard

Note: Depending on carbon chain length, chlorination degree and chlorine substitution pattern the physicochemical properties of polychlorinated alkanes can vary [55]. Thus, choice of isotope-labelled PCA standards used as internal standard for recovery correction can impact results and should be considered during method development.

$^{13}C_{10}$-1,5,5,6,6,10-hexachlorodecane (Cambridge Isotope Laboratories, Tewksbury, MA, USA) is one of the very few available, fully labelled single compound PCA standards and therefore considered suitable for recovery control for GC methods. However, this standard is not in all cases detectable using LC-ESI-MS or LC-APCI-MS [49,55]. A further available $^{13}C_{12}$-labelled PCA standard, i.e. 1,1,1,3,10,12,12,12-octachlorododecane, gives only poor signal in GC- or LC-applications. More recently, several partially carbon-labelled ($^{13}C_3$-) standards have been made commercially available, covering several carbon chain lengths between C_{10} and C_{21} (Chiron, Trondheim, Norway). This partial labelling has proven sufficient to separate the target mass from native PCA signals for both GC- and LC-based methods [56].

Schinkel *et al* [55] proposed some minimum requirements for internal standards, later expanded by Fernandes *et al [52]*:

- The standard should be isotope labelled (e.g. ^{13}C, deuterated,…)
- As PCAs are assumed to be mostly linear, the standard should also be based on a linear alkane
- Alternatively, the standard should at least be of aliphatic nature in order to ensure properties similar to PCAs during clean-up/sample preparation
- The standard should ideally be chlorinated to ensure similar behaviour to the analytes during sample preparation
- The chlorine atoms should be evenly distributed along the carbon chain, as standards with mostly terminal chlorination show low or no response on LC and GC

For the GC-EI-MS/MS monitoring method, the internal standard is only used for determination of internal standard recovery (relative to the syringe standard) which represents the primary decision tool for the reliability and usability of a sample analysis (**chapter 1.4.3**). Recovery correction is not recommended.

Note: Further information on available commercial standards, their advantages and disadvantages, and connected analytical conundrums when trying to quantify PCAs are discussed in a separate peer-reviewed publication written by members of the CWG CP [52].

2.4 Blank level management

2.4.1 General aspects to avoid contamination / high blank levels

Since CPs are in use in a variety of applications including as plasticisers in soft polymers, seals or computer components, it is vital to check the laboratory background and blank levels before sample preparation and analysis begins. Solvents, reagents and particularly equipment and automated systems should be checked during method development to ensure that the background is not significant. Consumables should also be tested for contribution to blanks. Known examples are vial caps and plastic pipette tips (if multi-pipettes are used where glass tips are not available). It is helpful to replace most plastic components and consumables in the laboratory with either PTFE, glass or metal alternatives and pre-rinse all glassware.

General aspects that should be considered:

- Measures must be taken to avoid cross-contamination at each stage of the sampling and analysis procedure in the laboratory.
- Glassware should be rinsed with solvents and/or heated at temperatures suitable to remove traces of PCAs from exposed surfaces and stored under conditions preventing re-contamination (e.g. cover open parts of glassware with aluminium foil, **Figure 11**).
- Reagents, glassware and other equipment used for analysis and sampling should be controlled to avoid possible introduction or loss of PCAs.
- All laboratory instrumentation (e.g., blenders, freeze dryers, evaporation or automated extraction systems) need to be checked for their blank contribution when setting up the method. Generally, older materials with high throughput of solvents present lower PCA blank levels than newer versions (due to washing out over time).
- Procedural blank analysis should be performed in each sample sequence by carrying out the entire analytical procedure omitting only the sample. The levels in the procedural blanks should be regularly monitored once the method is established, as blank levels might change over time and maintained within an acceptable range.

2.4.2 Pretreatment of consumables and glass ware

Solvents can be checked directly for contamination. In general, pesticide-grade or analysis-grade solvents have proven to be suitable for PCA analysis.

Persisting contamination issues might also be resolved by heating sorbents and pre-extracting filters and glass wool before use as exemplarily described in **Table 6**.

Table 6: Description of exemplary pre-treatment strategies for solvents, chemicals, consumables and glassware for PCA analysis.

	Description of pre-treatment
Solvents (Dichloromethane and *n*-hexane)	Can be used as bought (check each batch for contamination), recycling: pre-distillation through rotary evaporators to remove fat or glass wool residues is recommended before proper distillation
Sorbents (silica gel, Florisil®, sodium sulphate)	Heating in an oven at 600 °C (12 h), and then holding 130 °C for 6 h. Fill the heated sorbents while still at 130 °C into glass bottles for storage.

Glass wool, filters	Extraction with dichloromethane (3x 8 h, fresh solvent each day), then drying in a dust reduced environment
Glassware, spatulas	Heating at 435 °C overnight, and store covered with aluminium foil to keep dust out. Directly before use, rinse glassware with *n*-heptane.

Glassware might be treated by soaking, washing and heating, followed by storage with coverage of all open surfaces with aluminium foil and rinsing with solvent prior to use to minimize introduction of blanks due to dust. An exemplary treatment cycle for laboratory glassware is illustrated in **Figure 12**.

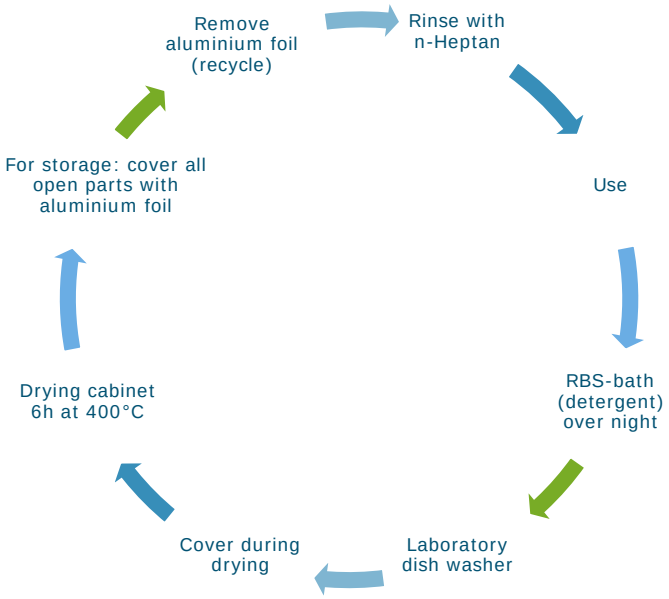


Figure 12 Circular treatment of glass ware used for PCA analysis to minimize contribution of blank levels.



Figure 11 Storage of glassware sealed with aluminium foil to keep out dust.

2.4.3 Precautions to avoid blank contribution from laboratory equipment

Besides consumables also laboratory equipment should be tested for contribution to sample contamination/blanks.

In particular, **freeze-drying** has been shown to introduce large amounts of SC/MC-PCAs (old instruments) or even LC-PCAs (especially new instruments) to the samples (**Figure 13**).

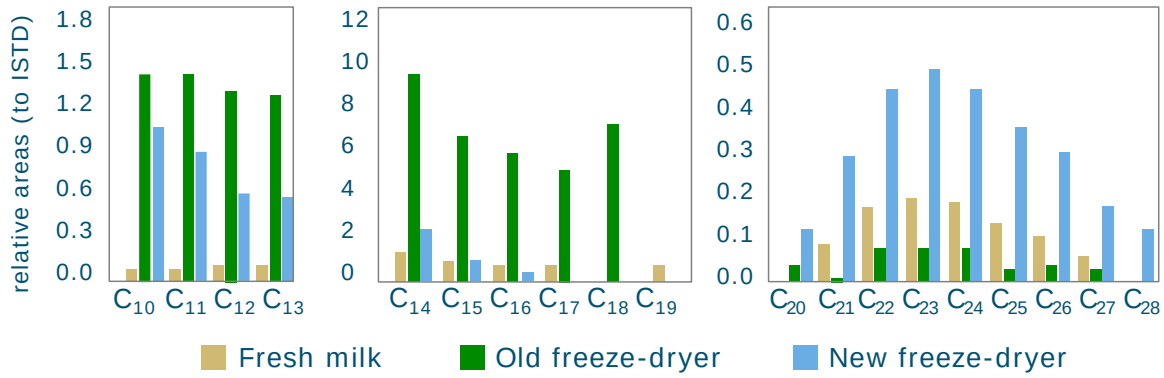


Figure 13 PCA homologue groups summed by carbon chain length found in milk without drying, drying in an old freeze dryer and drying in a newer freeze dryer. Data kindly provided by M. Mézière, LABERCA-Oniris, France.

Another known source of PCAs are **blenders**, which could be used for initial sample homogenisation. Commercial hand blenders for household use may "bleed" PCAs into the medium which is mixed [57]. This can be illustrated by the distribution of PCAs in the range C_{10}-C_{15} found in eggs homogenised with a new blender which shows strong similarities to an extract of the blender, suggesting this as source of the sample contamination (**Figure 14**).

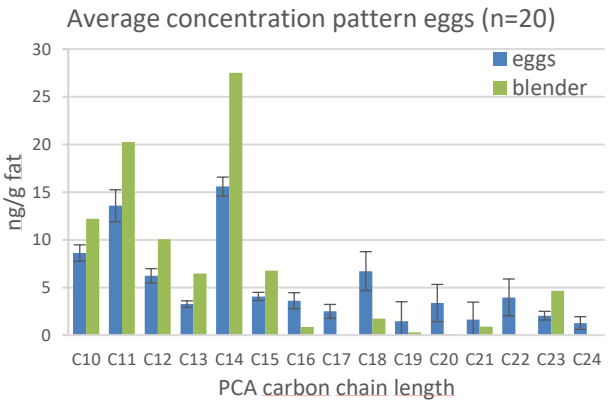


Figure 14 Average PCA carbon chain length specific concentration pattern found in 20 egg samples and a blank extract homogenized with the blender prior to analysis. Data and graphic provided by K. Krätschmer, WFSR, The Netherlands.

3 Analytical methods

This chapter gives examples for methods that are used within the EURL/NRL network for PCA analysis. Since all methods follow the same base principle (**Figure 15**), the methods are presented in a modular form. The three modules for sample preparation cover lipid extraction (**Module 1**), sample clean-up (**Module 2**) and measurement (**Module 3**). Due to their complexity particularly for characterisation analysis, the data processing/treatment and quantification strategies are discussed in a separate module (**Module 4**).

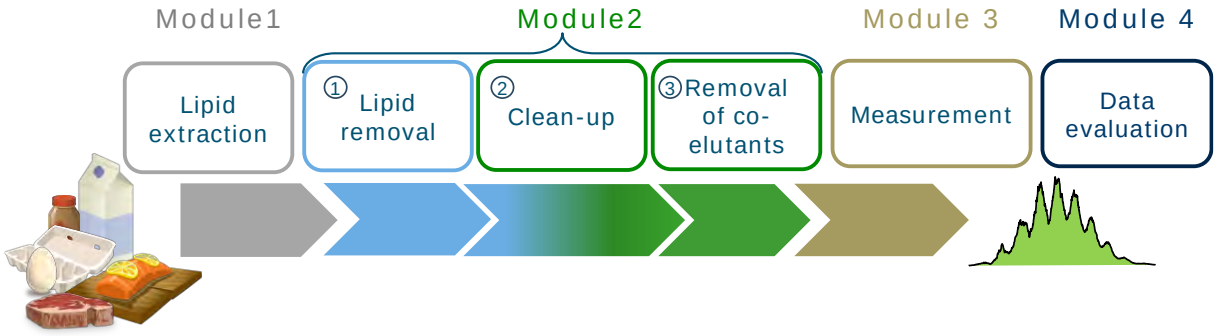


Figure 15 Schematic workflow of the base procedures of PCA analysis methods.

3.1 Module 1: Sample pre-treatment and extraction

Reagents and equipment that are used for PCA analysis are similar to those used for PCB and PCDD/F analysis and are commercially available. Different methods for sample preparation of food or feed samples are described in the literature. Basic schematics of the procedures used by participants of the first and second round of the EURL interlaboratory study on PCAs (2017 and 2018, Coconut fat and Lard) [29] barely differ from the methods summarized in a more recent literature review [27]. **Figure 16** gives an overview of different extraction methods that are applied for food and feed matrices in literature. The overlaps between the methods and matrices indicate that the choice of extraction modules is not considered to be a major influencing factor for PCA analysis.

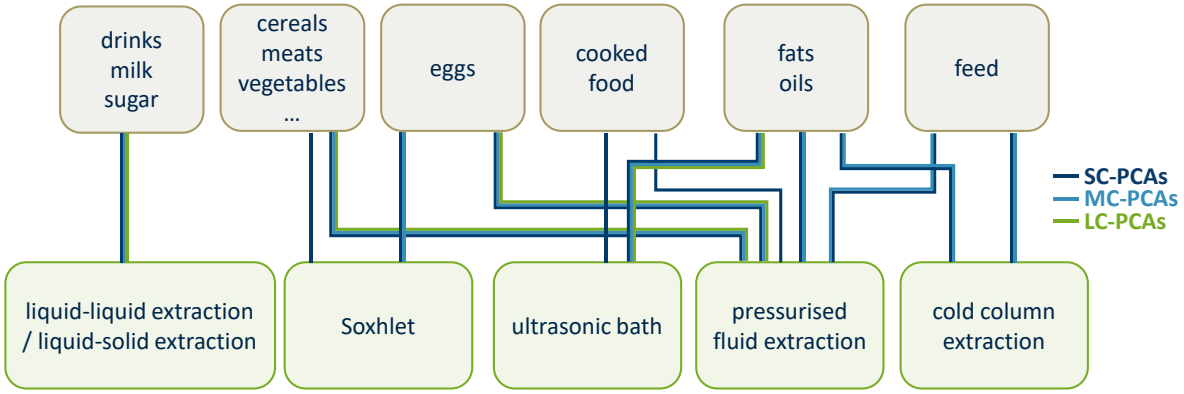


Figure 16 Schematic overview of sample extraction techniques for PCA analysis used for food and feed described in literature. Covered chain length described here for the matrices and methods are not exhaustive. For detailed information on the extraction method refer to the sections below or the review from Yuan *et al.* 2019 [25].

Note: Other extraction methods not presented in this module but commonly used for extraction of other halogenated POPs (e.g. PCDD/Fs and PCBs) such as extraction using heated solvents (e.g. Soxhlet or Twisselmann extraction) can also be applied for the extraction of PCAs from feed or food matrices. However, all methods, especially automated sample extraction should be verified regarding their impact on procedural blank levels and therefore also on samples.

Module 1A: Cold extraction

Cold extraction is a direct solid-liquid extraction method that relies on mechanical treatment of the dried sample to facilitate interaction with the extraction solvent. Advantage of this method is that almost all needed tools and containers can be replaced with glass or metal, which facilitates blank level management.

Example

10-20 g homogenised sample or an amount corresponding to approx. 1.5 g fat is weighed into a 150 mL beaker. Using a glass syringe, 100 µL internal standard is added to the sample. It is then mixed with anhydrous sodium sulphate using a glass stirring rod until the mixture is dry and flaky. The extraction solvent (DCM/*n*-hexane (1:1, v/v) or ethylacetate) is added to the beaker until there is approx. 1 cm supernatant, and then the mixture is strongly stirred for two minutes. After some resting time, the supernatant is decanted through a folded filter containing one spoon of sodium sulphate into a 250 mL round bottom flask. The extraction process is repeated at least two times. Alternatively, the mixture can be agitated for 30 min with an overhead-shaker and centrifuged (3600 rpm) to gain the supernatant.



Frozen samples

Dried samples with solvent and supernatant

Filtered sample extracts

Figure 17 Practical steps of an example workflow for cold extraction.

Module 1B: Extraction procedure using high-performance dispersion extraction

High-performance dispersion extraction works on the same principle as cold extraction (Module 1A). The extraction efficiency is enhanced due to the applied high shear forces.

Example

An aliquot of the sample corresponding to approx. 1.5 g fat for further clean-up is weighed and the internal standard is added. The sample is dried with a drying agent, e.g. Na_2SO_4. For high-performance dispersion extraction (using e.g. Ultra-Turrax®) hexane and dichloromethane (1:1, *v:v*) are added to the homogenised sample. Immiscible liquid/liquid mixtures or raw powders can also be homogenised, dispersed and extracted by high-speed stirring. After the sample is set the organic solvent is decanted over a filter containing a spoon of Na_2SO_4 in 250 mL round bottom flask. Fresh solvent mixture is added to the remaining sample and the

sample is extracted twice again. The combined organic extracts are subjected to further purification.



Figure 18 Egg sample during and after high-performance dispersion extraction.

Module 1C: Glass column extraction

Glass column extraction is a variant of cold extraction where the extraction solvent is penetrating the raw sample material filled in a column thereby dissolving and eluting the analytes of interest from the matrix.

Example

For samples with a fat content below 30 %, or when the fat content is unknown, up to 50 g of sample are weighed and mixed with anhydrous sodium sulphate until a free-flowing powder is obtained. Prior to extraction, 100 µL of internal standard are added. The sample is transferred onto a glass column packed with a plug of cotton wool and approximately 2 g of sea sand. The glass vessel is rinsed with approximately 100 mL cyclohexane/dichloromethane (1:1, v/v), and the solvent added onto the column for quantitative transfer. The analytes are extracted with a further 150 mL of the solvent mixture and eluted into a 500 mL round-bottom flask at a flow rate of approximately one drop per second. After complete elution, the column is dried under a gentle stream of nitrogen. The extract is concentrated to approximately 10 mL using a rotary evaporator.

For samples with a high fat content (≥ 30%; including fats and oils) extraction and clean-up can be combined in one step. Samples are therefore mixed with anhydrous sodium sulphate to obtain a free-flowing powder, spiked with 100 µL internal standard and can be directly transferred onto an acidic silica gel clean-up column and eluted using 150 ml extraction solvent.

The amount of the acidic silica and consequently the capacity of the column may need to be increased if the sample is directly transferred onto the column (**Module 2B, Note**).

Module 1D: Microwave extraction

Microwave-assisted extraction (MAE) is an automated extraction technique that allows shorter extraction times and reduced solvent consumption. During MAE, microwaves passing through the solvent rapidly heat the extraction mixture by direct interaction with the free water molecules present in the sample. This results in a drastic increase in the internal temperature (superheating), causing liquid vaporization within the cells. Rupture of the cell wall facilitates the release of fat and compounds of interest into the solvent.

Example: Extraction procedure using ETHOS X (Milestone)

Before extraction, the sample should be mixed with a drying agent (e.g. polyacrylate or sodium sulphate). The extraction solvents are similar to those used for cold extraction. An example of extraction conditions is summarized in **Table 7**.

Table 7 Example of extraction conditions for MAE using the ETHOS X system (Milestone)

Parameters	Applied conditions
Solvents	toluene/acetone (70/30, v/v)
Solvent volume	30 mL
Weight of sample	2-5 g
Temperature	110 °C
Static time	40 min
Cooling temperature	20 min

Module 1E: Pressurised liquid extraction

Hot pressurized extraction uses high temperature and high pressure, which decreases the extraction time and the quantity of solvent used. The high temperature increases solubility and diffusion and therefore enhances the extraction efficiency of the analytes of interest. The high pressure is used to keep the solvent in a liquid state as the temperature is increased above its boiling point, resulting in a higher solubility and higher diffusion rate of lipid solutes into the solvent, and higher penetration of the solvent into the matrix compared to other less drastic methods.

Example: Extraction procedure (ASE 350 Thermo Scientific)

The method is suitable for fatty fish, shellfish and fatty meat. Before extraction, the sample is mixed with a drying agent (e.g. polyacrylate or sodium sulphate). The dried sample is packed in the extraction cell. The extraction solvents are similar to those used for cold extraction. An example of extraction conditions is summarized in **Table 8**. The extract is filtered through sodium sulphate and the solvent is evaporated.

Table 8 Example of extraction conditions for pressurised liquid extraction using the ASE 350 system (Thermo Scientific).

Parameters	Applied conditions ASE 350 Thermo Scientific
Solvents	n-hexane/acetone (50/50, v/v)
Cell volume	34 mL
Weight of sample	10 g
Temperature	100 °C
Pressure	1500 PSI
Preheat time	0 min
Heat time	5 min
Static time	5 min
Flush	100 %
Cycles	3
Purge	120 sec

3.2 Module 2: Clean-up

The clean-up of extracts for PCA analysis can be divided into three steps: (i) lipid removal, (ii) general clean-up, and (iii) removal of co-eluting interferences. Depending on the type of instrumentation used for the analysis not all of these clean-up steps need to be applied. Matrix affects instrumental analysis differently: For GC-based methods, matrix effects occur mainly in the chromatographical system, while with LC-based methods matrix compounds mostly affect ionization.

The GC-EI-MS/MS monitoring method in particular, needs very pure sample extracts. Recommended combinations for the different steps for clean-up are given at the end of each example module description in this chapter.

It should be noted that HRMS methods independent of the type of chromatography are in most cases able to separate PCAs from (matrix) interferants, though too many interferences can also negatively impact method measurement reliability (e.g., through ion-suppression or overfilling the orbitrap). Such matrix overload is identifiable by missing chunks of extracted PCA homologue humps, or negative impressions of peaks within the humps (i.e., breakdown within the PCA hump).

Note: The described methodologies are usually applied for PCAs with a chain length of C_{10}-C_{17}. The covered range of chain lengths should be verified during validation. Should PCAs longer than C_{24} be targeted in the analysis, checking the elution for recovery of the longer chained homologue groups is recommended.

Good to know: Examples for material preparation

The consumables and sorbents used in most of the examples of this Module 2 need some preparation to lower the blank levels in the final sample extract (see also **chapter 2.4**) and for proper separation.

Florisil® is a syntethic amorphous magnesium silicate. It is possible that the quality and characteristics may differ notably between material batches.

