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Abstract

Chlorinated paraffins (CPs), or polychlorinated *n*-alkanes, form a complex family of chemicals as they exist as mixtures of several thousands of isomers. To facilitate their classification, they are subdivided into short-chains (C_{10}-C_{13}, SCCPs), medium-chains (C_{14}-C_{17}, MCCPs), and long-chains ($C_{\geq 18}$, LCCPs) and further subdivided according to their chlorination degree. Until recently, the most common strategy implemented for their analysis was GC-ECNI-LRMS, with the main disadvantage being the high dependence of the response to the chlorination degree and the incapability of analysing LCCPs. In this work, we developed a method based on liquid chromatography coupled with electrospray ionisation-Orbitrap mass spectrometry (LC-ESI-HRMS) to expand the analysis capabilities of CPs. Although the different physico-chemical properties of CPs have led to compromises on the choice of analytical parameters, the addition of a mixture of DCM/ACN post-column with appropriate LC-ESI(-)-HRMS parameters enabled optimal and simultaneous detection of SCCPs, MCCPs and LCCPs from 10 to 36 carbons in one single injection. The combination of both the optimised LC-ESI parameters and the high resolution of the mass spectrometer (R = 140,000 @200 *m/z*) allowed separation of CPs signals of interest from unwanted halogenated ones, leading to minimum interferences in the detection. The optimised method was then successfully applied to the characterization of three types of vegetable oil, which were mostly contaminated with MCCPs. Additionally, the implementation of the LC-HRMS strategy enabled the identification of highly chlorinated LCCPs in edible oil for the first time at dozens of $ng.g^{-1}$ lw, which demonstrates the need of such comprehensive methods to expand the knowledge about CPs occurrence in food and environmental matrices.

Keywords (*6 max*)

LC-ESI-Orbitrap HRMS; chlorinated modifier; halogenated compounds interferences; vegetable oils; emerging contaminants, Persistent Organic Pollutant

1. Introduction

Chlorinated paraffins (CPs) are a family of high production volume chemicals, whose chemical formula is $C_nH_{2n+2-x}Cl_x$ ($n \in [\![10,30]\!]$, %Cl = 40-70% *w/w*)[1]. The magnitude of this family leads to a wide variety of chemical properties, and hence numerous industrial applications such as cutting oil, lubricants, plasticizers and flame retardants. 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 1930s, but has been drastically growing in the past few decades, mainly due to the entry of China in the global production in the 2000s[2]. Nowadays, the worldwide production of CPs is estimated to be >1 million tons a year, which surpasses the cumulative former production volume of polychlorinated biphenyls[1,2]. Among CPs, SCCPs have received the highest attention, partly due to their toxicity towards aquatic life and mammals[3,4]. SCCPs have thus been registered as Persistent Organic Pollutants (POPs) in the Stockholm convention since 2017[5–7]. As a result of those restrictions, SCCPs are being replaced by MCCPs in almost all their applications, which consequently leads to a shift in environmental occurrences from SCCPs to MCCPs[8]. As MCCPs are under increasing scrutiny, it is besides expected that a shift from MCCPs to LCCPs will occur in the next decades for some applications[9,10], further increasing the complexity of CPs mixtures found in environmental and food matrices. Although CPs are now well known, studies on their toxicity and their occurrence, especially on MCCPs and LCCPs, are still scarce and are required to characterise fully the risk associated to CP exposure.

The lack of knowledge on SCCPs, MCCPs and especially on LCCPs occurrence arises from the high complexity of the compounds family. Indeed, the substitution of a hydrogen by a chlorine during the synthesis is not selective, leading to wide possibilities of constitutional isomers. For instance, more than 7000[11] isomers have been reported as constituting a SCCP technical mixture, and the complexity increases with the chain length. To date, complete separation between homologues groups (i.e. between two chemical formulas) by chromatographic techniques is considered a huge issue, and only homologue-specific identification and sum SCCPs, MCCPs or LCCPs quantification is possible[12,13]. Until the last decade, the predominant technique used to characterize CPs was gas chromatography coupled

to electron capture negative ionisation-low resolution mass spectrometry (GC-ECNI-LRMS)[14] for its capability of analysing CPs homologues with relatively high sensitivity[15,16]. However, although it is considered a soft ionisation inducing limited fragmentation compared to electron impact, numerous ions are formed in the source such as $[M - Cl]^-$, $[M - HCl]^-$, $[M + Cl]^-$, $[HCl_2]^-$, and $[Cl_2]^-$ [17], further increasing the complexity of the spectra. This leads to severe mass interferences and complex algorithms must be used to circumvent those interferences when using low resolution mass spectrometry (ex. $[C_{10}H_{14}Cl_8 - Cl]^-$, *m/z* 380.8886 and $[C_{10}H_{16}Cl_6 + Cl]^-$, *m/z* 380.9072)[18]. Moreover, as the ionisation efficiency depends on the chlorine content of the molecules, the quantification can vary drastically depending on the chlorination of the analytical standards used[19].

