Adduct ions behaviour with respect to source parameters for the comprehensive LC-HRMS analysis of chlorinated paraffins (CPs)

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Introduction

Chlorinated paraffins (CPs) are a family of high production volume chemicals with various industrial applications such as cutting oil, lubricants, plasticizers or flame retardants. Their chemical formula is $C_nH_{2n+2-x}Cl_x$ ($n \geq 10$, $x \leq n$) and it is usual to distinguish short-chain (SCCP, C_{10}-C_{13}) from medium-chain (MCCPs, C_{14}-C_{17}) and long-chain (LCCPs, $C_{\geq 18}$) CPs. Their production started in the early 1930's and has exponentially grown until reaching an annual production volume of more than a million metric tons in 2013[1]. However, CPs toxicity and environmental fate make them hazardous substances that are of growing concern. Particularly, SCCPs were pointed out as persistent, bio-accumulative, toxic, and with a potential of long-range environmental transport, leading to their classification as persistent organic pollutants (POPs) under the Stockholm Convention in 2017[2]. Yet, recent studies displaying MCCPs and LCCPs in soil maintain the scientific community concern toward this class of compounds[3].
The analysis of CPs is highly demanding due to the thousands of existing isomers. So far, the most widely used method has been gas chromatography coupled to low resolution mass spectrometry with electron capture negative ionization (GC-ECNI-LRMS). This strategy presents however several drawbacks as follows: i) better ionization of highly chlorinated CPs leading to a bias in the chlorination degree determination, ii) interferences between non-resolved homologues and other halogenated compounds, and iii) ineptitude in LCCPs analysis. The development of last generation high resolution mass spectrometers (HRMS) over the past decade (e.g. TOF, Orbitrap), allowed improving homologues discrimination[4]. Further, the combination of such HRMS instruments with new chromatographic techniques led to promising methods and the production of first CPs occurrence data in the environment and the food chain. Liquid chromatography (LC), specifically, offers the major advantage to analyse not only SCCPs, but also MCCPs and LCCPs in one single injection. However, among available recent analytical strategies, interlaboratory studies have highlighted huge qualitative and quantitative variations between participants[5], leading to the conclusion that such approaches are not equivalent and result in different outcomes in terms of CPs monitoring. Hence, it is now of prime importance to understand where those variations come from, and to develop a standardized and robust method for CPs analysis.
We have consequently explored the impact of mobile phase composition, ionization mode and tune parameters in the formation of different nature of adducts (mainly $[M+Cl]^-$ and $[M+C_2H_3O_2]^-$), and found that source parameters have significant effects on CP patterns. In light of this knowledge, we propose an optimized method that is suitable for comprehensive analysis of chlorinated paraffins.

Materials and methods

Technical mixtures

Four mixtures of SCCPs (low and high Cl content) and LCCPs (low and high Cl content), Chlorowax 500C, Paroil 179-HV, Unichlor 40-90 and CPW-100, respectively, were purchased from AccuStandard (New Haven, USA) and mixed in acetonitrile (ACN) at 10 $ng.\mu L^{-1}$ each in order to cover a wide range of C-chain length and Cl-content.

Fish oil extraction and purification

A pool of fish lipids, previously extracted by Pressurized Liquid Extraction and containing various species, was purified and spiked with the four CP mixtures to observe the matrix influence on source parameters and adduct ions response. Briefly, 8 aliquots of ~1.25 g were successively purified on acid-impregnated silica (40 g, 44% H_2SO_4) and Florisil® (6 g, 3% H_2O) columns to remove lipids and contaminants. Columns were loaded and rinsed with 100 mL *n*-hexane. Elution was performed with 120 mL of dichloromethane (DCM) for Florisil® or a mixture of DCM/*n*-hexane (1:1, *v/v*) for silica. The extracts were pooled, dried and reconstituted in 1 mL ACN containing 10 ng.μL^{-1} of each of the four CP mixtures.

LC-HRMS data acquisition

Standards and extracts were analysed by RPLC-HRMS with an Ultimate 3000 UHPLC system coupled to an Orbitrap Q-Exactive mass spectrometer (Thermo Fischer Scientific). Chromatographic separation was achieved on a Hypersil Gold column (Thermo Scientific, 100 mm × 2.1 mm, 1.9 µm) kept at 30 °C with a mixture of ACN/H_2O (from 70% to 100% ACN in 6 min) and a modifier depending on the strategy used. The flow rate was 0.4 mL.min^{-1} and the injection volume was 5 µL. Data were acquired in full scan mode over the *m/z* range 300-1500 at a resolving power of 140,000 full width half maximum at *m/z* 200.