Information on preparation of solvents and sorbents, consumables (filter, glass wool) and glassware are described in **chapter 2.4.2**. The following preparation protocols are examples on how to prepare sorbents needed for sample clean-up.

Silica gel/H_2SO_4	Mixture with 44% sulfuric acid: add for example 17.6 g (=9.6 mL) sulfuric acid to 22.4 g heated silica gel and shake immediately for approx. 5 min. The mixture is then shaken overnight in an overhead shaking device.
Florisil®/H_2O	Mixture with 1.5% water: add for example 3.0 g (=3.0 mL) deionised water to 197 g previously heated Florisil® and shake immediately for approx. 5 min. The mixture is then shaken overnight in an overhead shaking device. Elution behaviour of PCA from the Florisil® column should be checked regularly (e.g. for each batch). Based on experiences within the EURL/NRL network elution of $^{13}C_{10}$-1,5,5,6,6,10-Hexachlorodecane seems to be a suitable indicator of deviating elution behaviour.

Module 2A: Lipid removal - Direct acid treatment

Direct acid treatment is less work intensive than elution over acidified silica, but lacks the chromatographic retention action of an acid silica column.

To 10 mL sample extract (in *n*-hexane) (from Module 1) containing the lipid phase of the sample in a 25 mL tube, 5 mL concentrated sulphuric acid are added and the mixture is gently shaken. Then, the tube is agitated in an ultrasonic bath for one hour, before letting it rest overnight. The next day, the upper (hexane) layer is transferred to a 20 mL volumetric flask. The remaining sulphuric acid phase is extracted again with 8 mL *n*-hexane under gentle shaking and the hexane phases are combined and adjusted to 20 mL before further clean-up.

Note: For LRMS or MS/MS methods, further clean-up of this extract (at least **Module 2D**) is required before analysis to remove co-eluting organo-halogenated contaminants which might interfere during instrumental analysis! An additional general clean-up with a silica column (**Module 2C**) is recommended.

Module 2B: Lipid removal / clean-up - Acidic silica column

The sample extract from Module 1 is concentrated, e.g. using rotary evaporation until either pure oil or no more than approx. 1.5 mL solvent/fat mixture is left. For lipid removal, a glass column (no tap or glass/Teflon tap) is filled with glass wool, sodium sulphate, silica gel/H_2SO_4 (44%) and sodium sulphate again according to **Figure 19**. Since the silica gel mixture is added in suspension to the column, all further steps are to be performed without letting the column run dry to avoid cracks in the packaging.

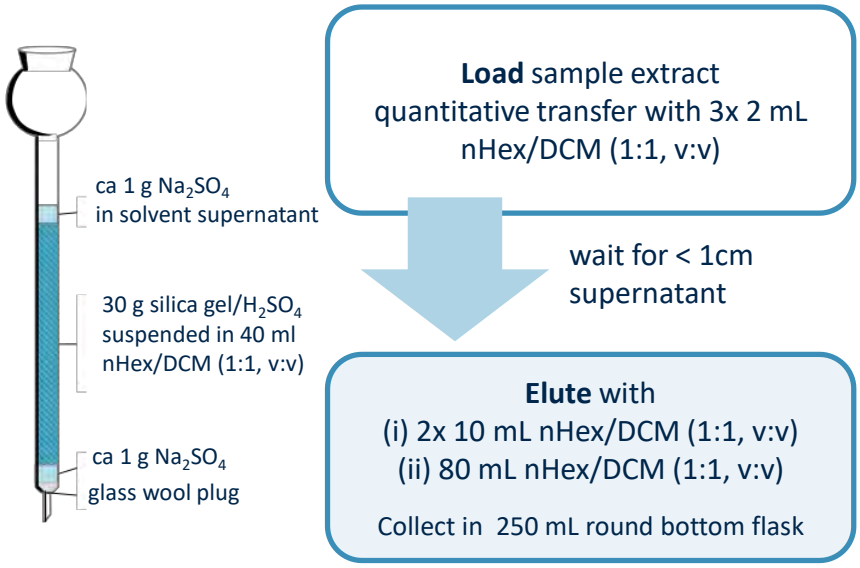


Figure 19 Example column set-up and workflow for acidic silica column clean-up according to [36]. Column dimensions: length: 48 cm, diameter 2 cm, inner diameter of outlet 0.4 cm.

The sample extract is added to the column with a glass pipette, while the round bottom flask is washed three times with 2 mL DCM/n-hexane (1:1, *v:v*) for a quantitative transfer. When there is less than 1 cm supernatant on the column, another 250 mL round bottom flask is placed below the column. Then 2x 10 mL and 80 mL DCM/*n*-hexane are added in sequence without letting the column run dry. After the last batch of DCM/n-hexane, the column can run dry. This column is able to handle up to 1.5 g lipid on-column comfortably.

Note: It is possible to drastically reduce the amount of acidic silica gel needed per sample, if an acid digestion with concentrated sulfuric acid followed by extraction with *n*-hexane is done beforehand (**Module 2A**). If the extraction is combined with acidic silica clean-up (**Module 1C**) the amount of acidic silica and consequently the capacity of the column may need to be increased. Generally, the amount of adsorbent used in any hand-packed column needs to be optimised for diameter of the column and of the column outlet.

Module 2C: General clean-up - Neutral silica column

For further clean-up after lipid removal (**Module 2A**), a short 30 mL glass column is filled dry with glass wool, 3 g silica deactivated with 30% water and 1 g sodium sulphate. The 20 mL hexane sample extract is added to the column, let soaked in, and then eluted with 60 mL hexane. If LC-PCAs (>C_{17}) are targeted the sample is eluted with 80 mL of *n*-hexane:dichloromethane (90:10, v/v).

Note: This extract is sufficiently clean for GC- or LC-HRMS analysis. With GC-ECNI-LRMS this extract may show interferences of organo-halogenated compounds which should be considered during evaluation. For monitoring by GC-EI-MS/MS this extract needs further Florisil® clean-up.

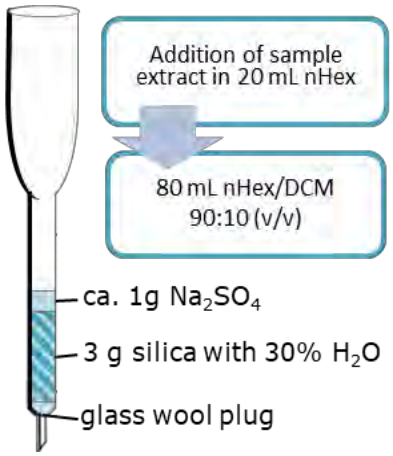


Figure 20 Example column set-up and workflow of neutral silica column.

Module 2D: Removal of co-eluting contaminants - Florisil® column

After reducing the extract of the silica column (**Module 2B** or **Module 2C**) to 1.5 mL using, e.g. rotary evaporation, the samples can be additionally cleaned using a glass column filled with glass wool, sodium sulphate, and Florisil®/H_2O according to **Figure 21**. All components of this column can be packed dry, so that light shaking or knocking of the column is necessary to ensure a tight packing of the sorbents.

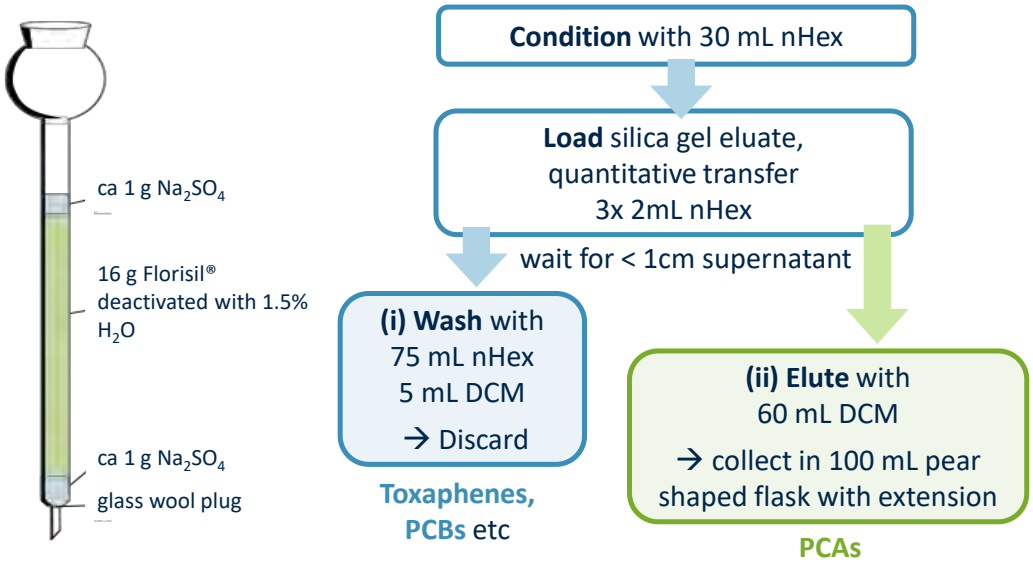


Figure 21 Example column setup and workflow for Florisil® column clean-up according to [36]. Column dimensions: length: 30 cm, diameter 1.5 cm, inner diameter of outlet 0.4 cm.

After pre-conditioning the column with 30 mL *n*-hexane, the sample extract is added (again with 3x 2 mL of the same solvent used to ensure quantitative transfer). The first fraction is eluted with 75 mL *n*-hexane followed by 5 mL dichloromethane and contains PCBs, toxaphenes and similar compounds. It is discarded. The second fraction is eluted with 60 mL dichloromethane into 100 mL pear shaped flasks with an extension. This fraction is evaporated almost to dryness using first the rotary evaporator and then a gentle nitrogen stream. Finally, 100 µL of the injection standard (in cyclohexane) is added to the last drop of the extract, quickly mixed and transferred to a vial for measurement.

Note: Florisil® is a synthetic amorphous magnesium silicate. It is possible that the quality and characteristics may differ notably between material batches. Also, preparation of the Florisil® (activation by heating and adjustment of water content) may have an impact on fractionation during clean-up (see **chapter 2.4.2** and introduction at the beginning of **Module 2**).

Elution behaviour of PCAs from the Florisil® column should be regularly checked (e.g., for each batch). Based on experiences within the EURL/NRL network elution of $^{13}C_{10}$-1,5,5,6,6,10-hexachlorodecane seems to be a suitable indicator of deviating elution behaviour. If the standard recovery is low (i.e. < 30%, **chapter 1.4.3**) it is recommended to check the waste fraction and if needed to adjust the elution volume.

Module 2E: Automated clean-up

There are currently no automated clean-up methods in use within the EURL/NRL network.

3.3 Module 3: Measurement

The choice of the measurement method should consider the overall aim of the analytical approach, i.e. monitoring/screening *vs.* characterisation analysis (see also **chapter 1.1 and 2.1/2.2**). While there are many different measurement methods available in the literature, with varying degrees of reported application to food and feed samples, the following modules only describe methods currently established within the EURL/NRL network. This is done to ensure that adequate support can be given to new laboratories getting started with PCA analysis.

Further information on optimising some GC- or LC-parameters for PCA analysis are described by Amoura *et al*. [45,48].

When using **GC-ECNI-based methods**, the state of the ion source is most important: Depending on the quality (purity) of the reaction gas in use, the source can quickly loose sensitivity and need cleaning. The warning sign for a dirty ion source in this set-up is the loss of signal for the lower chlorinated PCA homologues (most commonly, Cl_5- and Cl_6-homologues).

For **LC-based methods**, efficient ionisation of PCAs using ESI requires adduct formation and several types of ions can be formed. Deprotonated $[M\text{-}H]^-$ species provide poor sensitivity for low-chlorinated PCAs, thus enhancement of ionisation through addition of an additive to the mobile phase or post-column is often performed. There are several options for ionisation enhancement described in the literature, meant to reduce formation of different types of ion adducts by promoting favoured formation of distinct adducts. Depending on the applied additive different types of adducts are preferentially formed, e.g., $[M+Cl]^-$, $[M+Br]^-$ or $[M+Ac]^-$ adducts. However, formation of $[M\text{-}H]^-$-species still remains, particularly for highly chlorinated PCAs with shorter chain lengths.

For chlorine enhancement, the post-column addition of DCM or acetonitrile/DCM necessitates an extra valve at the instrument, which might not be available in every laboratory. Alternatively, ammonium chloride or tetramethylammonium chloride can be added directly to the mobile phase resulting in the formation of $[M+Cl]^-$ adducts. Among the members of CWG CP, the addition of ammonium chloride to the solvent showed reliable results comparable to post-column addition of DCM.

Formation of $[M+Ac]^-$ or $[M+Br]^-$ adducts can be favoured by addition of ammonium acetate or ammonium bromide to the mobile phase. Choice of adducts may also influence both sensitivity and fragmentation/selectivity which should be considered particularly when using LC-ESI-MS/MS [46].

Module 3A: GC-EI-MS/MS

Analysis goal:	Monitoring of the overall PCA level
Type of result:	ΣPCAs-C_{10-17}
Range of compounds:	PCAs-C_{17} or shorter, partial response for PCAs-C_{18-20}

The recommended method for monitoring is **gas chromatography electron ionisation tandem mass spectrometry ((GC-)EI-MS/MS)**. Literature describes the use of (5%-Phenyl)-methylpolysiloxane (e.g., DB-5) columns, with a length of 12.5 - 30 m and an ionisation energy of 70 eV [58–60]. Since PCAs cannot be chromatographically resolved, shorter columns are preferred (i.e., ≤ 15 m) to improve sensitivity. The comparatively hard ionisation leads to strong fragmentation, which prevents access to further homologue group or pattern specific information. The target mass transitions describe small, general chlorinated alkane fragments common to all PCAs such as $[C_5H_7Cl]^+$ and its primary non-chlorinated daughter ions $[C_5H_7]^+$ and $[C_5H_5]^+$, i.e. *m/z* 102→67, *m/z* 102→65 and *m/z* 91→53 [43,61,62]. While such fragments can also stem from other chlorinated compounds, tests have shown that sufficiently cleaned samples can be reliably analysed using these transitions to indicate their general PCA contamination.

Example method: GC-EI-MS/MS [43,62]	
Instrument	7890A GC coupled to a 7000B QQQ MS triple quadrupole system (Agilent)
Column	15 m length × 0.25 mm i.d., 0.25 µm film thickness HP5-MSUI fused silica capillary column (Agilent)
GC oven programme	60°C (1 min), increase at 50°C/min to 300°C (5 min); run time:11.8 min Helium flow: 1.2-1.4 ml/min Transfer line temperature: 280°C
Injector	Compressed air cooled PTV injector (Gerstel) in solvent vent mode (4.8 psi until 0.03 min) 5 µL sample is injected at 70 °C (hold 0.13 min), ramp at 720 °C/min to 300 °C (hold 5 min) (extract 100 µL in cyclohexane)
Detection	EI-MS/MS (MRM mode) Source electron energy 70eV, source temperature 280 °C, collision gas: nitrogen
Acquisition	Mass transitions PCAs: *m/z* 102→67 (CE 10 eV), *m/z* 102→65 (CE 20 eV), *m/z* 91→53 (CE 10 eV)

One major advantage of this method is that GC-EI-MS/MS is often used in other routine analyses and therefore likely available in most laboratories that analyse organic food contaminants. Unlike mineral oil hydrocarbon methods, this method does not use window-defining standards to define the range to be integrated for analysis. Instead, the retention time range covered by the calibration standard serves as basis for defining the integration window and the full resulting peak hump should be integrated for quantification. Visual guidance on integrating the PCA hump with examples can be found in **Annex I**. A compilation and complete description of all information and parameters of the screening/monitoring method for the sum parameter ΣPCAs-C_{10-17} can be found in **Annex I**.

Module 3B: GC-ECNI-LRMS

Analysis goal:	Monitoring/characterisation on PCA chain length level
Type of result:	ΣPCAs-C_n
Range of compounds:	PCAs-C_{17} or shorter, partial response for PCAs-C_{18-20}

In order to quantify PCA homologue groups separately, mass spectrometric detection with softer ionisation techniques is required. This can be achieved by **GC** (15-30 m, DB-5) with **negative chemical ionisation/electron capture negative ion low resolution MS (NCI-LRMS, ECNI-LRMS)** [44,54,60,63]. The most abundant $[M\text{-}Cl]^-$ or $[M\text{-}HCl]^-$ signals are used for quantification of PCA groups based on homologue group-specific masses. For coverage of all chosen mass signals, often several GC runs are necessary for the same sample due to the limited number of masses that can be monitored at once. Methane is most commonly used as reaction/moderating gas but ammonia or argon can also be used. The temperature of the ion source can also vary. With LRMS the produced ions between congener groups might overlap due to the same nominal mass, e.g. congener groups with five carbons more and two chlorines less (i.e. $C_{n+5}Cl_{m-2}$) as well as those with two carbons more and one chlorine less (i.e. $C_{n+2}Cl_{m-1}$) [44]. McGrath *et al*. [54] found evidence of strong matrix enhancement for PCAs which also needs to be accounted for during quantification, i.e. through use of suitable syringe standards (see chapter 2.3.3).

Example method GC-ECNI-LRMS [54]	
Instrument	8890 GC coupled to a 7000D triple quadrupole MS (Agilent)
Column	15 m length × 0.25 mm i.d., 0.1 µm film thickness DB5-MS capillary column (Agilent).
GC oven programme	90˚C (1.25 min), increase at 25˚C/min to 180˚C, increase at 10˚C/min to 325˚C, hold 6.5 min; run time: 25.85 min Helium gas flow at 1 mL/min (17.85 min), then 2.5 mL/min (6.5 min) Transfer line temperature: 300 °C
Injector	MultiMode Inlet (Agilent) fitted with 200 µL single taper dimpled inlet liner (Agilent). 2 µL pulsed splitless injections at 92˚C (hold 0.04 min), ramp at 700˚C/min to 300˚C (extract 100 µL in iso-octane)
Detection	ECNI-MS (SIM) ion source temperature: 150˚C, reaction gas: methane.
Acquisition	Monitoring of most abundant (quantification) and second or third most abundant (confirmation) isotopes of $[M\text{-}Cl]^-$ and $[M\text{-}HCl]^-$. Acquisition performed in 4 separate injections with grouping of monitored ions 1) C_{10} and C_{15}, 2) C_{11} and C_{16}, 3) C_{12} and C_{17}, 4) C_{13} and C_{14}. A detailed mass list can be found in Annex II.

Module 3C: LC-ESI-MS/MS

Analysis goal:	Monitoring/characterisation on PCA chain length level
Type of result:	ΣPCAs-C_n
Range of compounds:	only limited by available standards; PCAs-$C_{10\text{-}30+}$ possible

For coverage of PCAs with chain length >C_{17}, **liquid chromatography with electrospray ionisation mass spectrometry (LC-ESI-MS)** is needed. Under typically used ESI conditions efficient ionisation requires adduct formation which is usually achieved by chlorine or acetate enhancement (see general remarks at the beginning of Module 3) [46,48,50,54,64]. The use of MS/MS detection can increase the selectivity and sensitivity in case that no HRMS instrumentation is available, though it was shown that the increased selectivity is only observed for acetate adducts, while chlorine adducts suffer from the same mass interferences also observed with LRMS [46,65].

Example method: LC-ESI(-)-MS/MS [46]	
Instrument	Dionex UltiMate 3000 UHPLC system coupled to a TSQ Altis MS/MS system (Thermo Fisher Scientific)
Column	Kinetex Phenyl-hexyl, 100 mm × 2.1 mm, 2.6 µm (Phenomenex), 35 °C
LC gradient	Water(A) and methanol (B), both containing 5 mM ammonium acetate. Gradient: 50% B (1 min) →75% B (in1 min), 75% B → 95% B (in 11 min, using a concave gradient profile), 95% B → 100% B (in 1 min, hold 7 min), 100% B →50% B, hold until 22 min total run time. Flow rate 0.25 mL/min. Injection volume: 5 µl (final extract: 50 µl methanol)
Detection	Electrospray negative (ESI-), MRM mode, spray voltage 3500 V; sheath gas (N_2) 50 au; auxiliary gas (N_2) 10 au; sweep gas (N_2) 1 au; ion transfer tube temperature 325 °C; vaporizer temperature 350 °C; collision gas pressure 1.5 mTorr.
Acquisition	SRM mode (dwell time 200 ms): two transitions per PCA group, quantification on $[M+Ac]^-$ (**Annex III**). Data analysis in Thermo Scientific Xcalibur 4.7 and in the *R* environment.

In contrast to the GC-EI-MS/MS methodology (**Module 3A**), LC-ESI-MS/MS analysis is able to determine separate PCA homologue groups due to the softer ionisation technique and thus give more specific results than an overall sum parameter. However, restrictions on the number of mass transitions monitored at the same time still apply even with the use of scheduled SRM mode which is particularly relevant for the monitoring of internal standards or syringe standards with a “normal” peak width (approx.10 s) while for PCAs with a peak width of approx. 1 min, enough data points are acquired over the humps when monitoring 4 homologue groups per chain lengths $C_{10\text{-}17}$ (i.e. $C_nCl_{5\text{-}Cl8}$) in parallel [46].

Module 3D: GC- and LC-HRMS analysis

Analysis goal:	Characterisation of PCA homologue group patterns
Type of result:	ΣPCAs-C_n, homologue group pattern
Range of compounds:	PCAs-C_{17} or shorter (GC), no restriction (LC)

Mass spectrometric separation of overlaps between SC-, MC- and LC-PCAs groups needs high (R>20,000 FWHM) or even very high (R>100,000 FWHM) mass resolution, often in the form of Orbitrap [66–68], Iontrap, Fourier Transformation Ion Cyclotron Resonance (FT-ICR) [69] or TOF mass spectrometers for accurate measurement of PCA homologue pattern [27]. Due to the available configurations of these mass detectors, both gas chromatography and liquid chromatography are possible separation techniques. Generally, the literature describes homologue group patterns derived from soft ionisation techniques **(ECNI/NCI or APCI, ESI)** as smaller fragment ions do not allow specific enough conclusions on the corresponding homologue group [29,50,66,70–73].