During the past decade, the research dedicated to CPs analysis has grown significantly, mainly relying on recent technological innovations. Notably, the first CPs separation by two-dimensional gas chromatography was attempted in 2005 by Korytar et al.[20], and then Xia et al. managed to improve the separation of SCCPs by changing stationary phases and optimisation of GC parameters[21]. However, the volatility of CPs decreasing with increasing chain length, GC analysis is limited to SCCPs and MCCPs analysis. Hence, alternative comprehensive analytical methods such as direct introduction or liquid chromatography (LC) prior to MS analysis should be developed to study the LCCPs occurrence. The main difficulty related to such methods is the requirement of atmospheric pressure ionisation (API) sources, mainly electrospray ionisation (ESI), which is not suitable for nonpolar compounds such as CPs. However, Zencak and Oehme[22] demonstrated that adding a modifier to the mobile phase could enhance the formation of adducts, and thus facilitate LC-MS analysis. Since then, several strategies have been suggested. Bogdal et al.[23] proposed a chlorine-enhanced method by direct-introduction into an atmospheric pressure chemical ionisation source and analysis with a quadrupole time-of-flight high-resolution mass spectrometry (APCI-qTOF-HRMS) operated in full scan mode, with the advantage of being extremely fast (<1 min). Later, Li et al.[24] used liquid chromatography coupled with chlorine-enhanced electrospray ionisation-quadrupole time-of-flight mass spectrometry (ESI-qTOF-HRMS), and compared their observations with an APCI-Orbitrap approach[25], with the interesting findings that LC

coupling improved both the sensitivity and selectivity of the detection compared to direct introduction. In these cases, post-acquisition data-treatment of CPs signals from HRMS data sets remains highly demanding. More recently, Yuan et al.[26] developed a bromide-anion attachment APCI-MS method (Br-APCI-qTOF-MS), which simplified the spectra as only $[M + Br]^-$ anions are observed, improving thus the selectivity of the method.

Although these recent analytical strategies allow detecting LCCPs as well as SCCPs and MCCPs, their robustness has not been fully assessed. Indeed, although recent interlaboratory studies were performed for SCCPs and MCCPs[12,13] and showed improvement of the interlaboratory variabilities, results were mostly reported for GC-MS instruments with the exception of a few laboratories using APCI-qTOF-MS and LC-ESI-HRMS. Moreover, investigations are still on-going to understand the sources of the reported variabilities (CVs up to 137%), with one major reason being undeniably the challenges of CPs quantification. Indeed, because of the lack of labelled CPs standards, scientists are forced to develop alternatives to classical quantification strategies, which can induce strong over/underestimations of the concentrations[27,28].

We believe that one way of improving the robustness and comparability of the analytical methods is to get information on the differences of ionisation depending on the chain length and chlorination degree of CP standards. Thus, in this study, we take advantage of both the LC-Orbitrap-HRMS instrumentation, which allows detection of CPs with no mass interference, and an in-house software to perform semi-automatic post-acquisition data-treatment, to deeply investigate the behaviour of several ions of CPs in ESI and APCI, depending on the chlorination degree and chain length of the homologue groups. In light of those results, we propose an optimised chlorine-enhanced LC-ESI-HRMS method for comprehensive analysis of CP patterns in environmental and biota samples.

2. Material and methods

2.1. Chemicals

Hexane, dichloromethane (DCM) and magnesium silicate (Florisil) were purchased from LGC Promochem® (Picograde quality, Wesel, Germany). Sulphuric acid (98%) was purchased from Panreac (Barcelona, Spain). Silica gel (70/230 mesh, Fluka, Buchs, Switzerland), LC-MS grade water and acetonitrile (ACN), ammonium acetate, chloroform, dibromomethane and diodomethane were provided by Merck (Darmstadt, Germany). Bromoform was purchased from Fisher chemical.

In order to investigate the behaviour of CPs according to their chain length and chlorine content, a *CP standard mixture* was prepared by combining four technical standards from AccuStandard Inc. (New Haven, CT, USA) at equal proportions, including Chlorowax™ 500C (SCCPs, low chlorine content, *SCCPs Low %Cl*), Paroil™ 179-HV (SCCP, high chlorine content, *SCCPs High %Cl*), Unichlor™ 40-90 (LCCP, low chlorine content, *LCCPs Low %Cl*) and CPW-100 (LCCP, high chlorine content, *LCCPs High %Cl*) (Figure 1). Additionally, a MCCPs technical mixture produced by a Chinese company and kindly provided by the EMPA institute (Dübendorf, Switzerland), referred to as "I-42", was used for semi-quantification in the oil samples. A mixture of chlorinated contaminants including lindane, endosulfan sulfate, chlordecone hydrate and nonachlor was purchased from Cambridge Isotope Laboratories (Tewksbury, USA), tetrabromobisphenal A (TBBPA) and ^{13}C- and d_{18}-hexabromocyclododecane (^{13}C- and d_{18}-HBCDD) from Wellington Laboratories (Ontario, Canada) and octachlorobornane from LGC standards (Wesel, Germany).