Three strategies were compared as follows: (1) ESI-Acetate-enhanced: 10 mM of ammonium acetate was added in the mobile phase. The $[M+C_2H_3O_2]^-$ adducts formed in an ion max source equipped with a Heated Electrospray (HESI) probe were monitored. (2) ESI-Cl-enhanced: 10% (final) of DCM was added in the mobile phase with a T-connection after chromatography. The $[M+Cl]^-$ adducts were monitored. (3) APCI-Cl-enhanced: The latter mobile phase was kept and the probe was changed to Atmospheric Pressure Chemical Ionization (APCI). The corona was set to 5 µA and the $[M+Cl]^-$ adducts were monitored.

Post-acquisition data treatment

Raw LC-HRMS data (*.raw*) were converted to the open format *.mzXML* using the open access *msConvert* software (ProteoWizard) through the open source programming R environment. Theoretical isotopic patterns of the respective ions for all CP homologue groups within C_8-C_{36} chain length and Cl_4-Cl_{26} chlorine number, excluding homologue groups with x > n+2, were computed from enviPat 2.2 (Eawag) in the centroid mode using the Q-Exactive R140,000@200 settings. A list of *m/z* features (n = 1147) corresponding to the two most intense ions (quantifier & qualifier) from each homologue group was compiled. Their intensities were then computed in the R environment using the *rawEIC* function from *xcms* package to generate Extracted Ion Chromatograms (EIC) at ± 5 ppm tolerance and the *trapz* function from the *pracma* package to integrate areas within the 1-14 min retention time range. Only homologue groups complying at 20% tolerance compared to the theoretical ion ratios were considered.

Results and discussion

Optimisation of source parameters for the ESI-Cl- and ESI-acetate-enhanced strategies

As CPs exhibit a wide range of chemical properties depending on their chemical structure, it can be expected that optimal ionization conditions vary among the whole range of CPs. Consequently, using a spiked fish matrix, we have explored the influence of three major source parameters for both strategies ($[M+Cl]^-$ or $[M+C_2H_3O_2]^-$ adducts monitoring) on a Q-Exactive mass spectrometer: the optical lens (S-Lens), the auxiliary gas temperature and the capillary temperature. For all adducts it was observed that greater S-Lens favoured ionisation of highly chlorinated but disadvantaged detection of lowly chlorinated CP, meaning that a compromise is needed (Fig. 1).

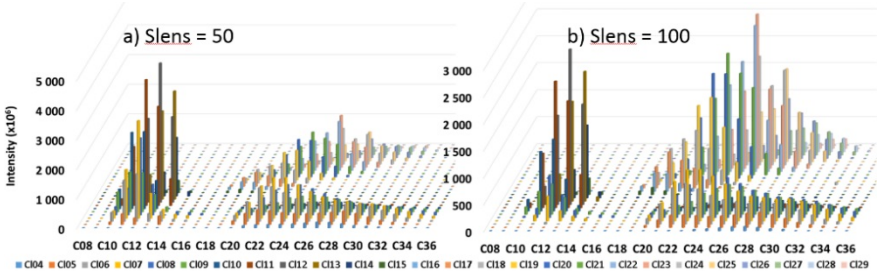

Figure 1. Influence of the S-Lens on the $[M+Cl]^-$ adducts response.

Likewise, the longer the CPs chain, the stronger its intensity was measured using high S-Lens values, meaning that the overall observed profile is highly influenced by the lenses set up. An optimal S-Lens value of 70 and 50 was selected for the strategy ESI-Cl-enhanced and ESI-acetate-enhanced respectively.

For both strategies, higher temperatures for the capillary as well as for the auxiliary gas led to higher intensities (Fig. 2). However, the LCCPs with high %Cl exhibited opposite behaviour to the low %Cl SCCPs when varying the capillary temperature, demonstrating that the resulting profiles vary depending on the chosen source parameters. Furthermore, the temperature of the auxiliary gas and of the capillary had different influences depending of the strategy used, showing that those parameters are component dependant. Hence, the optimal values for the two strategies were different and are shown on Fig. 2.

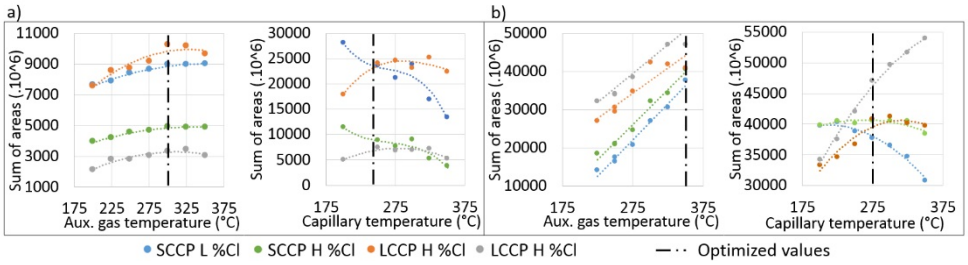

Figure 2. Influence of the auxiliary gas and the capillary temperatures of an ESI source for the (a) ESI-acetate-enhanced and (b) ESI-Cl-enhanced methods.