Example method: GC-ECNI-Orbitrap-HRMS [70]	
Instrument	TRACE 1310 GC system coupled to a Q-Exactive mass spectrometer (Thermo Fisher Scientific)
Column	15 m × 0.25 mm, 0.25 µm HP-5MS UI capillary column connected to 1 m pre-column (Agilent)
GC oven programme	60 °C (2 min), then 50 °C/min to 300 °C (11 min); run time: 17.8 min Helium flow: 1.4 ml/min Transfer line temperature: 280 °C
Injector	PTV injector in splitless mode 1 µL sample is injected at 70 °C (hold 0.1 min) ramp at 12 °C/sec to 300 °C (hold 5 min) (extract 100 µL in cyclohexane)
Detection	Electron capture negative ion (ECNI) ion source temperature: 180 °C, reaction gas: methane
Acquisition	Full scan mode (*m/z* 250-810), R=120,000 (FWHM), AGC target 1E6 Extraction of three most abundant isotopologues of $[M\text{-}Cl]^-$ and $[M\text{-}HCl]^-$ adduct ions for each homologue (**Annex IV**) *via* TraceFinder software (Thermo Fisher Scientific)

Example method: LC-ESI-QTOF-MS [50,64]	
Instrument	Dionex UltiMate 3000 UHPLC system (Thermo Fisher Scientific) coupled to a Compact Q-TOF mass spectrometer (Bruker)
Column	Kinetex C18, 50 mm × 3 mm, 2.6 µm (Phenomenex), 30 °C
LC gradient	Water (A) and acetonitrile (B), Gradient: 70 → 100% in 3 min (hold 11 min), flow rate 0.4 mL/min
Detection	Electrospray (ESI-), 4.5 kV; dry gas flow 10 L/min (200 °C); heater temperature 325 °C. post column addition of ACN/DCM (1:1, *v/v*) at 0.08 mL/min
Acquisition	Full scan mode (*m/z* 100-1500), R=12,000 (FWHM), extraction of three most abundant isotopologues of $[M+Cl]^-$ adduct ions for each homologue (**Annex IV**) via *MZmine 2 (version 2.37)* software [74]

Example method: LC-HESI-Orbitrap-HRMS [66]	
Instrument	Dionex UltiMate 3000 UHPLC system coupled to a Q-Exactive mass spectrometer (Thermo Fisher Scientific)
Column	Hypersil Gold, 100 mm × 2.1 mm, 1.9 µm (Thermo Fisher Scientific), 30 °C
LC gradient	Water (A) and acetonitrile (B), Gradient 70 → 100% in 3 min (hold 11 min), flow rate 0.4 mL/min
Detection	Heated electrospray in negative mode (HESI-), 2.5 kV; Heater: 350 °C, capillary temperature 245 °C post column addition of ACN/DCM (1:1, *v/v*) at 0.08 mL/min
Acquisition	Full scan (*m/z* 300-1500), R=140,000 (FWHM), AGC target 5.5E5 extraction of two most abundant isotopologues of $[M+Cl]^-$ adduct ions for each homologue group (**Annex IV**) via *R* programming environment

3.4 Module 4: Quantification methods

Monitoring method

Module 4A: Quantification strategy for GC-EI-MS/MS monitoring method

Quantification of the sum parameter ΣPCAs-C_{10-17} determined using the GC-EI-MS/MS monitoring/screening method (**Module 3A**) should be done by linear regression (concentration against area ratio analyte/syringe standard).

To determine the amount of ΣPCAs-C_{10-17}, the following quantification strategy is currently the most appropriate and is strongly recommended:

1) The peak hump areas integrated for the three monitored PCA mass transitions are summed up. This pertains to both the calibration standards and the samples.
2) Normalize the summed-up areas to the area of the syringe standard (ε-HCH).
3) Use linear calibration of the type $y=m \cdot x+b$ to calculate the result, whereas x is the ΣPCAs-C_{10-17} concentration and y is the summed area of the three transitions divided by the area of the syringe standard.

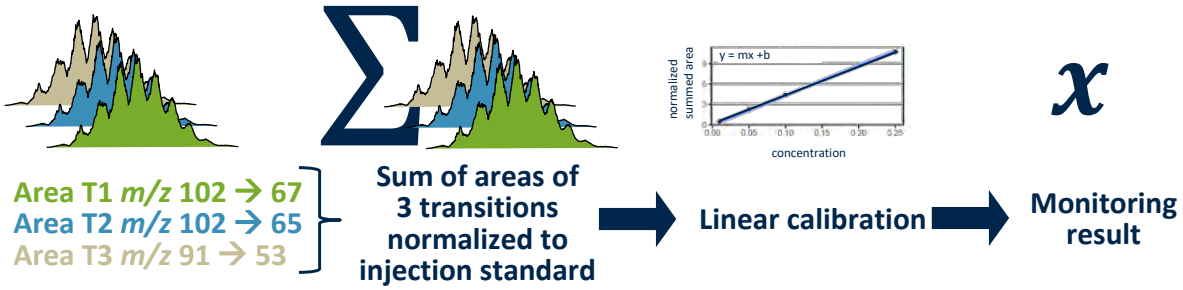


Figure 22 Scheme of the quantification strategy for determination of ΣPCAs-C_{10-17} by GC-EI-MS/MS.

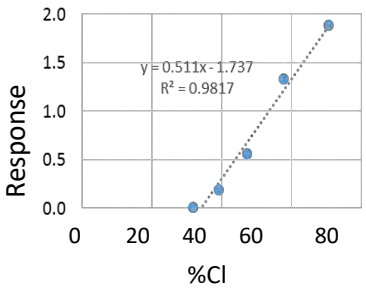
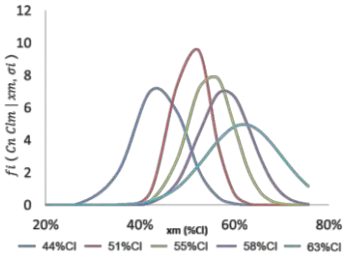
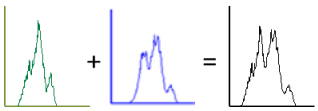
Note: Results are not to be corrected for internal standard recovery! Should the recovery not fulfil requirements (i.e. < 30%, see **chapter 1.4.3**), the analysis is to be repeated.

Characterisation methods

In recent years, high resolution mass spectrometers (HRMS), interfaced to gas chromatography (GC) [43,50,72,75] or high performance liquid chromatography (LC) [50,64,66,76] with soft ionisation are increasingly used for PCA analysis. This is also combined with the application of increasingly complex data processing regimens. The quantification methods presented here (**Table 9**) are suitable for use with HRMS instruments but can also be adapted for use with GC-ECNI-LRMS, LC-ESI-MS/MS or other MS techniques applying soft ionisation for characterization of homologue groups (**Module 3B, Module 3C, Module 3D**).

The methods shown in **Table 9** were tested on three different instrument set-ups (GC-ECNI-HRMS, LC-ESI-HRMS and LC-ESI-TOF-MS) and were found to be comparable both to each other and to spiked amounts in fat samples. All methods presented have been applied to food samples and provided satisfactory results in interlaboratory exercises.

Table 9 Overview of possible quantification methods used for PCA analysis within the EURL/NRL network. Modified based on Mézière *et al*, 2020 [50].

Module	Calibration based on chlorination degree 4B	Gaussian curve model 4C	Deconvolution of spectra 4D
Quan. level	ΣPCAs-$C_{a\text{-}z}$, ΣPCAs-C_n	ΣPCAs-C_n, C_nCl_m	ΣPCAs-C_n, C_nCl_m
Model	RFs calculated from linear or exponential correlation between response and chlorine content of standards 	RRFs calculated from Gaussian distribution assumption 	Concentrations calculated from linear combination of standards 
Weakness	• Linear regression does not always describe the correlation • Needs large array of standards spanning different chlorination degrees	• Non-Gaussian data • Not enough chain length standards available • Atypical behaviour of fringe homologues	• Dependence on fit of standards/technical mixtures • Not enough chain length standards available

Module 4B: Calibration corrected for chlorination degree

Instrumental set-up needed: soft ionisation, LC-MS or GC-MS
Minimum requirements for standards: all standards applicable
Type of possible results: ΣPCAs-$C_{a\text{-}z}$, ΣPCAs-C_n

This method was primarily developed to account for GC-ECNI-MS instrument response dependency on the chlorination degree of the sample or standard analysed.

The method first described by Reth *et al.* [53] derives the total response factors and calculated chlorine contents for a set of CP mixture standards using the GC-ECNI-MS response of each analysed homologue group divided by an internal standard. Depending on the used standard mixtures, chlorine-dependent response factors can be determined for ΣPCAs-$C_{a\text{-}z}$ or ΣPCAs-C_n. A linear regression is then typically fit to the relationship between these parameters, although some laboratories have reported improved results using an exponential curve [54,77].

PCA concentrations in samples are determined by calculating the chlorine content in samples and deriving the corresponding total response factor from the calibration equation.

$$total\ response\ factor\ (sample)=mx+b \quad \text{(Eq. 1)}$$

Where *m* is the slope of the linear regression, *x* the chlorine content calculated from ECNI analysis and *b* the axis intercept. The PCA amount of the sample is then directly calculated as division of the summed relative total peak area from each of the analysed homologue groups by the calculated total response factor of that sample (**Equation 2**).

$$PCA\ amount\ (sample)=\frac{relative\ total\ area\ (sample)}{total\ response\ factor\ (calculated\ for\ the\ sample)} \quad \text{(Eq. 2)}$$

The method can also be applied for quantitation of results acquired by LC-ESI-MS and was adapted using a concentration range for the different calibration mixtures with different chain lengths and chlorination degrees [46,69]. The following scheme illustrates the basic workflow of this approach, here, as an example for a chain length specific calculation in the PCA-$C_{10\text{-}13}$ range (**Figure 23**, [78]).

This method was also adapted in a modified version for the ISO 12010 method on PCA analysis in drinking water, which uses multiple linear regression of the sum of SC-PCAs and peak areas of two distinct PCA masses [79], although no applications of the ISO method to food or feed matrices have been reported yet.

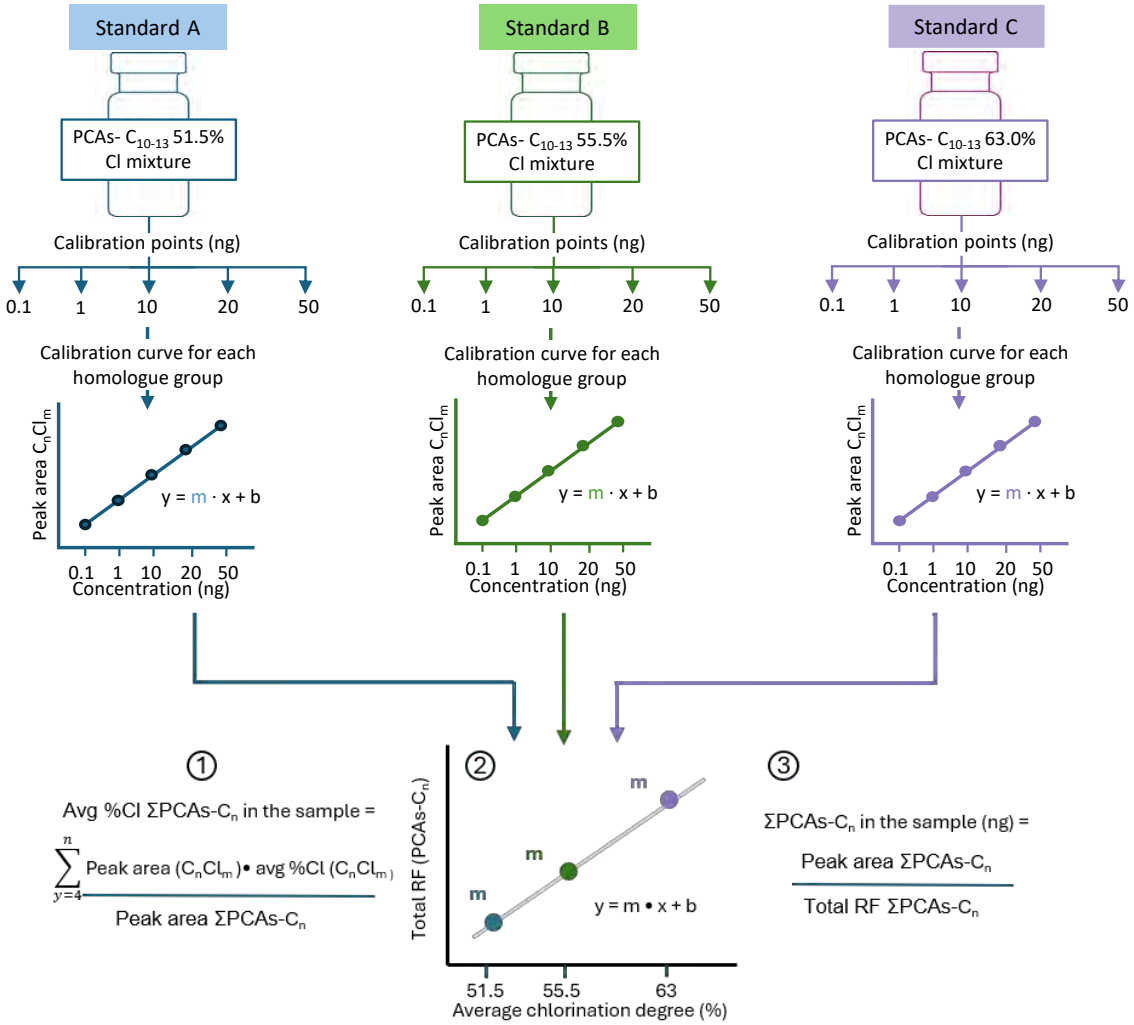


Figure 23 Scheme describing the workflow of chlorination degree-based linear quantification. [74]
Figure adapted based on the original version from Beloki Ezker *et al. 2025, Environ Sci Technol,* https://doi.org/10.1021/acs.est.5c04928 *licensed under CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/).*

Module 4C: Gaussian curve method

Instrumental set-up needed: soft ionisation, HRMS (detection of homologue groups)
Minimum requirements for standards: single chain length standards
Type of possible results: ΣPCAs-C_{a-z}, ΣPCAs-C_n, C_nCl_m

The Gaussian curve method of Yuan *et al.* was published in 2017 [80] and has already been used for occurrence studies in food [70,81]. It was slightly modified for the purpose of integrating MC-PCAs [70].

In brief, the base assumption of the underlying mathematical model is a Gaussian distribution of the abundance of homologue groups within the same carbon chain length. For a considered chain length, the homologue group-specific relative response factors (RRFs) are derived from observed responses of several single-chain CP standards of varying chlorination degrees after modulation in a way that matches as closely as possible the observed and theoretical Gaussian distributions of all standards **(Equations 3** and **4)**. An Excel sheet for calculating these RRFs is available from the EURL upon request.

$$f_i(C_nCl_m|x_m,\sigma_i)=\frac{1}{\sigma_i\sqrt{2\pi}}*e^{\frac{(x_m-\%Cl_i)^2}{2\sigma_i^2}} \quad \text{(Eq. 3)}$$

$$RRF_i(C_nCl_m)=\frac{relative\ area_i(C_nCl_m)}{concentration_i(C_n)*\frac{f_i(C_nCl_m|x_m,\sigma_i)}{\sum_1^m f_i(C_nCl_m|x_m,\sigma_i)}} \quad \text{(Eq. 4)}$$

x_m is the %Cl of C_nCl_m (determined from the molecular formula)
σ_i is the standard deviation describing how close the congener groups are clustered

In the modified method, the calculated RRFs are not directly used for quantification but rather as a base to calculate theoretical concentrations of individual homologue groups for a set of calibration mixtures. Quantification itself is then done as a simple linear calibration with a mixed standard, which accounts for daily changes in analysis conditions by including calibration solutions in every measured batch. In general, the use of RRFs instead of RFs is recommended to correct for these daily changes, even if the calibration is not repeated with each batch.

Module 4D: Deconvolution method

Instrumental set-up needed: soft ionisation, HRMS (detection of homologue groups)
Minimum requirements for standards: all standards applicable (wide range needed)
Type of possible results: ΣPCAs-$C_{a\text{-}z}$, ΣPCAs-C_n, C_nCl_m

The deconvolution method of Bogdal *et al*. [73] on technical CP mixtures is already well-integrated in the PCA-analysis community [82,83]. It was further adapted from technical mixtures to single-chain length standards by Pērkons *et al.* [64].

Briefly, single-chain PCA mixtures are injected within the same sequence as the sample extracts. Each chain length profile of the samples *s* is then matched with a combination of two single-chain standards of different %Cl (y_1 and y_2), e.g. using the *R* programming environment. The quantification of each chain length is performed using the corresponding combination of RRFs, assuming that the response factor is independent of the concentration.

$$\begin{pmatrix} y_{1,1} & y_{2,1} \\ \vdots & \vdots \\ y_{1,n} & y_{2,n} \end{pmatrix} * \begin{pmatrix} x_1 \\ x_2 \end{pmatrix} = \begin{pmatrix} s_1 \\ \vdots \\ s_n \end{pmatrix} \quad \text{(Eq. 5)}$$

Beloki Ezker *et al*. [78] published a variation of this quantification approach that combines the method originally proposed by Bogdal *et al*. [73], incorporating the first step of the response factor calculation for each homologue group from Reth *et al.* [53], as applied by Perkons *et al*. [64], and applies a non-negative least squares (NNLS) algorithm to solve for the contributions of all standard mixtures. An example workflow of this method is shown in the following scheme (**Figure 24**)) from the supplementary material of [78].

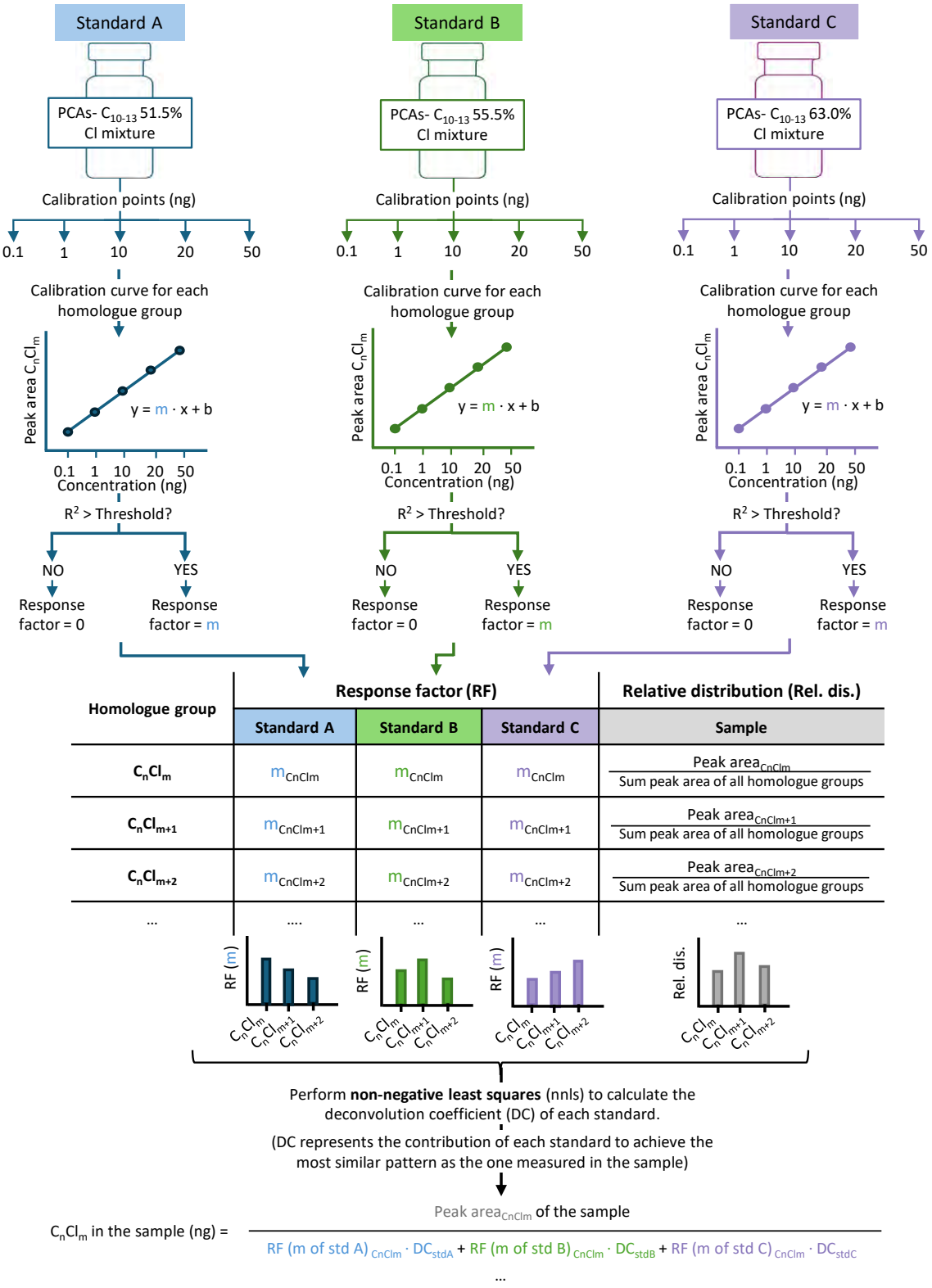


Figure 24 Schematic workflow for quantification of PCA homologue groups applying deconvolution after taking chlorination degree dependent response factors into account [74].
Figure adapted for consistent nomenclature from the supplementary material from Beloki Ezker *et al. 2025, Environ Sci Technol, https://doi.org/10.1021/acs.est.5c04928 licensed under CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/).*

Applications and scripts for quantification

In the **Module 4B**, **Module 4C** and **Module 4D**, the general principles and applications of the three tested quantification strategies are described. These can be applied using MS Excel calculation sheets with macros, but the trend has gone towards scripts or applications in the *R* environment. The following, expressly non-exhaustive, list gives examples of scripts or apps developed or used within the EURL/NRL network for PCA analysis.