2.2. Sample preparation

A pool of fish muscles (pickerel, trout, sardine, carp and arctic char) available from previous studies was lyophilised and extracted by Pressurised Liquid Extraction (SpeedExtractor E-914, Büchi, Switzerland) with a mixture of toluene/acetone (7:3, *v/v*) pending three static cycles. The extract was split prior to purification steps.

Clean-up of the fish extracts and vegetable oils were performed by loading about 1 g of fat on a first column packed with 20 g of neutral and 40 g of acidic (44% H_2SO_4) silica gel. Elution was performed with 120 mL of a mixture of DCM/Hexane (1:1, *v/v*). After reconstitution in 1 mL of hexane, the extracts

were further purified with 6 g of deactivated Florisil® (3% H_2O). Rinsing was achieved with 20 mL of hexane, and then elution was performed with 20 mL of DCM. The extracts were reconstituted in 50 µL of acetonitrile and stored at -20 °C until analysis.

For instrumental optimisation purposes (cf. §3.3), the fish extracts were spiked with the *CP standard mixture* at a final concentration of 10 ng.µL^{-1} per standard. For semi-quantification of vegetable oils, 10 ng of ^{13}C-and d_{18}-HBCDD were added in the samples before clean-up and before final extraction reconstitution, as internal and external standards, respectively.

2.3. Data acquisition

Analytical standards and extracts were analysed with an UltiMate 3000 UHPLC pumping system coupled to a Q-Exactive mass spectrometer fitted with either a Heated ElectroSpray (HESI) or an Atmospheric Pressure Chemical Ionisation (APCI) source (Thermo Fischer Scientific, San José, CA, USA). The sample injection volume was 5 µL, and chromatographic separation was achieved using reversed phase chromatography on a Hypersil Gold analytical column (100 mm × 2.1 mm, 1.9 µm) (Thermo Fischer Scientific) maintained at 30 °C. Mobile phase consisted in 70% to 100% ACN in water (6 min gradient, and then 6 min isocratic at 100% ACN) at a flow rate of 0.4 µL.min^{-1}. The modifier added post-column will be discussed later.

The source parameters and monitored ions will also be discussed hereafter. Briefly, the ionisation of CPs was performed with an ESI or APCI probe in negative acquisition mode with a voltage of 2.5 kV. The sheath gas and the auxiliary gas flow rates were 50 and 5 arbitrary units (AU), respectively. The capillary temperature, the probe temperature as well as the optical lenses were modified to achieve the highest efficiency of the monitored ions. HRMS data were acquired in full scan mode over the *m/z* range [300-1500], with the AGC target set at 5.10^5 and at a resolving power set at 140,000 full width at half maximum at *m/z* 200.

2.4. Automatic data-treatment

Raw LC-HRMS data (*raw*) were converted to an open format (*mzXML*) using the open access *msConvert* software (ProteoWizard) through the open source programming R environment. Theoretical isotopic patterns of selected ions for all CP homologue groups within C_8-C_{36} chain length and Cl_4-Cl_{30} chlorine number were computed through the open R environment using the *isopattern*, *envelope* and *vdetect* functions from the *enviPat* package[29]. The targeted homologues list started from C_8 in order to identify potentially occurring "very-short" CPs[30], and homologue groups with $n_{Cl} > n_C+2$ were excluded as substitution of 2 chlorines per carbon is unlikely from the synthesis mechanism[31]. A list of *m/z* features (n = 1146) corresponding to the two most intense ions (quantifier & qualifier) from each homologue group was compiled (Table S1). The data were computed in the centroid mode with R=140,000@200 as resolution and threshold = 0.001. Their corresponding intensities were then computed in the R environment using the *rawEIC* function from *xcms* package[32] to generate Extracted Ion Chromatograms at ± 5 ppm tolerance according to the sequence and the *trapz* function from the *pracma* package[33] to integrate areas within 2-14 min retention time (RT) range. As the LC provides only limited separation of CPs, the RTs were not used as identification criteria. However, only homologue groups featuring an area >1 000 000 AU and complying 20% tolerance compared to the theoretical ion ratio between the two most intense ions of the isotopic profile were considered. Intensities were then normalised to total homologue isotopic patterns to correct for the isotopic contribution of the quantifier ion. For comparison purposes during the instrumental development, the homologue peak areas were summed according to the four standards from the *CP standard mixture*.