Comparison of ESI-Acetate- and ESI-Cl-enhanced strategies

The two optimised ESI strategies were compared in terms of detection sensitivity for the different types of CPs. Although the ESI-Acetate-enhanced strategy was optimised, it led to less sensitive detection of highly chlorinated CPs in comparison to lowly chlorinated CPs (Fig. 3a). The optimised ESI-Cl-enhanced strategy, in contrast, conducted to improved detection sensitivity, providing besides more equilibrated detection of the different technical mixtures respective CP profiles (Fig. 3b).

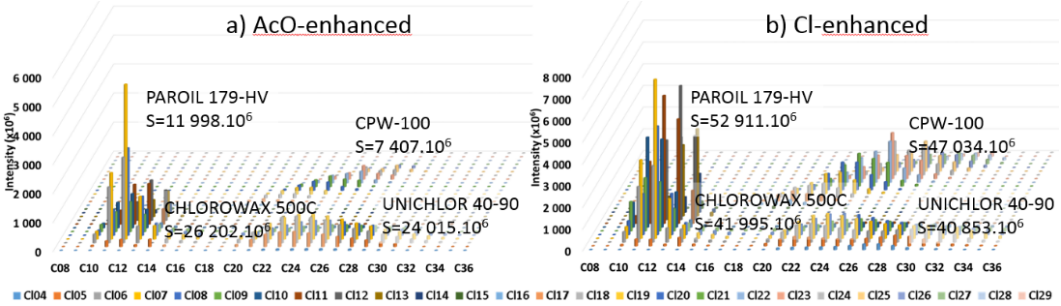

Figure 3. Comparison of the profiles obtained with the ESI-AcO- and ESI-Cl-enhanced strategies.

Performance of APCI and ESI sources for [M+Cl]⁻ adducts monitoring

The APCI-Cl-enhanced strategy was optimized with the same procedure as for ESI-Cl-enhanced. Then, the sensitivity and selectivity of both sources were studied for the analysis of CPs. For both methods, [M+Cl]- adducts were ionized preferentially in comparison to any other possible ions, proving the efficiency of DCM addition in post-column (Fig. 4a). Although ionization competition known to occur in ESI sources should limit detection sensitivity, here ESI appeared significantly more sensitive for both the standards and the fortified matrix (Fig. 4b). This could possibly be explained by the corresponding total ion current, which was not as stable in APCI as in ESI, possibly meaning that the gas phase was not optimal in APCI.

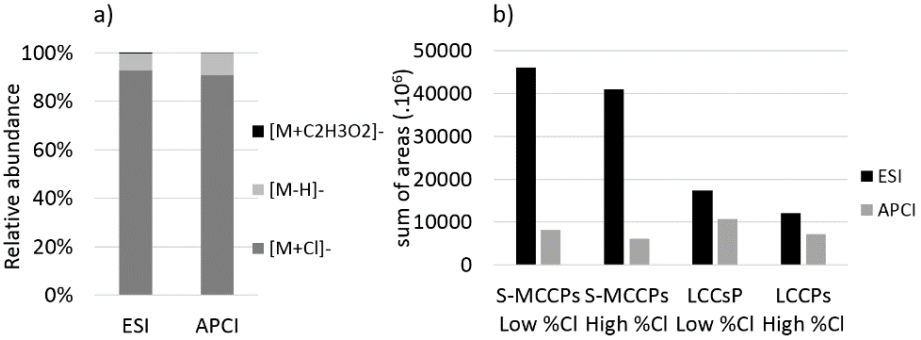

Figure 4. Comparison of ESI and APCI performances when monitoring $[M+Cl]^-$ adducts on a mix of technical mixtures:
a) relative abundance of adduct ions
b) sensitivity toward SCCPs, MCCPs and LCCPs detection.

Conclusion and perspectives

The source parameters tested in this study demonstrated to be CPs homologue dependant and to strongly affectCPs ionization. Although these parameters could be further optimized for specific CP groups investigation, the ESI-Cl-enhanced optimised protocol presented here enables comprehensive analysis of a wide range of CPs with high sensitivity.

In the present work, the results on the spiked fish extract were only qualitative. Future work directions include the selection of internal standards to study more precisely the response factors and to quantify CPs in biotic and abiotic samples.

Acknowledgements

The authors are grateful to the French General Directorate for Food (DGAl), Ministry of Agriculture and Food, for financial support.

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