Table 10: Examples of applications or R scripts that make use of the described quantification strategies.

Name	CPseeker	CPquantR	CP-Crawler	CPxplorer
Developer	LABERCA (NRL France)	Ingus Pērkons (BIOR, NRL Latvia)	Ingus Pērkons (BIOR, NRL Latvia)	Idoia Beloki Ezker (Linköping University)
Reference	[84]	-	[64]	[78]
Source	Available upon request contact.cpseeker@oniris-nantes.fr	https://github.com/ingusperkons/CP-QuantR	https://github.com/ingusperkons/CP-Crawler	https://github.com/WBS-TW/CPxplorer
Function	Qualitative deconvolution, much information about patterns	Linear calibration based on chlorination degree	Deconvolution on mixed or chain-length specific standards (2 modes) taking %Cl into account	Deconvolution taking %Cl dependent response factors into account Adds 2 functions to R: • **CPions()**: opens a shiny app to generate a target list of PCAs and analogues for ion extraction. • **CPquant()**: opens a shiny app to analyze and quantify output from Skyline
Extras	Can give insight into bromo- or bromo-chloro alkanes and olefins; does not quantify by itself (yet)	• Works with Skyline data (open source) • Documentation available • Produces QC reports, PCA pattern graphs and result tables	• Works with Skyline data (open source) • Extensive documentation and example data available	• Can give insight into bromo- or bromo-chloro alkanes and olefins • Automated peak detection, integration and quantification
Module	**4D**	**4B**	**4D**	**4D**

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5 ANNEXES

ANNEX I Guidance for monitoring of sum parameter ΣPCAs-C_{10-17} by GC-EI-MS/MS

ANNEX II Additional information for GC-ECNI-LRMS analysis (Module 3B)

ANNEX III Additional information for LC-ESI-MS/MS analysis (Module 3C)

ANNEX IV Additional information for GC-ECNI-HRMS and LC-ESI-HRMS analysis (Module 3D)

Guidance Document on the Analysis of Polychlorinated Alkanes

ANNEXES

VERSION 2.0

23 November 2026

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ANNEX I Recommended method for monitoring of ΣPCAs-C_{10-17} by GC-EI-MS/MS

version. 1.0

References to chapters of the main guidance document are highlighted in bold. References to chapters within this annex are highlighted in blue. Abbreviations, terms and definitions explained in the main guidance document apply.

This Annex summarises all **mandatory** and some recommended aspects for quantification of the sum parameter ΣPCAs-C_{10-17} using the recommended GC-EI-MS/MS monitoring method as mentioned in the *Commission Recommendation (EU) 2026/2335 on monitoring the presence of chlorinated paraffins in food* [1].
General information on standards (**chapter 2.3**), sample preparation and clean-up (**chapter 1.4.2/Module 1 and Module 2**), instrumentation (**chapter 2.1**) and quality control (**chapter 1.4**) can be found in separate chapters of the **main guidance document.**
The mandatory aspects of the method described here were developed and tested by several members of the CWG CP of the EURL/NRL network using different food samples and within an ILS.

Please note that the following method description is not comparable to a method published by a normalisation body. Outside of the mandatory aspects, it retains the flexibility of the modular approach followed throughout this guidance document.

AI) 1 Standards

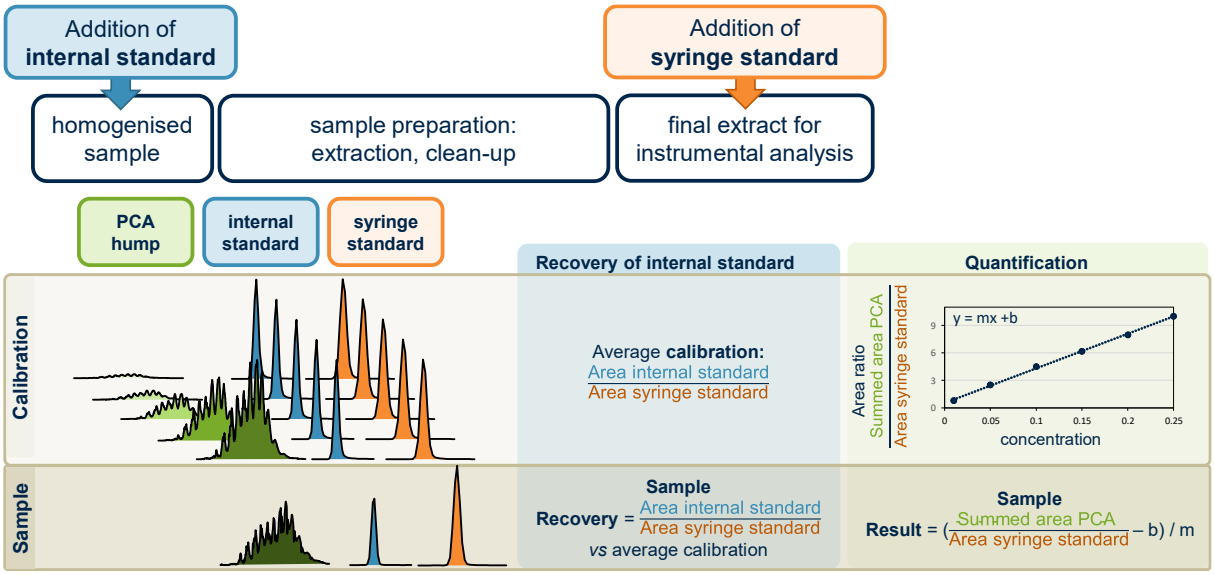

Figure AI.1 Overview on addition and use of internal standard and syringe standard for PCA analysis using the quantitative GC-EI-MS/MS screening method for monitoring of ΣPCAs-C_{10-17}.

AI) 1.1 Quantification standards

The used native standards for quantification are essential for a comparable monitoring result. It is therefore mandatory to use the mixed standards **Chloroparaffin C_{10-13} 55.5%Cl and Chloroparaffin C_{14-17} 57% in a 1:1 mixture** (from Dr. Ehrenstorfer) for quantification.

Dr. Ehrenstorfer standards, Augsburg, Germany	
Chloroparaffin C_{10}-C_{13} 55.5%Cl **DRE-X23105500CY**	**Chloroparaffin C_{14}-C_{17} 57%Cl** **DRE-X23145700CY**

AI) 1.2 Syringe standard

Syringe standard *also referred to as injection standard or volumetric standard: A standard that is added at the very end of sample preparation, immediately before instrumental analysis. It does not have to be structurally similar to the analyte as it accounts only for losses caused by problems with injection or other steps of instrumental analysis/measurement.*

Based on several tested compounds, **ε-hexachlorocyclohexane (ε-HCH)** is required as syringe standard. While it does not elute outside the PCA hump, its influence on the overall peak hump area is negligible (and is considered during quantification as it is added to samples as well as calibration standards).

Note: Unlike isotope-dilution analysis of other POPs (e.g. PCDD/Fs and PCBs) this syringe standard is used for normalization of the summed areas of the native PCA transitions (Figure AI.1).

Note: While most of the standard suppliers in Europe offer this standard from the Dr. Ehrenstorfer brand, this standard can, unlike the quantification standards, be obtained from any supplier available.

AI) 1.3 Internal standards

Internal standard: *A standard that is added to the sample during the first step of preparation to monitor or account for losses during sample preparation and the whole analytical procedure. Often an isotope-labelled compound is used as it should not be naturally present in the sample but possess the same physicochemical properties as the analyte.*

Monitoring results should not be corrected for internal standard recovery. Thus, the choice on which standard is used for this purpose is free. It is recommended to use a compound which is structurally similar to PCAs, ideally an isotope-labelled PCA congener. Examples for suitable standards are **$^{13}C_{10}$-1,5,5,6,6,10-hexachlorodecane** (Cambridge Isotope Laboratories, Tewksbury, MA, USA) or the **$^{13}C_3$-labelled PCAs** from Chiron (Chiron, Trondheim, Norway). More information about internal standards can be found in **chapter 2.3.3 of the main guidance document**.

AI) 2 Sample preparation and clean-up

Sample preparation and clean-up can be mostly oriented on workflows and instrumentation already in place for other lipophilic halogenated contaminants. The principle remains the same: 1) lipid extraction, 2) lipid removal, 3) clean-up and fractionation to remove co-elutants. While the first two steps can be chosen among the examples given in **module 1 and 2 of chapter 3** of the main guidance document, it is mandatory for the samples analysed by the GC-EI-MS/MS monitoring method to be cleaned up using a **Florisil® column** (see also **Module 2D**). The following protocol describes an example of a Florisil® clean-up; it can be adapted to available glassware, sample amount, and type of cleaning done before this step. For more general information on how to prepare the solvents, sorbents, and glassware to minimise blank levels, see **chapter 2.4** and **Module 2 in chapter 3**.

An acidic silica gel pre-cleaned extract of 1.5 mL is added to a glass column containing glass wool, sodium sulphate, and Florisil®/H_2O according to the scheme in Figure AI.2. All components of this column can be packed dry, so that light shaking or knocking of the column is necessary to ensure tight packed sorbents. After pre-conditioning the column with 30 mL *n*-hexane, the sample extract is added (with 3x 2 mL of the same solvent to ensure quantitative transfer).
The first fraction of this column is eluted with 75 mL *n*-hexane followed by 5 mL dichloromethane and contains PCBs, toxaphenes and similar compounds. It is discarded. The second fraction is eluted with 60 mL dichloromethane from the column. This fraction is then evaporated almost to dryness using first a rotary evaporator and then a gentle nitrogen stream. Finally, to the last drop of extract the syringe standard is added and quickly mixed. This mix is then transferred to a vial for GC-EI-MS/MS measurement.

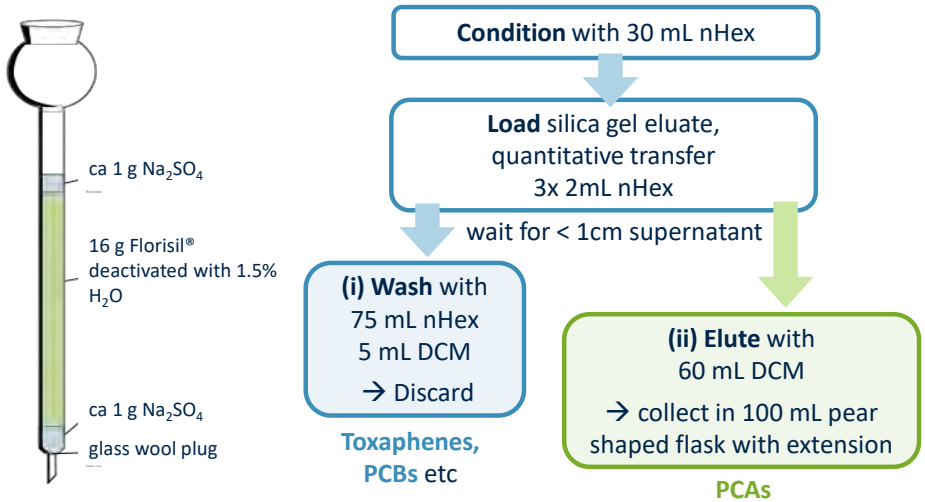

Figure AI.2 Example column set-up and workflow for Florisil® column clean-up.

Note: The quality and characteristics of Florisil® may differ notably between material batches. Elution behaviour of PCAs from the Florisil® column should thus be regularly checked (e.g., for each batch). Based on experiences within the EURL/NRL network elution of $^{13}C_{10}$-1,5,5,6,6,10-hexachlorodecane seems to be a suitable indicator of deviating elution behaviour. If the standard recovery is low (i.e. < 30%) it is recommended to check the wash fraction and if needed to adjust the elution volume.

AI) 3 GC-EI-MS/MS measurement method

For maximum adaptability, only essential parts of the measurement method are fixed requirements with the rest being stated as recommendations to allow flexible use of available instrumentation. For more information on GC-EI-MS/MS analysis see **Module 3A**. The mandatory parameters are marked **green** in the following overview.

Table AI.1 The most important method parameters for the recommended monitoring method. Mandatory parameters are marked in green.

<table>
<tr><th colspan="2">Method parameters</th></tr>
<tr><td>Technique</td><td>GC-EI-MS/MS</td></tr>
<tr><td>Column</td><td>DB5 column, ≤ 15 m length (e.g., HP5-MSUI fused silica capillary column (Agilent Technologies)), transfer line temperature: 280-340°C</td></tr>
<tr><td>Injector</td><td>PTV or SSL (220 °C)</td></tr>
<tr><td>Detection</td><td>EI-MS/MS (MRM mode), collision gas: Nitrogen
Source electron energy 70eV, source temperature 250-280 °C
Mass transitions:
<table>
<tr><th>Target analyte</th><th>Mass transition [m/z]</th><th>CE [eV]</th></tr>
<tr><td>PCAs-C_{10-17} (1)</td><td>102 → 67</td><td>10*</td></tr>
<tr><td>PCAs-C_{10-17} (2)</td><td>102 → 65</td><td>20*</td></tr>
<tr><td>PCAs-C_{10-17} (3)</td><td>91 → 53</td><td>10*</td></tr>
<tr><td>ε-HCH (syringe standard)</td><td>181 → 145
181 → 109</td><td>15*
31*</td></tr>
<tr><td>$^{13}C_{10}$-hexachlorodecane #</td><td>142 → 106
142 → 78</td><td>5*
11*</td></tr>
<tr><td>$^{13}C_{3}$-hexachlorotridecane #</td><td>284 → 248
286 → 176</td><td>2*
10*</td></tr>
<tr><td>$^{13}C_{3}$-hexachlorotetradecane #</td><td>298 → 262
300 → 190</td><td>5*
10*</td></tr>
</table>
*The applied CE should be optimized on the used GC-EI-MS/MS system
Depending on the choice of internal standard
The dwell time should be adjusted for acquisition of enough data points over the peaks of the internal standard and syringe standard</td></tr>
</table>

AI) 4 Identification and Quantification

Identification of the PCA hump should be carried out by comparison to the calibration standards, with regards to retention time range and visual match (check for visible interfering peaks within the hump), using the three required mass transitions. Visual guidance on integration is given exemplary: see examples in **chapter 8** of this annex.

Quantification should be done by linear regression (**concentration against area ratio sum PCA/syringe standard**).

To determine the amount of ΣPCAs-C_{10-17}, the following **quantification strategy** is currently the most appropriate and strongly recommended (Figure AI.3) has to be followed:

1) The peak hump areas integrated for the three monitored PCA mass transitions are summed up. This pertains to both the calibration standards and the samples.
2) Normalize the summed-up areas to the area of the syringe standard (ε-HCH).
3) Use linear calibration of the type $y=m \cdot x+b$ to calculate the result, whereas x is the ΣPCAs-C_{10-17} concentration and y is the summed area of the three transitions divided by the area of the syringe standard.

Note: Results are not to be corrected for internal standard recovery! Should the recovery not fulfil requirements (i.e. < 30%, see section 5), the analysis is to be repeated.

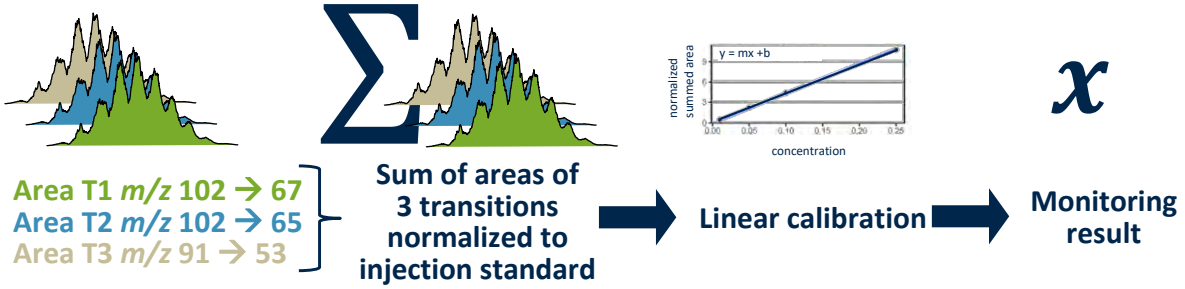

Figure AI.3 Scheme of the quantification strategy for determination of ΣPCAs-C_{10-17} by GC-EI-MS/MS.

AI) 5 Performance criteria

AI) 5.1 Internal standard recovery

The internal standard recovery, determined relative to the syringe standard, represents the primary decision tool for the reliability and usability of a sample analysis.

The following criteria describe the lower end of the recommended recovery range. An upper limit for recovery is yet to be defined.

Recovery	Action
> 50%	Sample can be used
30% ≤ x ≤ 50%	Consider repeat the analysis
< 30%	Repeat the analysis

AI) 5.2 Limit of quantification (LOQ)

For the different methods of estimation of LOD or LOQ, please refer to the Joint Guidance Documents of the four Contaminant EURLs *"Guidance Document on the Estimation of LOD and LOQ for Measurements in the Field of Contaminants in Feed and Food"* [2] and *"Guidance Document on the Estimation of LOQ for Measurements in the Field of Contaminants in Feed and Food"* [3]. The application of the lowest validated level approach is recommended.

For monitoring methods, LOQs should be established in relation to the thresholds defined in the Monitoring Recommendation (see **chapter 7**) to ensure reliable monitoring results.

- LOQ ≤ ½ threshold level per matrix(group) on [wet weight] basis
- LOQ ≤ ⅓ threshold level per matrix(group) on [fat weight] basis

If no threshold is defined for the matrix category, then fortification experiments in matrix should be carried out to determine a target working range before validating the LOQ.
For the purpose of monitoring, laboratories should strive to establish LOQs as low as possible.

AI) 5.3 Working range

The working range of the method should cover the monitoring thresholds taking into account the minimum required LOQ (see **chapter 5.2**).

AI) 6 Quality control

To ensure consistent performance and quality of analytical results certain continuous quality control or quality assurance measures should be followed:

- Regular participation in interlaboratory study or proficiency test schemes (EURL, Quasimeme, …) or co-analysis of samples with at least one other laboratory if no such test is available
- Regular check of blank levels (ideally with each sample batch)
- Use of PT/ILS material as QC samples, ideally with each batch
- Use of certified reference material (CRM), where applicable

AI) 7 Reporting and threshold principle

The *Commission Recommendation (EU) 2026/2335 on monitoring the presence of chlorinated paraffins in food* requests the analysis of a unified sum parameter: the sum of short and medium-chain PCAs, i.e. ΣPCAs-C_{10-17} in comparison to a threshold value (Figure AI.4). The specified thresholds (Table AI.2) serve as trigger for a more thorough characterisation of a monitoring sample: within the EU official food control network, such samples should be communicated to EURL POPs, who then advises on an expert laboratory that will do the characterisation analysis. The EURL POPs should also be informed if the determined level is very high (i.e. > 50 ng/g wet weight or 300 ng/g fat) and no threshold is defined for the analysed sample matrix. The full procedure is described in detail in the sample distribution scheme document [4].



Figure AI.4 Workflow of analysis, reporting and further sample distribution for characterisation analysis in the context of the *Commission Recommendation (EU) 2026/2335 on monitoring the presence of chlorinated paraffins in food.*

Reporting of occurrence data (i.e., sum parameter ΣPCAs-C_{10-17}) acquired in the context of the *Commission recommendation (EU) 2026/2335 on monitoring the presence of chlorinated paraffins in food* and beyond should include:

- A brief description of the used methodology
- Fat content of the sample (for feed samples moisture content)
- Concentration of sum parameter ΣPCAs-C_{10-17} in the same unit as the threshold value. If no threshold value is defined for the category, report in ng/g wet weight.
  Note: Feed is not specifically included in this recommendation, but reporting should be done in µg/kg product (12% moisture content) or µg/kg product with additional of report of the moisture content
- LOQ (in particular in case of concentrations below LOQ)
- Recovery and identity of internal standard
- (Expanded) measurement uncertainty

Table AI.2 Threshold values for the sum parameter ΣPCAs-C_{10-17} as trigger for further characterisation analysis in accordance with the *Commission Recommendation (EU) 2026/2335 on monitoring the presence of chlorinated paraffins in food.*

Food category	Threshold for ΣPCAs-C_{10-17}
Fish	100 ng/g wet weight
Milk and dairy products	20 ng/g wet weight
Oils and fats	1000 ng/g fat
Other products of animal origin (e.g. meat, eggs, …)	300 ng/g fat
Processed foods (whole meals)	50 ng/g wet weight
Babyfood*	20 ng/g wet weight

* In the context of the *Commission Recommendation (EU) 2026/2335 on monitoring the presence of chlorinated paraffins in food* the threshold for the category "babyfood" is recommended to be applied to all kinds of food intended for infants and young children including infant formula.

AI) 8 Visual guidance on integration of PCA hump

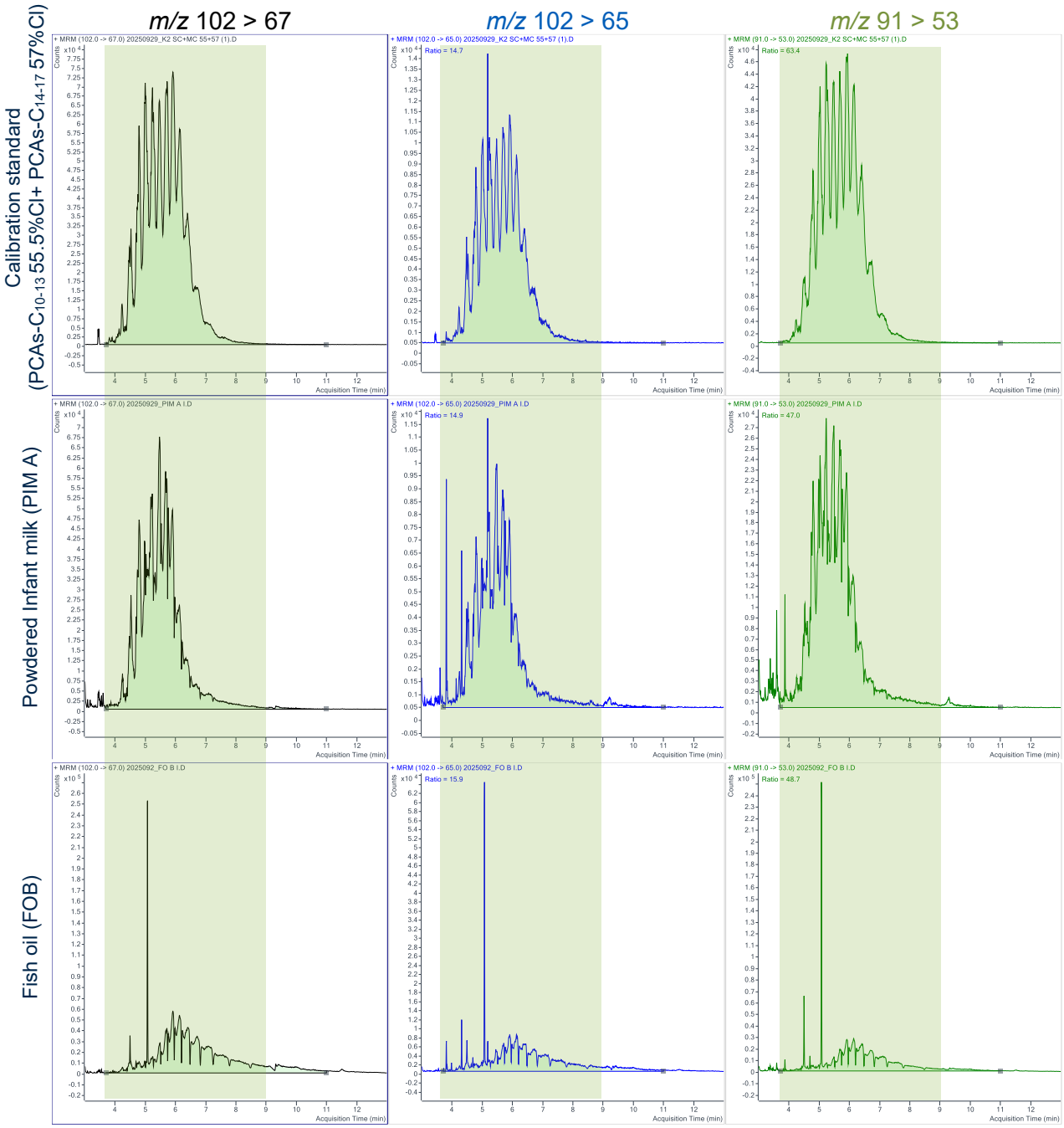

Figure AI.5 Visual Guidance on peak integration in GC-EI-MS/MS. Shown is for all three mass transitions the chromatograms of (top) the calibration standards, (middle) of a sample extract from powdered infant milk (24-03 PIM A) and (bottom) of a sample extract from fish oil (22-02 FOB). The green area represents the integrated area.

References

[1] Commission Recommendation (EU) 2026/2335 of 21 October 2026 on monitoring the prescence of chlorinated paraffins in food. http://data.europa.eu/eli/reco/2026/2335/oj

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[3] J. J. Sloth, H. Mol, A. Fromberg, A. Schächtele, Guidance Document on the Estimation of LOQ for Measurements in the Field of Contaminants in Feed and Food (2022), version 1.0. https://eurl-pops.eu/user/pages/05.news/12.guidance-document-loq-2022/Guidance_Document_on_the_Estimation_of_LOQ_for_Measurements_in_the_Field_of_Contaminants_in_Feed_and_Food_V1.0_2022.pdf?g-66352cfe

[4] Sample distribution scheme for Commission Recommendation on monitoring the presence of Chlorinated Paraffins in Food, 2026, https://eurl-pops.eu/news/sample-distribution-scheme-for-pca-monitoring-recommendation/pca-sample-distribution-scheme

ANNEX II: Monitored ions and visual guidance for identification of interferences in GC-LRMS (Module 3B)

version. 2.0

AII) 1 Distribution of injections

For analysis of PCA homologue groups by GC-ECNI-LRMS 4 injections are necessary due to limited monitoring of the number of traces in parallel.

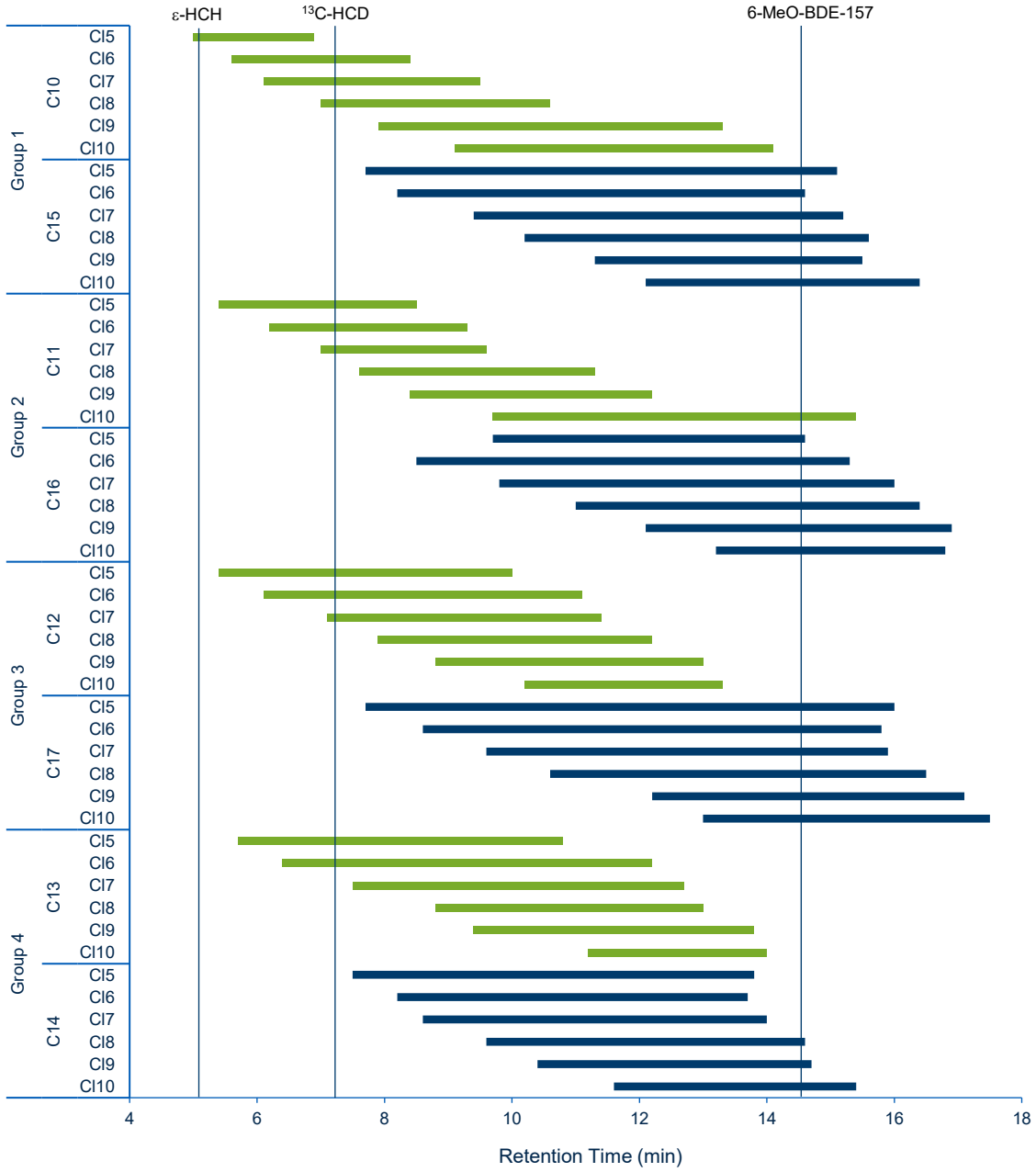


Figure AII.1 Gas chromatograph retention time ranges of PCAs-$C_{10\text{-}13}$ (green) and PCAs-$C_{14\text{-}17}$ (dark blue) measured in individual mixture standards of Chloroparaffin $C_{10\text{-}13}$ 55.5%Cl and Chloroparaffin $C_{14\text{-}17}$ 57%Cl. Retention times of internal and recovery standards are indicated by vertical lines. Groups 1-4 indicate the four separate injection groups. Figure taken from McGrath *et al*, 2020 [1].

AII) 2 Monitored Ions

GC-ECNI-methods (**Module 3B and 3D**) usually monitor $[M\text{-}Cl]^-$ or $[M\text{-}HCl]^-$ adduct ions depending on the PCA homologue groups. This is based on experiments monitoring the abundance of different adduct ions in ECNI, which showed that especially with lowering the overall chlorination degree, the $[M\text{-}HCl]^-$ adduct ions became as abundant as $[M\text{-}Cl]^-$ [2].

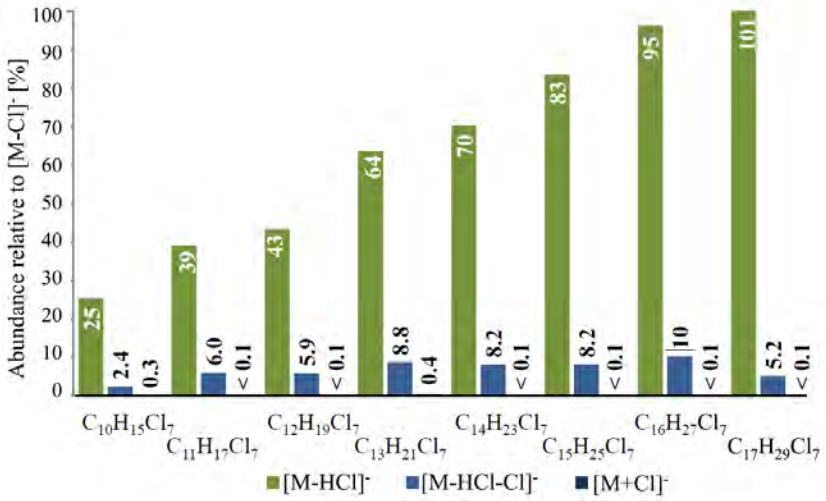


Figure AII.2 Abundance of $[M\text{-}HCl]^-$, $[M\text{-}HCl\text{-}Cl]^-$ and $[M+Cl]^-$ fragment ions relative to the signal of $[M\text{-}Cl]^-$. Figure taken from Krätschmer *et al* [2].

Table AII.1 Adduct ions monitored in SIM mode for **GC-ECNI-LRMS analysis** of PCAs-C_{10-17}.

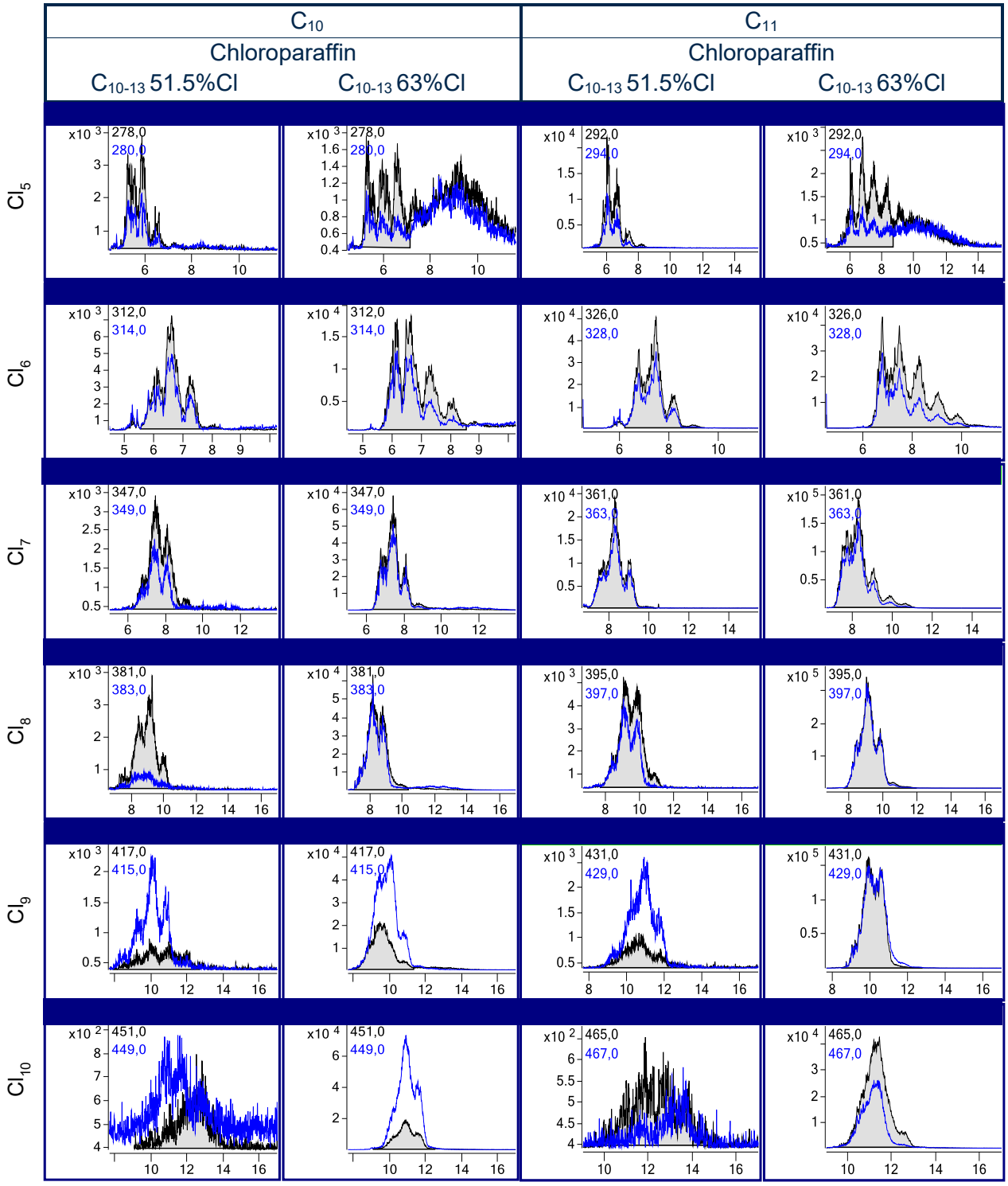
Homologue group			Adduct Ion	Quantification *m/z*	Confirmation *m/z*
C_{10}	Cl_5	$C_{10}H_{17}Cl_5$	$[M\text{-}HCl]^-$	278	280
	Cl_6	$C_{10}H_{16}Cl_6$	$[M\text{-}HCl]^-$	312	314
	Cl_7	$C_{10}H_{15}Cl_7$	$[M\text{-}Cl]^-$	347	349
	Cl_8	$C_{10}H_{14}Cl_8$	$[M\text{-}Cl]^-$	381	383
	Cl_9	$C_{10}H_{13}Cl_9$	$[M\text{-}Cl]^-$	417	415
	Cl_{10}	$C_{10}H_{12}Cl_{10}$	$[M\text{-}Cl]^-$	451	449
C_{11}	Cl_5	$C_{11}H_{19}Cl_5$	$[M\text{-}HCl]^-$	292	294
	Cl_6	$C_{11}H_{18}Cl_6$	$[M\text{-}HCl]^-$	326	328
	Cl_7	$C_{11}H_{17}Cl_7$	$[M\text{-}Cl]^-$	361	363
	Cl_8	$C_{11}H_{16}Cl_8$	$[M\text{-}Cl]^-$	395	397
	Cl_9	$C_{11}H_{15}Cl_9$	$[M\text{-}Cl]^-$	431	429
	Cl_{10}	$C_{11}H_{14}Cl_{10}$	$[M\text{-}Cl]^-$	465	467
C_{12}	Cl_5	$C_{12}H_{21}Cl_5$	$[M\text{-}HCl]^-$	306	304
	Cl_6	$C_{12}H_{20}Cl_6$	$[M\text{-}HCl]^-$	340	342
	Cl_7	$C_{12}H_{19}Cl_7$	$[M\text{-}Cl]^-$	375	377
	Cl_8	$C_{12}H_{18}Cl_8$	$[M\text{-}Cl]^-$	409	411
	Cl_9	$C_{12}H_{17}Cl_9$	$[M\text{-}Cl]^-$	445	443
	Cl_{10}	$C_{12}H_{16}Cl_{10}$	$[M\text{-}Cl]^-$	479	481
C_{13}	Cl_5	$C_{13}H_{23}Cl_5$	$[M\text{-}HCl]^-$	320	318
	Cl_6	$C_{13}H_{22}Cl_6$	$[M\text{-}HCl]^-$	354	356
	Cl_7	$C_{13}H_{21}Cl_7$	$[M\text{-}Cl]^-$	389	391
	Cl_8	$C_{13}H_{20}Cl_8$	$[M\text{-}Cl]^-$	423	425
	Cl_9	$C_{13}H_{19}Cl_9$	$[M\text{-}Cl]^-$	459	457
	Cl_{10}	$C_{13}H_{18}Cl_{10}$	$[M\text{-}Cl]^-$	493	491
C_{14}	Cl_5	$C_{14}H_{25}Cl_5$	$[M\text{-}Cl]^-$	335	337
	Cl_6	$C_{14}H_{24}Cl_6$	$[M\text{-}Cl]^-$	369	371
	Cl_7	$C_{14}H_{23}Cl_7$	$[M\text{-}Cl]^-$	403	405
	Cl_8	$C_{14}H_{22}Cl_8$	$[M\text{-}Cl]^-$	437	439
	Cl_9	$C_{14}H_{21}Cl_9$	$[M\text{-}Cl]^-$	473	471
	Cl_{10}	$C_{14}H_{20}Cl_{10}$	$[M\text{-}Cl]^-$	507	505
C_{15}	Cl_5	$C_{15}H_{27}Cl_5$	$[M\text{-}Cl]^-$	349	347
	Cl_6	$C_{15}H_{26}Cl_6$	$[M\text{-}Cl]^-$	383	385
	Cl_7	$C_{15}H_{25}Cl_7$	$[M\text{-}Cl]^-$	417	419
	Cl_8	$C_{15}H_{24}Cl_8$	$[M\text{-}Cl]^-$	451	453
	Cl_9	$C_{15}H_{23}Cl_9$	$[M\text{-}Cl]^-$	487	485
	Cl_{10}	$C_{15}H_{22}Cl_{10}$	$[M\text{-}Cl]^-$	521	519
C_{16}	Cl_5	$C_{16}H_{29}Cl_5$	$[M\text{-}Cl]^-$	363	361
	Cl_6	$C_{16}H_{28}Cl_6$	$[M\text{-}Cl]^-$	397	395
	Cl_7	$C_{16}H_{27}Cl_7$	$[M\text{-}Cl]^-$	431	429
	Cl_8	$C_{16}H_{26}Cl_8$	$[M\text{-}Cl]^-$	465	467
	Cl_9	$C_{16}H_{25}Cl_9$	$[M\text{-}Cl]^-$	501	499
	Cl_{10}	$C_{16}H_{24}Cl_{10}$	$[M\text{-}Cl]^-$	535	537
C_{17}	Cl_5	$C_{17}H_{31}Cl_5$	$[M\text{-}Cl]^-$	377	375
	Cl_6	$C_{17}H_{30}Cl_6$	$[M\text{-}Cl]^-$	411	413
	Cl_7	$C_{17}H_{29}Cl_7$	$[M\text{-}Cl]^-$	445	447
	Cl_8	$C_{17}H_{28}Cl_8$	$[M\text{-}Cl]^-$	479	481
	Cl_9	$C_{17}H_{27}Cl_9$	$[M\text{-}Cl]^-$	515	513
	Cl_{10}	$C_{17}H_{26}Cl_{10}$	$[M\text{-}Cl]^-$	549	547
Internal standard	$^{13}C_{10}$-HCD	$^{13}C_{10}H_{16}Cl_6$	$[M\text{-}Cl]^-$	323	321
Syringe standard	ε-HCH	$C_6H_6Cl_6$	$[M\text{-}Cl]^-$	255	257
Syringe standard	6-MeO-BDE-157	$C_{13}H_6Br_6O_2$	$[Br]^-$	81	79

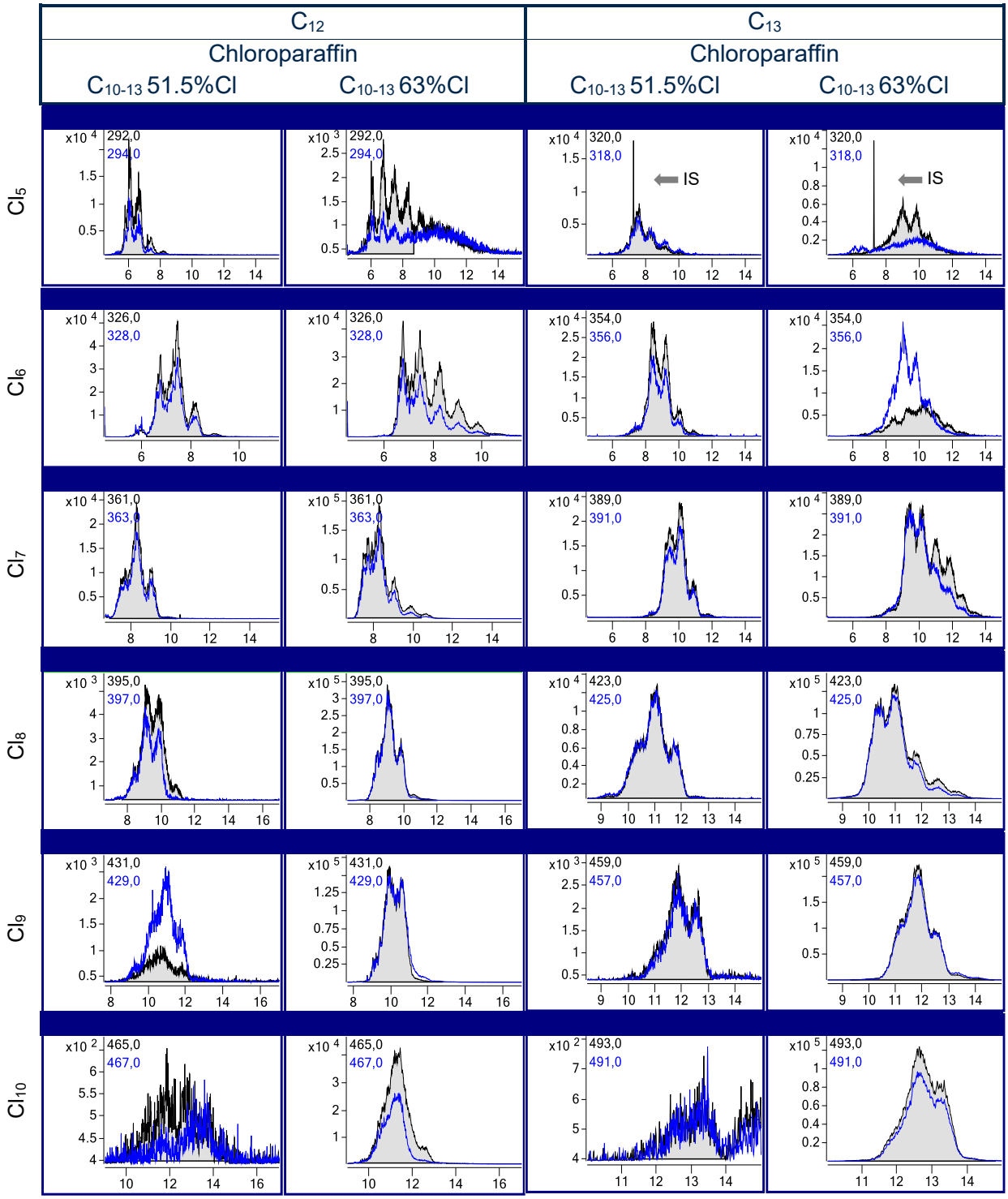
AII) 3 Visual Guidance

The separation of SC-PCAs and MC-PCAs in GC-ECNI-LRMS by nominal mass and retention time alone is not always possible, leading to mass interferences in the resulting chromatograms. While attempts at manually separating interfering PCAs by cropping the peak humps or similar methods is inadvisable, instrument operators are nevertheless able to at least estimate the severity of interference in the sample.

To help with this very subjective task, in the following extracted ion chromatograms (XICs) are shown for SC-PCAs and MC-PCAs in separate technical standards of Chloroparaffin $C_{10\text{-}13}$ 51.5%Cl, $C_{10\text{-}13}$ 63%Cl, $C_{14\text{-}17}$ 42%Cl and $C_{14\text{-}17}$ 57%Cl at 10 µg/mL, respectively. Analyses were performed on an Agilent 7000D gas chromatograph-triple quadrupole mass spectrometer operated in electron capture negative ionization mode (GC-ECNI-LRMS) using single ion monitoring (SIM) according to McGrath et al., 2021 [1]. Observations were confirmed by a second laboratory using a similar instrument set-up but slightly different analysis methods.

Vertical axes of chromatograms show instrument response and horizontal axes show retention time in minutes. Values written in black and blue indicate quantitative and qualitative *m/z*, respectively, as corresponds to the colours of chromatograms, while grey shading indicates integrated area of quantitative peak. IS peaks labelled in $C_{13}Cl_5$ chromatograms indicate internal standard $^{13}C_{10}$-1,3,3,5,6,10-hexachlorodecane, which shares isotopic overlap at this *m/z*. For further information, please refer to the original publication [1].




C12
Chloroparaffin
C10-13 51.5%Cl
C10-13 63%Cl
C13
Chloroparaffin
C10-13 51.5%Cl
C10-13 63%Cl
Cl5
Cl6
Cl7
Cl8
Cl9
Cl10
292,0
294,0
320,0
318,0
IS
326,0
328,0
354,0
356,0
361,0
363,0
389,0
391,0
395,0
397,0
423,0
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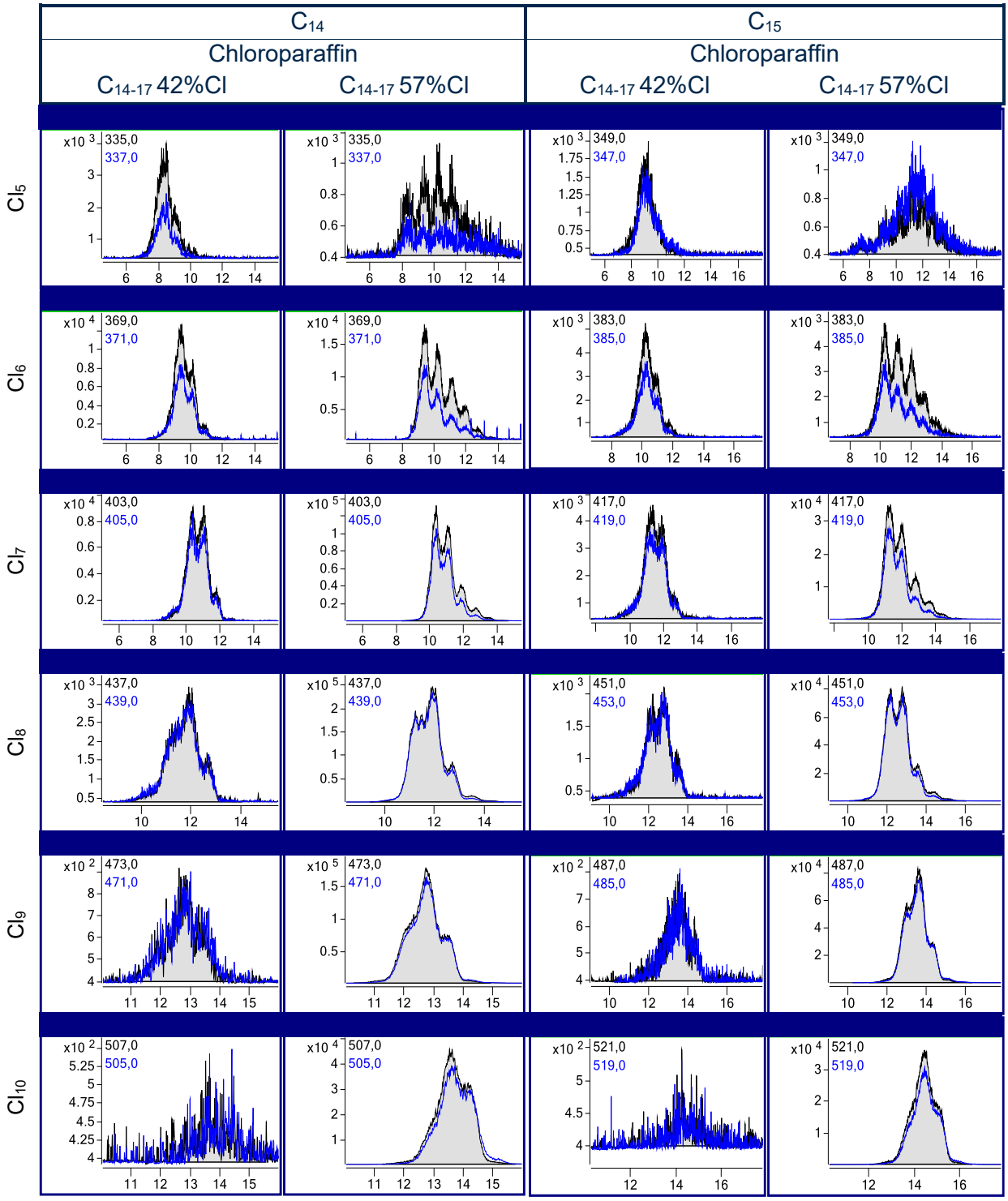
467,0
493,0
491,0


C14
Chloroparaffin
C14-17 42%Cl
C14-17 57%Cl
C15
Chloroparaffin
C14-17 42%Cl
C14-17 57%Cl
Cl5
Cl6
Cl7
Cl8
Cl9
Cl10
335,0
337,0
349,0
347,0
369,0
371,0
383,0
385,0
403,0
405,0
417,0
419,0
437,0
439,0
451,0
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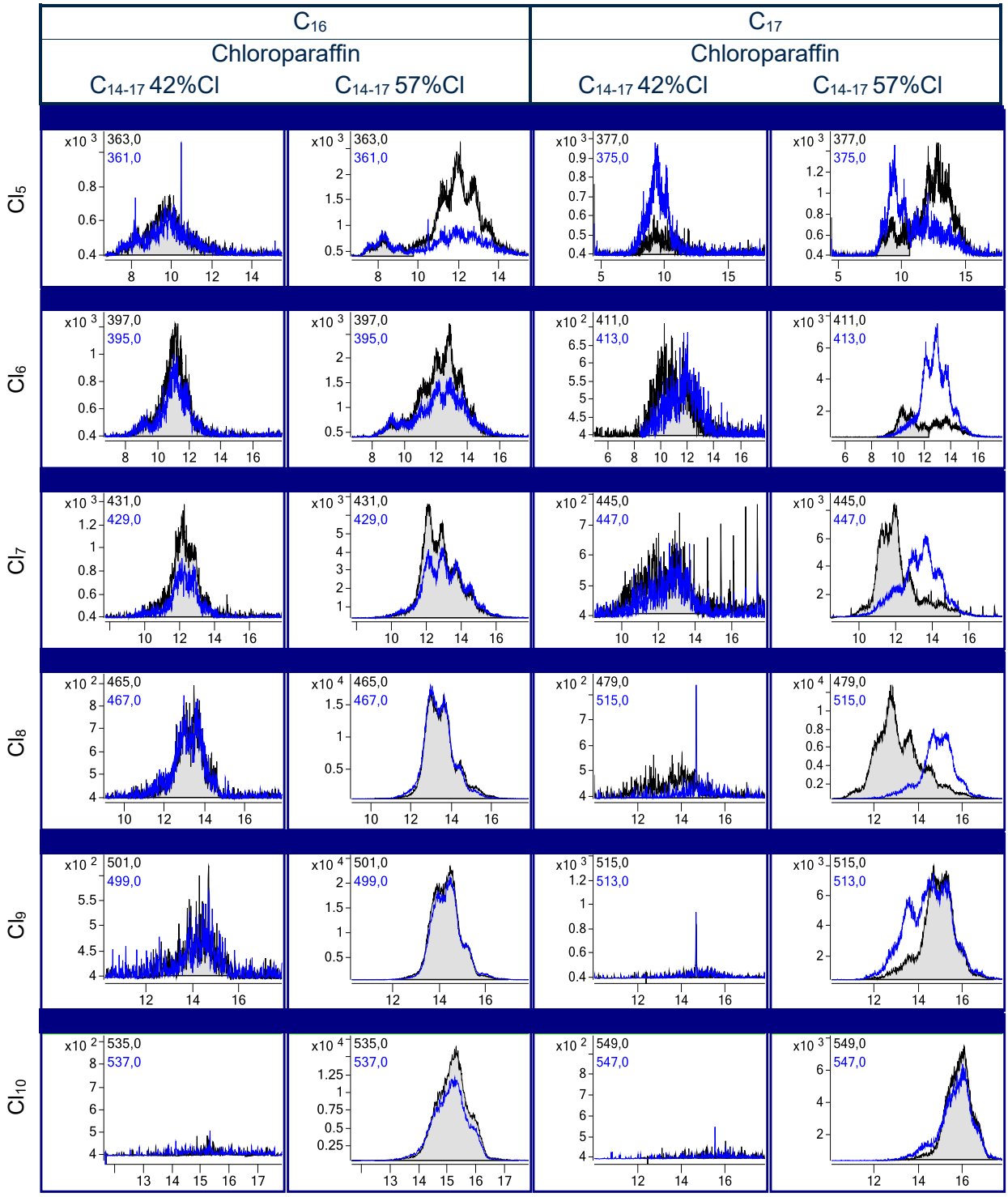
505,0
521,0
519,0


C16
Chloroparaffin
C14-17 42%Cl
C14-17 57%Cl
C17
Chloroparaffin
C14-17 42%Cl
C14-17 57%Cl
Cl5
Cl6
Cl7
Cl8
Cl9
Cl10
363,0
361,0
377,0
375,0
397,0
395,0
411,0
413,0
431,0
429,0
445,0
447,0
465,0
467,0
479,0
515,0
501,0
499,0
515,0
513,0
535,0
537,0
549,0
547,0

References

[1] T.J. McGrath, G. Poma, J. Bombeke, F. Limonier, E. van Hoeck, L. Joly, A. Covaci, Optimization and validation of an analytical method for the quantification of short- and medium-chained chlorinated paraffins in food by gas chromatography-mass spectrometry, *Food Control* 119 (2021) 107463. https://doi.org/10.1016/j.foodcont.2020.107463.

[2] K. Krätschmer, C. Cojocariu, A. Schächtele, R. Malisch, W. Vetter, Chlorinated paraffin analysis by gas chromatography Orbitrap high-resolution mass spectrometry: Method performance, investigation of possible interferences and analysis of fish samples, *J. Chromatogr. A* 1539 (2018) 53–61. https://doi.org/10.1016/j.chroma.2018.01.034.

ANNEX III: Monitored ions for LC-ESI-MS/MS analysis (Module 3C)

version 1.0

Table AIII.1 Mass transitions and collision energies for monitoring PCAs-$C_{10\text{-}17}$ by LC-ESI-MS/MS analysis according to Pērkons *et al* 2005 [1].

PCA homologue group	RT [min]	SRM 1		SRM 2	
		m/z	CE [eV]	*m/z*	CE [eV]
$C_{10}H_{17}Cl_5$	11.3	373 > 313	5	373 > 59	10
$C_{10}H_{16}Cl_6$	11.7	407 > 347	5	407 > 59	12.5
$C_{10}H_{15}Cl_7$	12.3	441 > 381	5	441 > 59	12.5
$C_{10}H_{14}Cl_8$	12.5	477 > 417	5	477 > 59	12.5
$^{13}C_{10}H_{16}Cl_6$ (internal standard)	12.7	417 > 59	7.5	419 > 59	7.5
$C_{11}H_{19}Cl_5$	12.3	387 > 327	5	387 > 59	10
$C_{11}H_{18}Cl_6$	12.5	421 > 361	5	421 > 59	12.5
$C_{11}H_{17}Cl_7$	12.9	455 > 395	5	455 > 59	15
$C_{11}H_{16}Cl_8$	13.1	491 > 431	7.5	491 > 59	15
$C_{12}H_{21}Cl_5$	12.5	401 > 341	5	401 > 59	10
$C_{12}H_{20}Cl_6$	13.3	435 > 375	5	435 > 59	14
$C_{12}H_{19}Cl_7$	13.5	469 > 409	7.5	469 > 59	15
$C_{12}H_{18}Cl_8$	13.7	505 > 445	7.5	505 > 59	15
$C_{13}H_{23}Cl_5$	13.6	415 > 355	5	415 > 59	12.5
$C_{13}H_{22}Cl_6$	13.8	449 > 389	5	449 > 59	15
$C_{13}H_{21}Cl_7$	14.8	483 > 423	7.5	483 > 59	15
$C_{13}H_{20}Cl_8$	14.9	519 > 459	7.5	519 > 423	10
$C_{14}H_{25}Cl_5$	14.9	429 > 369	5	429 > 59	14
$C_{14}H_{24}Cl_6$	15.5	463 > 403	7.5	463 > 59	15
$C_{14}H_{23}Cl_7$	15.5	497 > 437	7.5	497 > 59	15
$C_{14}H_{22}Cl_8$	15.7	533 > 473	7.5	533 > 437	10
$C_{15}H_{27}Cl_5$	15.7	443 > 383	5	443 > 59	12.5
$C_{15}H_{26}Cl_6$	15.8	477 > 417	5	477 > 59	12.5
$C_{15}H_{25}Cl_7$	16	511 > 451	7.5	511 > 59	15
$C_{15}H_{24}Cl_8$	16.1	547 > 487	7.5	547 > 451	10
$C_{16}H_{29}Cl_5$	16.4	457 > 397	5	457 > 59	10
$C_{16}H_{28}Cl_6$	16.4	491 > 431	7.5	491 > 59	15
$C_{16}H_{27}Cl_7$	16.5	525 > 465	7.5	525 > 59	15
$C_{16}H_{26}Cl_8$	16.7	561 > 501	7.5	561 > 465	10
$C_{17}H_{31}Cl_5$	16.8	471 > 411	7.5	471 > 59	12.5
$C_{17}H_{30}Cl_6$	17	505 > 445	7.5	505 > 59	15
$C_{17}H_{29}Cl_7$	17.2	539 > 479	7.5	539 > 59	15
$C_{17}H_{28}Cl_8$	17.2	575 > 515	10	575 > 479	10

References

[1] I. Pērkons, L. Lazdina, D. Zacs, Analysis of polychlorinated alkanes in food by liquid chromatography-tandem mass spectrometry, Anal. Bioanal. Chem. 417 (2025) 5533–5544. https://doi.org/10.1007/s00216-025-06070-0.

ANNEX IV: Monitored ions for LC-ESI-HRMS and GC-ECNI-HRMS analysis (Module 3D)

version 2.0

AIV) 1 LC-ESI-HRMS

The exact masses given here can be used for Orbitrap and TOF-MS applications [1,2]. The target adduct ions differ depending on whether chlorine enhancement is used (**Tables AIV.1 – 3**) or not (**Tables AIV4 – 6**).

AIV) 1.1 Chlorine-enhanced analysis

Table AIV.1 Exact masses of extracted ions for determination of PCAs-$C_{10\text{-}13}$ by **chlorine-enhanced LC-HESI-HRMS** with quantification ion (Q) and confirmation ions (CF1, CF2) and relative abundance of the isotopologues.

Homologue group		Adduct	Q *m/z*	CF1 *m/z*	Ion Ratio [%]	CF2 *m/z*	Ion Ratio [%]
Internal standard	$^{13}C_{10}H_{16}Cl_6$	$[M+Cl]^-$	392.93722	394.93427	96.5		
Internal standard	$^{13}C_3C_{10}H_{22}Cl_6$	$[M+Cl]^-$	427.96178	429.95883	96.5		
Internal standard	$^{13}C_3C_{13}H_{28}Cl_6$	$[M+Cl]^-$	470.00873	472.00578	96.5		
Internal standard	$^{13}C_3C_{18}H_{36}Cl_8$	$[M+Cl]^-$	610.00609	612.00314	77.8		
Syringe standard	diuron	$[M+H]^-$	231.0098	233.0069	63.2		
	^{13}C-β-HBCDD	$[M+Cl]^-$	688.6542	690.651	84.4		
C_{10}	Cl_4	$[M+Cl]^-$	314.98271	316.97976	64.4	312.98566	62.1
	Cl_5	$[M+Cl]^-$	348.94374	350.94079	80.4	346.94669	51.8
	Cl_6	$[M+Cl]^-$	382.90477	384.90182	96.5	386.89887	51.7
	Cl_7	$[M+Cl]^-$	418.86285	416.86580	88.9	420.85990	64.3
	Cl_8	$[M+Cl]^-$	452.82387	450.82682	77.8	454.82092	75.0
	Cl_9	$[M+Cl]^-$	486.78490	488.78195	85.7	484.78785	69.2
	Cl_{10}	$[M+Cl]^-$	520.74593	522.74298	96.4	518.74888	62.3
C_{11}	Cl_4	$[M+Cl]^-$	328.99836	330.99541	64.4	327.00131	62.1
	Cl_5	$[M+Cl]^-$	362.95939	364.95644	80.4	360.96234	51.8
	Cl_6	$[M+Cl]^-$	396.92042	398.91747	96.5	400.91452	51.7
	Cl_7	$[M+Cl]^-$	432.87850	430.88145	88.9	434.87555	64.3
	Cl_8	$[M+Cl]^-$	466.83952	464.84247	77.8	468.83657	75.0
	Cl_9	$[M+Cl]^-$	500.80055	502.79760	85.7	498.80350	69.2
	Cl_{10}	$[M+Cl]^-$	534.76158	536.75863	96.3	532.76453	62.3
	Cl_{11}	$[M+Cl]^-$	570.71966	568.72261	93.4	572.71671	72.2
C_{12}	Cl_4	$[M+Cl]^-$	343.01401	345.01106	64.4	341.01696	62.1
	Cl_5	$[M+Cl]^-$	376.97504	378.97209	80.4	374.97799	51.8
	Cl_6	$[M+Cl]^-$	410.93607	412.93312	96.5	414.93017	51.7
	Cl_7	$[M+Cl]^-$	446.89415	444.89710	88.9	448.89120	64.3
	Cl_8	$[M+Cl]^-$	480.85517	478.85812	77.8	482.85222	75.0
	Cl_9	$[M+Cl]^-$	514.81620	516.81325	85.7	512.81915	69.2
	Cl_{10}	$[M+Cl]^-$	548.77723	550.77428	96.3	546.78018	62.3
	Cl_{11}	$[M+Cl]^-$	584.73531	582.73826	93.4	586.73236	72.2
	Cl_{12}	$[M+Cl]^-$	618.69633	616.69928	85.0	620.69338	80.2
C_{13}	Cl_4	$[M+Cl]^-$	357.02966	359.02671	64.3	355.03261	62.2
	Cl_5	$[M+Cl]^-$	390.99069	392.98774	80.4	388.99364	51.8
	Cl_6	$[M+Cl]^-$	424.95172	426.94877	96.4	428.94582	51.7
	Cl_7	$[M+Cl]^-$	460.90980	458.91275	88.9	462.90685	64.3
	Cl_8	$[M+Cl]^-$	494.87082	492.87377	77.8	496.86787	75.0
	Cl_9	$[M+Cl]^-$	528.83185	530.82890	85.6	526.83480	69.2
	Cl_{10}	$[M+Cl]^-$	562.79288	564.78993	96.3	560.79583	62.3
	Cl_{11}	$[M+Cl]^-$	598.75096	596.75391	93.4	600.74801	72.2
	Cl_{12}	$[M+Cl]^-$	632.71198	630.71493	85.0	634.70903	80.2
	Cl_{13}	$[M+Cl]^-$	666.67301	668.67006	88.2	664.67596	77.9

Table AIV.2 Exact masses of extracted ions for determination of PCAs-$C_{14\text{-}17}$ by **chlorine-enhanced LC-HESI-HRMS** with quantification ion (Q) and confirmation ions (CF1, CF2) and relative abundance of the isotopologues.

Homologue group	Adduct	Q *m/z*	CF1 *m/z*	Ion ratio [%]	CF2 *m/z*	Ion ratio [%]
C_{14} Cl_4	$[M+Cl]^-$	371.04531	373.04236	64.3	369.04826	62.2
Cl_5	$[M+Cl]^-$	405.00634	407.00339	80.4	403.00929	51.8
Cl_6	$[M+Cl]^-$	438.96737	440.96442	96.4	442.96147	51.7
Cl_7	$[M+Cl]^-$	474.92545	472.92840	88.9	476.92250	64.3
Cl_8	$[M+Cl]^-$	508.88647	506.88942	77.8	510.88352	74.9
Cl_9	$[M+Cl]^-$	542.84750	544.84455	85.6	540.85045	69.2
Cl_{10}	$[M+Cl]^-$	576.80853	578.80558	96.3	574.81148	62.3
Cl_{11}	$[M+Cl]^-$	612.76661	610.76956	93.5	614.76366	72.2
Cl_{12}	$[M+Cl]^-$	646.72763	644.73058	85.0	648.72468	80.2
Cl_{13}	$[M+Cl]^-$	680.68866	682.68571	88.3	678.69161	77.9
Cl_{14}	$[M+Cl]^-$	714.64969	716.64675	96.3	712.65264	71.9
C_{15} Cl_4	$[M+Cl]^-$	385.06096	387.05801	64.3	383.06391	62.2
Cl_5	$[M+Cl]^-$	419.02199	421.01904	80.4	417.02494	51.8
Cl_6	$[M+Cl]^-$	452.98302	454.98007	96.4	456.97712	51.6
Cl_7	$[M+Cl]^-$	488.94110	486.94405	88.9	490.93815	64.3
Cl_8	$[M+Cl]^-$	522.90212	520.90507	77.8	524.89917	74.9
Cl_9	$[M+Cl]^-$	556.86315	558.86020	85.6	554.86610	69.2
Cl_{10}	$[M+Cl]^-$	590.82418	592.82123	96.3	588.82713	62.3
Cl_{11}	$[M+Cl]^-$	626.78226	624.78521	93.5	628.77931	72.2
Cl_{12}	$[M+Cl]^-$	660.74329	658.74624	85.0	662.74034	80.2
Cl_{13}	$[M+Cl]^-$	694.70431	696.70136	88.2	692.70726	77.9
Cl_{14}	$[M+Cl]^-$	728.66534	730.66239	96.3	726.66830	72.0
Cl_{15}	$[M+Cl]^-$	764.62342	762.62638	96.0	766.62047	77.0
C_{16} Cl_4	$[M+Cl]^-$	399.07661	401.07366	64.3	397.07956	62.2
Cl_5	$[M+Cl]^-$	433.03764	435.03469	80.4	431.04059	51.8
Cl_6	$[M+Cl]^-$	466.99867	468.99572	96.4	470.99277	51.6
Cl_7	$[M+Cl]^-$	502.95675	500.95970	88.9	504.95380	64.2
Cl_8	$[M+Cl]^-$	536.91777	534.92072	77.8	538.91482	74.9
Cl_9	$[M+Cl]^-$	570.87880	572.87585	85.6	568.88175	69.2
Cl_{10}	$[M+Cl]^-$	604.83983	606.83688	96.3	602.84278	62.3
Cl_{11}	$[M+Cl]^-$	640.79791	638.80086	93.5	642.79496	72.2
Cl_{12}	$[M+Cl]^-$	674.75894	672.76189	85.0	676.75599	80.2
Cl_{13}	$[M+Cl]^-$	708.71997	710.71701	88.2	706.72292	77.9
Cl_{14}	$[M+Cl]^-$	742.68100	744.67804	96.2	740.68394	71.9
Cl_{15}	$[M+Cl]^-$	778.63908	776.64203	96.0	780.63614	77.1
Cl_{16}	$[M+Cl]^-$	812.60012	810.60306	89.0	814.59717	83.4
C_{17} Cl_4	$[M+Cl]^-$	413.09226	415.08931	64.3	411.09521	62.2
Cl_5	$[M+Cl]^-$	447.05329	449.05034	80.3	445.05624	51.9
Cl_6	$[M+Cl]^-$	481.01432	483.01137	96.4	485.00842	51.6
Cl_7	$[M+Cl]^-$	516.97240	514.97535	89.0	518.96945	64.2
Cl_8	$[M+Cl]^-$	550.93342	548.93637	77.9	552.93047	74.9
Cl_9	$[M+Cl]^-$	584.89445	586.89150	85.6	582.89740	69.2
Cl_{10}	$[M+Cl]^-$	618.85548	620.85253	96.3	616.85843	62.3
Cl_{11}	$[M+Cl]^-$	654.81356	652.81651	93.5	656.81061	72.2
Cl_{12}	$[M+Cl]^-$	688.77459	686.77754	85.0	690.77164	80.3
Cl_{13}	$[M+Cl]^-$	722.73562	724.73267	88.3	720.73857	77.9
Cl_{14}	$[M+Cl]^-$	756.69665	758.69371	96.3	754.69959	71.9
Cl_{15}	$[M+Cl]^-$	792.65474	790.65768	95.8	794.65180	77.1
Cl_{16}	$[M+Cl]^-$	826.61577	824.61872	89.1	828.61282	83.4
Cl_{17}	$[M+Cl]^-$	860.57690	862.57388	89.1	858.57981	82.8

Table AIV.3 Exact masses of extracted ions for determination of PCAs-C_{18-20} by **chlorine-enhanced LC-HESI-HRMS** with quantification ion (Q) and confirmation ions (CF1, CF2) and relative abundance of the isotopologues.

Homologue group	Adduct	Q *m/z*	CF1 *m/z*	Ion ratio [%]	CF2 *m/z*	Ion ratio [%]
C_{18}	Cl_4	$[M+Cl]^-$	427.10791	429.10496	64.3	425.11086	62.2
	Cl_5	$[M+Cl]^-$	461.06894	463.06599	80.3	459.07189	51.9
	Cl_6	$[M+Cl]^-$	495.02997	497.02702	96.4	499.02407	51.6
	Cl_7	$[M+Cl]^-$	530.98805	528.99100	89.0	532.98510	64.2
	Cl_8	$[M+Cl]^-$	564.94907	562.95202	77.9	566.94612	74.9
	Cl_9	$[M+Cl]^-$	598.91010	600.90715	85.6	596.91305	69.2
	Cl_{10}	$[M+Cl]^-$	632.87113	634.86818	96.3	630.87408	62.3
	Cl_{11}	$[M+Cl]^-$	668.82921	666.83216	93.5	670.82626	72.2
	Cl_{12}	$[M+Cl]^-$	702.79024	700.79319	85.0	704.78729	80.2
	Cl_{13}	$[M+Cl]^-$	736.75127	738.74832	88.2	734.75422	78.0
	Cl_{14}	$[M+Cl]^-$	770.71230	772.70936	96.4	768.71525	72.0
	Cl_{15}	$[M+Cl]^-$	806.67039	804.67333	95.9	808.66744	77.0
	Cl_{16}	$[M+Cl]^-$	840.63142	838.63438	89.2	842.62848	83.5
	Cl_{17}	$[M+Cl]^-$	874.59256	876.58966	90.2	872.59547	82.8
	Cl_{18}	$[M+Cl]^-$	908.55358	910.55068	96.6	906.55649	77.6
C_{19}	Cl_4	$[M+Cl]^-$	441.12356	443.12061	64.3	439.12651	62.2
	Cl_5	$[M+Cl]^-$	475.08459	477.08164	80.3	473.08754	51.9
	Cl_6	$[M+Cl]^-$	509.04562	511.04267	96.4	513.03972	51.6
	Cl_7	$[M+Cl]^-$	545.00370	543.00665	89.0	547.00075	64.2
	Cl_8	$[M+Cl]^-$	578.96472	576.96767	77.9	580.96177	74.9
	Cl_9	$[M+Cl]^-$	612.92575	614.92280	85.6	610.92870	69.2
	Cl_{10}	$[M+Cl]^-$	646.88678	648.88383	96.3	644.88973	62.3
	Cl_{11}	$[M+Cl]^-$	682.84486	680.84781	93.5	684.84191	72.2
	Cl_{12}	$[M+Cl]^-$	716.80589	714.80884	84.9	718.80294	80.2
	Cl_{13}	$[M+Cl]^-$	750.76693	752.76397	88.2	748.76987	77.8
	Cl_{14}	$[M+Cl]^-$	784.72796	786.72502	96.3	782.73091	71.9
	Cl_{15}	$[M+Cl]^-$	820.68604	818.68900	96.0	822.68312	77.2
	Cl_{16}	$[M+Cl]^-$	854.64709	852.65011	89.9	856.64416	83.6
	Cl_{17}	$[M+Cl]^-$	888.60823	890.60533	90.3	886.61113	82.8
	Cl_{18}	$[M+Cl]^-$	922.56925	924.56635	96.7	920.57216	77.6
	Cl_{19}	$[M+Cl]^-$	958.52736	956.53027	97.0	960.52446	80.6
C_{20}	Cl_4	$[M+Cl]^-$	455.13921	457.13626	64.3	453.14216	62.2
	Cl_5	$[M+Cl]^-$	489.10024	491.09729	80.3	487.10319	51.9
	Cl_6	$[M+Cl]^-$	523.06127	525.05832	96.4	527.05537	51.6
	Cl_7	$[M+Cl]^-$	559.01935	557.02230	89.0	561.01640	64.2
	Cl_8	$[M+Cl]^-$	592.98037	590.98332	77.9	594.97742	74.9
	Cl_9	$[M+Cl]^-$	626.94140	628.93845	85.6	624.94435	69.2
	Cl_{10}	$[M+Cl]^-$	660.90243	662.89948	96.3	658.90538	62.3
	Cl_{11}	$[M+Cl]^-$	696.86051	694.86346	93.5	698.85756	72.2
	Cl_{12}	$[M+Cl]^-$	730.82154	728.82449	85.1	732.81859	80.3
	Cl_{13}	$[M+Cl]^-$	764.78258	766.77963	88.3	762.78552	77.9
	Cl_{14}	$[M+Cl]^-$	798.74362	800.74067	96.2	796.74656	71.9
	Cl_{15}	$[M+Cl]^-$	834.70172	832.70464	95.6	836.69877	77.0
	Cl_{16}	$[M+Cl]^-$	868.66276	866.66578	89.8	870.65983	83.6
	Cl_{17}	$[M+Cl]^-$	902.62390	904.62101	90.3	900.62680	82.7
	Cl_{18}	$[M+Cl]^-$	936.58492	938.58202	96.7	934.58782	77.6
	Cl_{19}	$[M+Cl]^-$	972.54303	970.54593	97.0	974.54014	80.6
	Cl_{20}	$[M+Cl]^-$	1006.50405	1004.50695	91.3	1008.50115	86.0

AIV) 1.2 Analysis without chlorine dopant

Without chlorine enhancement monitoring of the $[M-H]^-$ ion species is more reliable for LC-ESI-HRMS analysis. The **Tables AIV4 – 6** summarize the corresponding *m/z* values and isotopologue ratios.

Table AIV. 4 Exact masses of extracted ions for determination of PCAs-C_{10-13} by **LC-HESI-HRMS** with quantification ion (Q) and confirmation ions (CF1, CF2) and relative abundance of the isotopologues.

Homologue groups		Adduct	Q *m/z*	CF1 *m/z*	Ion ratio [%]	CF2 *m/z*	Ion ratio [%]
C_{10}	Cl_4	$[M-H]^-$	279.00603	277.00898	77.6	281.00308	48.3
	Cl_5	$[M-H]^-$	312.96706	314.96411	64.4	310.97001	62.1
	Cl_6	$[M-H]^-$	346.92809	348.92514	80.4	344.93104	51.8
	Cl_7	$[M-H]^-$	380.88912	382.88617	96.5	384.88322	51.7
	Cl_8	$[M-H]^-$	416.84720	414.85015	88.9	418.84425	64.3
	Cl_9	$[M-H]^-$	450.80822	448.81117	77.8	452.80527	75.0
	Cl_{10}	$[M-H]^-$	484.76925	486.76630	85.7	482.77220	69.2
C_{11}	Cl_4	$[M-H]^-$	293.02168	291.02464	77.6	295.01873	48.3
	Cl_5	$[M-H]^-$	326.98271	328.97976	64.4	324.98566	62.1
	Cl_6	$[M-H]^-$	360.94374	362.94079	80.4	358.94669	51.8
	Cl_7	$[M-H]^-$	394.90477	396.90182	96.5	398.89887	51.7
	Cl_8	$[M-H]^-$	430.86285	428.86580	88.9	432.85990	64.3
	Cl_9	$[M-H]^-$	464.82387	462.82682	77.8	466.82092	75.0
	Cl_{10}	$[M-H]^-$	498.78490	500.78195	85.7	496.78785	69.2
	Cl_{11}	$[M-H]^-$	532.74593	534.74298	96.3	530.74888	62.3
C_{12}	Cl_4	$[M-H]^-$	307.03733	305.04029	77.6	309.03438	48.3
	Cl_5	$[M-H]^-$	340.99836	342.99541	64.4	339.00131	62.1
	Cl_6	$[M-H]^-$	374.95939	376.95644	80.4	372.96234	51.8
	Cl_7	$[M-H]^-$	408.92042	410.91747	96.5	412.91452	51.7
	Cl_8	$[M-H]^-$	444.87850	442.88145	88.9	446.87555	64.3
	Cl_9	$[M-H]^-$	478.83952	476.84247	77.8	480.83657	75.0
	Cl_{10}	$[M-H]^-$	512.80055	514.79760	85.7	510.80350	69.2
	Cl_{11}	$[M-H]^-$	546.76158	548.75863	96.3	544.76453	62.3
	Cl_{12}	$[M-H]^-$	582.71966	580.72261	93.4	584.71671	72.2
C_{13}	Cl_4	$[M-H]^-$	321.05299	319.05594	77.6	323.05003	62.3
	Cl_5	$[M-H]^-$	355.01401	357.01106	64.4	353.01696	72.2
	Cl_6	$[M-H]^-$	388.97504	390.97209	80.4	386.97799	80.2
	Cl_7	$[M-H]^-$	422.93607	424.93312	96.4	426.93017	48.3
	Cl_8	$[M-H]^-$	458.89415	456.89710	88.9	460.89120	62.2
	Cl_9	$[M-H]^-$	492.85517	490.85812	77.8	494.85222	51.8
	Cl_{10}	$[M-H]^-$	526.81620	528.81325	85.6	524.81915	51.7
	Cl_{11}	$[M-H]^-$	560.77723	562.77428	96.3	558.78018	64.3
	Cl_{12}	$[M-H]^-$	596.73531	594.73826	93.5	598.73236	75.0
	Cl_{13}	$[M-H]^-$	630.69633	628.69928	85.0	632.69338	69.2

Table AIV.5 Exact masses of extracted ions for determination of PCA-C_{14-17} by **LC-HESI-HRMS** with quantification ion (Q) and confirmation ions (CF1, CF2) and relative abundance of the isotopologues.

Homologue group	Adduct	Q *m/z*	CF1 *m/z*	Ion ratio [%]	CF2 *m/z*	Ion ratio [%]
C_{14}	Cl_4	[M-H]$^-$	335.06864	333.07159	77.7	337.06569	48.3
	Cl_5	[M-H]$^-$	369.02966	371.02671	64.3	367.03261	62.2
	Cl_6	[M-H]$^-$	402.99069	404.98774	80.4	400.99364	51.8
	Cl_7	[M-H]$^-$	436.95172	438.94877	96.4	440.94582	51.7
	Cl_8	[M-H]$^-$	472.90980	470.91275	88.9	474.90685	64.3
	Cl_9	[M-H]$^-$	506.87082	504.87377	77.8	508.86787	75.0
	Cl_{10}	[M-H]$^-$	540.83185	542.82890	85.6	538.83480	69.2
	Cl_{11}	[M-H]$^-$	574.79288	576.78993	96.3	572.79583	62.3
	Cl_{12}	[M-H]$^-$	610.75096	608.75391	93.5	612.74801	72.2
	Cl_{13}	[M-H]$^-$	644.71198	642.71493	85.0	646.70903	80.2
	Cl_{14}	[M-H]$^-$	678.67301	680.67006	88.2	676.67596	77.9
C_{15}	Cl_4	[M-H]$^-$	349.08429	347.08724	77.7	351.08134	48.3
	Cl_5	[M-H]$^-$	383.04531	385.04236	64.3	381.04826	62.2
	Cl_6	[M-H]$^-$	417.00634	419.00339	80.4	415.00929	51.8
	Cl_7	[M-H]$^-$	450.96737	452.96442	96.4	454.96147	51.6
	Cl_8	[M-H]$^-$	486.92545	484.92840	88.9	488.92250	64.3
	Cl_9	[M-H]$^-$	520.88647	518.88942	77.8	522.88352	74.9
	Cl_{10}	[M-H]$^-$	554.84750	556.84455	85.6	552.85045	69.2
	Cl_{11}	[M-H]$^-$	588.80853	590.80558	96.3	586.81148	62.3
	Cl_{12}	[M-H]$^-$	624.76661	622.76956	93.5	626.76366	72.2
	Cl_{13}	[M-H]$^-$	658.72764	656.73058	85.0	660.72468	80.2
	Cl_{14}	[M-H]$^-$	692.68866	694.68571	88.2	690.69161	77.9
	Cl_{15}	[M-H]$^-$	726.64969	728.64675	96.3	724.65264	71.9
C_{16}	Cl_4	[M-H]$^-$	363.09994	361.10289	77.7	365.09699	48.3
	Cl_5	[M-H]$^-$	397.06096	399.05801	64.3	395.06391	62.2
	Cl_6	[M-H]$^-$	431.02199	433.01904	80.4	429.02494	51.8
	Cl_7	[M-H]$^-$	464.98302	466.98007	96.4	468.97712	51.6
	Cl_8	[M-H]$^-$	500.94110	498.94405	88.9	502.93815	64.2
	Cl_9	[M-H]$^-$	534.90212	532.90507	77.8	536.89917	74.9
	Cl_{10}	[M-H]$^-$	568.86315	570.86020	85.6	566.86610	69.2
	Cl_{11}	[M-H]$^-$	602.82418	604.82123	96.3	600.82713	62.3
	Cl_{12}	[M-H]$^-$	638.78226	636.78521	93.5	640.77931	72.2
	Cl_{13}	[M-H]$^-$	672.74329	670.74623	85.0	674.74034	80.2
	Cl_{14}	[M-H]$^-$	706.70432	708.70136	88.2	704.70726	77.9
	Cl_{15}	[M-H]$^-$	740.66535	742.66239	96.2	738.66829	71.9
	Cl_{16}	[M-H]$^-$	776.62343	774.62638	95.9	778.62048	77.0
C_{17}	Cl_4	[M-H]$^-$	377.11559	375.11854	77.7	379.11264	48.2
	Cl_5	[M-H]$^-$	411.07661	413.07366	64.3	409.07956	62.2
	Cl_6	[M-H]$^-$	445.03764	447.03469	80.3	443.04059	51.9
	Cl_7	[M-H]$^-$	478.99867	480.99572	96.4	482.99277	51.6
	Cl_8	[M-H]$^-$	514.95675	512.95970	89.0	516.95380	64.2
	Cl_9	[M-H]$^-$	548.91777	546.92072	77.9	550.91482	74.9
	Cl_{10}	[M-H]$^-$	582.87880	584.87585	85.6	580.88175	69.2
	Cl_{11}	[M-H]$^-$	616.83983	618.83688	96.3	614.84278	62.3
	Cl_{12}	[M-H]$^-$	652.79791	650.80086	93.5	654.79496	72.2
	Cl_{13}	[M-H]$^-$	686.75894	684.76189	85.0	688.75599	80.2
	Cl_{14}	[M-H]$^-$	720.71997	722.71702	88.2	718.72291	77.9
	Cl_{15}	[M-H]$^-$	754.68100	756.67805	96.3	752.68394	71.9
	Cl_{16}	[M-H]$^-$	790.63908	788.64203	95.9	792.63613	77.0
	Cl_{17}	[M-H]$^-$	824.60011	822.60307	89.1	826.59718	83.6

Table AIV.6 Exact masses of extracted ions for determination of PCAs-C_{18-20} by **LC-HESI-HRMS** with quantification ion (Q) and confirmation ions (CF1, CF2) and relative abundance of the isotopologues.

Homologue group	Adduct	Q *m/z*	CF1 *m/z*	Ion ratio [%]	CF2 *m/z*	Ion ratio [%]
C_{18}	Cl_4	[M-H]$^-$	391.13124	389.13419	77.7	393.12829	48.2
	Cl_5	[M-H]$^-$	425.09226	427.08931	64.3	423.09521	62.2
	Cl_6	[M-H]$^-$	459.05329	461.05034	80.3	457.05624	51.9
	Cl_7	[M-H]$^-$	493.01432	495.01137	96.4	497.00842	51.6
	Cl_8	[M-H]$^-$	528.97240	526.97535	89.0	530.96945	64.2
	Cl_9	[M-H]$^-$	562.93342	560.93637	77.9	564.93047	74.9
	Cl_{10}	[M-H]$^-$	596.89445	598.89150	85.6	594.89740	69.2
	Cl_{11}	[M-H]$^-$	630.85548	632.85253	96.3	628.85843	62.3
	Cl_{12}	[M-H]$^-$	666.81356	664.81651	93.5	668.81061	72.2
	Cl_{13}	[M-H]$^-$	700.77459	698.77754	85.0	702.77164	80.2
	Cl_{14}	[M-H]$^-$	734.73562	736.73267	88.2	732.73857	77.9
	Cl_{15}	[M-H]$^-$	768.69665	770.69370	96.2	766.69960	71.9
	Cl_{16}	[M-H]$^-$	804.65474	802.65768	95.8	806.65180	77.1
	Cl_{17}	[M-H]$^-$	838.61578	836.61873	89.2	840.61284	83.5
	Cl_{18}	[M-H]$^-$	872.57691	874.57401	90.2	870.57982	82.8
C_{19}	Cl_4	[M-H]$^-$	405.14689	403.14984	77.8	407.14394	48.2
	Cl_5	[M-H]$^-$	439.10791	441.10496	64.3	437.11086	62.2
	Cl_6	[M-H]$^-$	473.06894	475.06599	80.3	471.07189	51.9
	Cl_7	[M-H]$^-$	507.02997	509.02702	96.4	511.02407	51.6
	Cl_8	[M-H]$^-$	542.98805	540.99100	89.0	544.98510	64.2
	Cl_9	[M-H]$^-$	576.94907	574.95202	77.9	578.94612	74.9
	Cl_{10}	[M-H]$^-$	610.91010	612.90715	85.6	608.91305	69.2
	Cl_{11}	[M-H]$^-$	644.87113	646.86818	96.3	642.87408	62.3
	Cl_{12}	[M-H]$^-$	680.82921	678.83216	93.5	682.82626	72.2
	Cl_{13}	[M-H]$^-$	714.79024	712.79319	85.0	716.78729	80.3
	Cl_{14}	[M-H]$^-$	748.75128	750.74833	88.2	746.75422	77.9
	Cl_{15}	[M-H]$^-$	782.71230	784.70936	96.3	780.71526	72.0
	Cl_{16}	[M-H]$^-$	818.67039	816.67333	95.8	820.66745	77.1
	Cl_{17}	[M-H]$^-$	852.63142	850.63438	89.1	854.62851	83.7
	Cl_{18}	[M-H]$^-$	886.59258	888.58968	90.3	884.59548	82.8
	Cl_{19}	[M-H]$^-$	920.55360	922.55070	96.7	918.55651	77.6
C_{20}	Cl_4	[M-H]$^-$	419.16254	417.16549	77.8	421.15959	48.2
	Cl_5	[M-H]$^-$	453.12356	455.12061	64.3	451.12651	62.2
	Cl_6	[M-H]$^-$	487.08459	489.08164	80.3	485.08754	51.9
	Cl_7	[M-H]$^-$	521.04562	523.04267	96.4	525.03972	51.6
	Cl_8	[M-H]$^-$	557.00370	555.00665	89.0	559.00075	64.2
	Cl_9	[M-H]$^-$	590.96472	588.96767	77.9	592.96177	74.9
	Cl_{10}	[M-H]$^-$	624.92575	626.92280	85.6	622.92870	69.2
	Cl_{11}	[M-H]$^-$	658.88678	660.88383	96.3	656.88973	62.3
	Cl_{12}	[M-H]$^-$	694.84486	692.84781	93.5	696.84191	72.2
	Cl_{13}	[M-H]$^-$	728.80589	726.80884	85.0	730.80294	80.2
	Cl_{14}	[M-H]$^-$	762.76692	764.76397	88.2	760.76987	77.9
	Cl_{15}	[M-H]$^-$	796.72796	798.72502	96.3	794.73090	71.9
	Cl_{16}	[M-H]$^-$	832.68605	830.68901	96.0	834.68310	76.9
	Cl_{17}	[M-H]$^-$	866.64711	864.65013	89.7	868.64417	83.4
	Cl_{18}	[M-H]$^-$	900.60825	902.60536	90.3	898.61115	82.7
	Cl_{19}	[M-H]$^-$	934.56927	936.56637	96.7	932.57217	77.6
	Cl_{20}	[M-H]$^-$	970.52738	968.53028	97.0	972.52449	80.6

AIV) 2 GC-ECNI-HRMS

GC-ECNI-methods (**Module 3B and 3D**) usually monitor $[M\text{-}Cl]^-$ or $[M\text{-}HCl]^-$ adduct ions depending on the PCA homologue groups. This is based on experiments monitoring the abundance of different adduct ions in ECNI, which showed that especially with lowering the overall chlorination degree, the $[M\text{-}HCl]^-$ adduct ions became as abundant as $[M\text{-}Cl]^-$ [3]. For more information, please refer to **Figure AII.2** in **Annex II**.

Furthermore, this annex also supplied theoretical masses and ion ratios for some PCAs with chain length > C_{17}. Please note that only some of them will be visible when using GC-ENCI-HRMS, and then only in much higher concentrations than PCAs with shorter carbon chains.

Table AIV.7 Exact masses of extracted ions for determination of PCAs-$C_{10\text{-}13}$ by **GC-ECNI-HRMS** with quantification ion (Q) and confirmation ions (CF1, CF2) and relative abundance of the isotopologues.

Homologue group		Adduct	Q *m/z*	CF1 *m/z*	Ion ratio [%]	CF2 *m/z*	Ion ratio [%]
Syringe standard	ε-HCH	$[M\text{-}Cl]^-$	254.88881	256.88586	64.5	252.89176	62.0
Internal standard	$^{13}C_{10}H_{16}Cl_6$	$[M\text{-}Cl]^-$	323.00061	324.99766	63.9	321.00356	62.6
Internal standard	$^{13}C_3C_8H_{18}Cl_6$	$[M\text{-}Cl]^-$	329.99278	331.98983	63.9	327.99573	62.6
Internal standard	$^{13}C_3C_9H_{20}Cl_6$	$[M\text{-}HCl]^-$	343.00060	344.99765	63.9	341.00355	62.6
Internal standard	$^{13}C_3C_{10}H_{22}Cl_6$	$[M\text{-}HCl]^-$	357.01625	359.01330	63.9	355.019200	62.6
Internal standard	$^{13}C_3C_{11}H_{24}Cl_6$	$[M\text{-}HCl]^-$	371.03190	373.02895	63.9	369.034850	62.6
Internal standard	$^{13}C_3C_{12}H_{26}Cl_6$	$[M\text{-}HCl]^-$	385.04755	387.04460	63.9	383.050500	62.6
Internal standard	$^{13}C_3C_{13}H_{28}Cl_6$	$[M\text{-}HCl]^-$	399.06320	401.06025	63.9	397.06615	62.6
C_{10}	Cl_5	$[M\text{-}HCl]^-$	277.99821	276.00116	77.6	279.99526	48.3
	Cl_6	$[M\text{-}HCl]^-$	312.96706	314.96411	64.4	310.97001	62.1
	Cl_7	$[M\text{-}Cl]^-$	346.92809	348.92514	80.5	344.93104	51.8
	Cl_8	$[M\text{-}Cl]^-$	380.88912	382.88617	96.5	384.88322	51.7
	Cl_9	$[M\text{-}Cl]^-$	416.8472	414.85015	88.9	418.84425	64.3
	Cl_{10}	$[M\text{-}Cl]^-$	450.80822	448.81117	77.8	452.80527	75.0
C_{11}	Cl_5	$[M\text{-}HCl]^-$	292.01386	290.01681	77.6	294.01091	48.3
	Cl_6	$[M\text{-}HCl]^-$	325.97489	327.97194	64.4	323.97784	62.1
	Cl_7	$[M\text{-}Cl]^-$	360.94374	362.94079	80.4	358.94669	51.8
	Cl_8	$[M\text{-}Cl]^-$	394.90477	396.90182	96.5	398.89887	51.7
	Cl_9	$[M\text{-}Cl]^-$	430.86285	428.8658	88.9	432.8599	64.3
	Cl_{10}	$[M\text{-}Cl]^-$	464.82387	462.82682	77.8	466.82092	75.0
	Cl_{11}	$[M\text{-}Cl]^-$	498.7849	500.78195	85.7	496.78785	69.2
C_{12}	Cl_5	$[M\text{-}HCl]^-$	306.02951	304.03246	77.6	308.02656	48.3
	Cl_6	$[M\text{-}HCl]^-$	339.99054	341.98759	64	337.99349	62.5
	Cl_7	$[M\text{-}Cl]^-$	374.95939	376.95644	80.4	372.96234	51.8
	Cl_8	$[M\text{-}Cl]^-$	408.92042	410.91747	96.5	412.91452	51.7
	Cl_9	$[M\text{-}Cl]^-$	444.8785	442.88145	88.9	446.87555	64.3
	Cl_{10}	$[M\text{-}Cl]^-$	478.83952	476.84247	77.8	480.83658	75.0
	Cl_{11}	$[M\text{-}Cl]^-$	512.80055	514.79761	85.7	510.8035	69.2
	Cl_{12}	$[M\text{-}Cl]^-$	546.76158	548.75863	96.3	544.76453	62.3
C_{13}	Cl_5	$[M\text{-}HCl]^-$	320.04516	318.04811	77.7	322.04221	48.3
	Cl_6	$[M\text{-}HCl]^-$	354.00619	356.00324	64.4	352.00914	62.6
	Cl_7	$[M\text{-}Cl]^-$	388.97504	390.97209	80.4	386.97799	51.8
	Cl_8	$[M\text{-}Cl]^-$	422.93607	424.93312	96.4	426.93017	51.7
	Cl_9	$[M\text{-}Cl]^-$	458.89415	456.8971	88.9	460.8912	64.3
	Cl_{10}	$[M\text{-}Cl]^-$	492.85517	490.85812	77.8	494.85222	75.0
	Cl_{11}	$[M\text{-}Cl]^-$	526.8162	528.81325	85.7	524.81915	69.2
	Cl_{12}	$[M\text{-}Cl]^-$	560.77723	562.77428	96.3	558.78018	62.3
	Cl_{13}	$[M\text{-}Cl]^-$	596.73531	594.73826	93.5	598.73236	72.2

Table AIV.8 Exact masses of extracted ions for determination of PCAs-C_{14-17} by **GC-ECNI-HRMS** with quantification ion (Q) and confirmation ions (CF1, CF2) and relative abundance of the isotopologues.

Homologue group		Adduct	Q *m/z*	CF1 *m/z*	Ion ratio [%]	CF2 *m/z*	Ion ratio [%]
C_{14}	Cl_5	$[M\text{-}HCl]^-$	334.06081	332.06376	77.7	336.05786	48.3
	Cl_6	$[M\text{-}HCl]^-$	368.02184	370.01889	64.3	366.02479	62.2
	Cl_7	$[M\text{-}Cl]^-$	402.99069	404.98774	80.4	400.99364	51.8
	Cl_8	$[M\text{-}Cl]^-$	436.95172	438.94877	96.4	440.94582	51.7
	Cl_9	$[M\text{-}Cl]^-$	472.9098	470.91275	88.9	474.90685	64.3
	Cl_{10}	$[M\text{-}Cl]^-$	506.87082	504.87377	77.8	508.86787	74.9
	Cl_{11}	$[M\text{-}Cl]^-$	540.83185	542.8289	85.6	538.8348	69.2
	Cl_{12}	$[M\text{-}Cl]^-$	574.79288	576.78993	96.3	572.79583	62.3
	Cl_{13}	$[M\text{-}Cl]^-$	610.75096	608.75391	93.5	604.75981	72.2
	Cl_{14}	$[M\text{-}Cl]^-$	644.71198	642.71493	85.0	646.70903	80.2
C_{15}	Cl_5	$[M\text{-}HCl]^-$	348.07646	346.07941	77.7	350.07351	48.3
	Cl_6	$[M\text{-}HCl]^-$	382.03749	384.03454	64.3	380.04044	62.2
	Cl_7	$[M\text{-}Cl]^-$	417.00634	419.00339	80.4	415.00929	51.8
	Cl_8	$[M\text{-}Cl]^-$	450.96737	452.96442	96.4	454.96147	51.6
	Cl_9	$[M\text{-}Cl]^-$	486.92545	484.9284	88.9	488.9225	64.3
	Cl_{10}	$[M\text{-}Cl]^-$	520.88647	518.88942	77.8	522.88352	74.9
	Cl_{11}	$[M\text{-}Cl]^-$	554.8475	556.84455	85.6	552.85045	69.2
	Cl_{12}	$[M\text{-}Cl]^-$	588.80853	590.80558	96.3	586.81148	62.3
	Cl_{13}	$[M\text{-}Cl]^-$	624.76661	622.76956	93.5	626.76366	72.2
	Cl_{14}	$[M\text{-}Cl]^-$	658.72763	656.73058	85.0	660.72468	80.2
	Cl_{15}	$[M\text{-}Cl]^-$	692.68866	694.68571	88.3	690.69161	77.9
C_{16}	Cl_5	$[M\text{-}HCl]^-$	362.09211	360.09506	77.7	364.08916	48.3
	Cl_6	$[M\text{-}HCl]^-$	396.05314	398.05019	64.3	394.05609	62.2
	Cl_7	$[M\text{-}Cl]^-$	431.02199	433.01904	80.4	429.02494	51.8
	Cl_8	$[M\text{-}Cl]^-$	464.98302	466.98007	96.4	468.97712	51.6
	Cl_9	$[M\text{-}Cl]^-$	500.9411	498.94405	88.9	502.93815	64.3
	Cl_{10}	$[M\text{-}Cl]^-$	534.90212	532.90507	77.8	536.89917	74.9
	Cl_{11}	$[M\text{-}Cl]^-$	568.86315	570.8602	85.6	566.8661	69.2
	Cl_{12}	$[M\text{-}Cl]^-$	602.82418	604.82123	96.3	600.82713	62.3
	Cl_{13}	$[M\text{-}Cl]^-$	638.78226	636.78521	93.5	640.77931	72.2
	Cl_{14}	$[M\text{-}Cl]^-$	672.74328	670.74623	85.0	674.74033	80.2
	Cl_{15}	$[M\text{-}Cl]^-$	706.70431	708.70136	88.2	704.70726	77.9
	Cl_{16}	$[M\text{-}Cl]^-$	740.66534	742.66239	96.3	738.66829	71.9
C_{17}	Cl_5	$[M\text{-}HCl]^-$	376.10776	374.11071	77.7	378.10481	48.3
	Cl_6	$[M\text{-}HCl]^-$	410.06879	408.07174	62.2	412.06584	64.3
	Cl_7	$[M\text{-}Cl]^-$	445.03764	447.03469	80.4	443.04059	51.8
	Cl_8	$[M\text{-}Cl]^-$	478.99867	480.99572	96.4	482.99277	51.6
	Cl_9	$[M\text{-}Cl]^-$	514.95675	512.9597	88.9	516.9538	64.2
	Cl_{10}	$[M\text{-}Cl]^-$	548.91777	546.92072	77.8	550.91482	74.9
	Cl_{11}	$[M\text{-}Cl]^-$	582.8788	584.87585	85.6	580.88175	69.2
	Cl_{12}	$[M\text{-}Cl]^-$	616.83983	618.83688	96.3	614.84278	62.3
	Cl_{13}	$[M\text{-}Cl]^-$	652.79791	650.80086	93.5	654.79496	72.2
	Cl_{14}	$[M\text{-}Cl]^-$	686.75893	684.76189	85.0	688.75599	80.2
	Cl_{15}	$[M\text{-}Cl]^-$	720.71996	722.71701	88.2	718.72291	77.9
	Cl_{16}	$[M\text{-}Cl]^-$	754.68099	756.67804	96.3	752.68394	71.9
	Cl_{17}	$[M\text{-}Cl]^-$	790.63907	788.64202	95.9	792.63613	77.0

Table AIV.9 Exact masses of extracted ions for the determination of some PCAs-C_{18-20} by **GC-ECNI-HRMS** with quantification ion (Q) and confirmation ions (CF1, CF2) and relative abundance of the isotopologues.
Note that not all of these compounds will actually be visible when using this method!

Homologue group		Adduct	Q *m/z*	CF1 *m/z*	Ion ratio [%]	CF2 *m/z*	Ion ratio [%]
C_{18}	Cl_5	$[M\text{-}HCl]^-$	390.1231	388.12636	78.1	392.12046	48.0
	Cl_6	$[M\text{-}HCl]^-$	424.08444	426.08149	64.0	422.08739	62.5
	Cl_7	$[M\text{-}Cl]^-$	459.05329	461.05034	80.0	457.05624	52.1
	Cl_8	$[M\text{-}Cl]^-$	493.01432	495.01137	96.0	497.00842	51.2
	Cl_9	$[M\text{-}Cl]^-$	528.97239	526.97535	89.3	530.96945	64.0
	Cl_{10}	$[M\text{-}Cl]^-$	562.93342	560.93637	78.1	564.93047	74.7
	Cl_{11}	$[M\text{-}Cl]^-$	596.89445	598.8915	85.3	594.8974	69.4
	Cl_{12}	$[M\text{-}Cl]^-$	630.85545	632.85253	96.0	628.85843	62.5
	Cl_{13}	$[M\text{-}Cl]^-$	666.81356	664.81651	93.8	668.81061	72.0
	Cl_{14}	$[M\text{-}Cl]^-$	700.77458	698.77753	85.2	702.77163	80.0
	Cl_{15}	$[M\text{-}Cl]^-$	734.73561	736.73266	88.0	732.73856	78.1
	Cl_{16}	$[M\text{-}Cl]^-$	768.69664	770.69369	96.0	766.69959	72.1
C_{19}	Cl_5	$[M\text{-}HCl]^-$	404.13906	402.14201	78.1	406.13611	48.0
	Cl_6	$[M\text{-}HCl]^-$	438.10009	440.09714	64.0	436.10304	62.5
	Cl_7	$[M\text{-}Cl]^-$	473.06894	475.06599	80.0	471.07189	52.1
	Cl_8	$[M\text{-}Cl]^-$	507.02997	509.02702	96.0	511.02407	51.2
	Cl_9	$[M\text{-}Cl]^-$	542.98804	540.99099	89.3	544.98509	64.0
	Cl_{10}	$[M\text{-}Cl]^-$	576.94907	574.95202	78.1	578.94612	74.7
	Cl_{11}	$[M\text{-}Cl]^-$	610.9101	612.90715	85.3	608.91305	69.4
	Cl_{12}	$[M\text{-}Cl]^-$	644.87113	646.86818	96.0	642.87408	62.5
	Cl_{13}	$[M\text{-}Cl]^-$	680.82921	678.83216	93.8	682.82626	72.0
	Cl_{14}	$[M\text{-}Cl]^-$	714.79023	712.79318	85.2	716.78728	80.0
	Cl_{15}	$[M\text{-}Cl]^-$	748.75126	750.74831	88.0	746.75421	78.1
	Cl_{16}	$[M\text{-}Cl]^-$	782.71229	784.70934	96.0	780.71524	72.1
C_{20}	Cl_5	$[M\text{-}HCl]^-$	418.15471	416.15766	78.1	420.15176	48.0
	Cl_6	$[M\text{-}HCl]^-$	452.11574	454.11279	64.0	450.11869	62.5
	Cl_7	$[M\text{-}Cl]^-$	487.08459	489.08164	80.0	485.08754	52.1
	Cl_8	$[M\text{-}Cl]^-$	521.04456	523.04267	96.0	525.03972	51.2
	Cl_9	$[M\text{-}Cl]^-$	557.00369	555.00665	89.3	559.00075	64.0
	Cl_{10}	$[M\text{-}Cl]^-$	590.96472	588.96767	78.1	592.96177	74.7
	Cl_{11}	$[M\text{-}Cl]^-$	624.92575	626.9228	85.3	622.9287	69.4
	Cl_{12}	$[M\text{-}Cl]^-$	658.88678	660.88383	96.0	656.88973	62.5
	Cl_{13}	$[M\text{-}Cl]^-$	694.84486	692.84781	93.8	696.84191	72.0
	Cl_{14}	$[M\text{-}Cl]^-$	728.80588	726.80883	85.2	730.80293	80.0
	Cl_{15}	$[M\text{-}Cl]^-$	762.76691	764.76396	88.0	760.76986	78.1
	Cl_{16}	$[M\text{-}Cl]^-$	796.72794	798.72499	96.0	794.73089	72.1

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