For interferences considerations, theoretical *m/z* values of all the isotopologues of the $[M + Cl]^-$, $[M - H]^-$, and $[M + C_2H_3O_2]^-$ ions were computed for each homologue from C_8 to C_{36} and Cl_4 to Cl_{n+2} in the *m/z* [300-1500] range (according to *enviPat 2.2*, Table S2). Then, the mass-to-charge ratios of the quantifier and qualifier ions ($[M + Cl]^-$ two most intense isotopes) were compared to the obtained list of *m/z*. When potentially interfering ions exhibited the same nominal mass, the theoretical resolution necessary to discriminate was calculated and compared to the reached resolution of the Orbitrap at the same mass (Table S3).

194

195 *2.5. Semi-quantification of CPs in vegetable oils*

196 An external calibration curve was built from the 4 technical mixtures used for method development as
197 well as the MCCPs mixture I-42. The dynamic range was 0.1-15 ng.µL^{-1} for each technical mixture. The
198 sum of the signal areas for each technical mixture normalised by the internal standard area was
199 expressed as a function of the mixture concentration. The procedural blank contribution was
200 subtracted from the samples before quantification.

201 In order to assess the similarity between the samples and each technical mixture, a least-squares
202 approximation with a non-negative constraint was calculated using the function *lsqnonneg* of the
203 *pracma* package in the *R* open source environment[23,33]. The profiles were considered as similar when
204 the parameter *a* of the equation $[S] = \mathrm{a} \times [M] + \mathrm{b}$ was equal to 1 (± 0.1), where $[S]$ and $[M]$ are the
205 vectors of the homologues detected in the sample and the corresponding technical mixture,
206 respectively.

207

208 **3. Results and discussion**

209 *3.1. Ionisation of CPs in ESI without modifier*

210 Analysis by LC-MS couplings being most commonly achieved with API sources such as ESI or APCI, the
211 ESI probe was first selected to investigate which type of ions would be created in this source. The
212 ionisation of the molecules depending on their chemical structure, and by extension, their chemical
213 properties, the impact of CP chain length and chlorine content on the ionisation was studied with four
214 standards: two SCCPs and two LCCPs standards, with low of high chlorine content. No additional
215 MCCPs standards was added in the mixture, as it was hypothesised that their behaviour would be
216 intermediate between SCCPs and LCCPs.

217 *The CP standard mixture* at 1 ng.µL^{-1} was thus injected in the LC-MS system with no modifier in the
218 mobile phase, according to parameters described above (§ 2.3). The temperatures of the capillary and
219 ESI probe were 300 °C and 150 °C, respectively, and the optical lenses tension was set to 50 AU. Three

ion types were monitored during the data treatment, according to CP ions previously reported in the literature when using ESI: $[M - H]^-$ [25], $[M + Cl]^-$ [24], and $[M + C_2H_3O_2]^-$ [34]. Distinct profiles could be observed, highlighting that the ions formation varies according to CP chemical formula (Figure 2, Figure S1). SCCPs with low chlorine content were more prone to form $[M + C_2H_3O_2]^-$ ions whereas SCCPs with high chlorine content were better ionised in the $[M + Cl]^-$ or $[M - H]^-$ forms. Regarding LCCPs, the $[M + Cl]^-$ ions were formed predominantly, even if the $[M + C_2H_3O_2]^-$ ions were observed at low intensity for LCCPs of low chlorine content. Interestingly, whereas $[M - H]^-$ ions were relatively intense for SCCPs and would explain the choice of those ions when monitoring SCCPs[35], they weren't formed for LCCPs.

The strong decrease of $[M + C_2H_3O_2]^-$ ions signal with increasing chlorine content as already reported and explained by the difference of acidity between acetate and chloride[36], was confirmed in the present study. Regarding the competition between $[M - H]^-$ and $[M + Cl]^-$ ions, it could also be related to the acidity of the analytes that drives the affinity for one or the other ion formation. Indeed, $[M - H]^-$ are formed when molecules can lose a proton, which occurs mostly for acidic molecules ($pKa < 5$)[37]. Concerning CPs, as the hydrophilicity of the CPs decreases with increasing chain length, it can be expected that the capability of CPs for losing a proton decreases accordingly, thus favouring $[M + Cl]^-$ ions formation. Overall, the $[M + Cl]^-$ ions were formed regardless of the chlorine content or the chain length of CPs homologues. As SCCPs and LCCPs could ionise in their $[M + Cl]^-$ form, it is expected that an ionisation efficiency in between will also occur for MCCPs.

It is worth noting that $[M - HCl + Cl]^-$ and $[M - H]^-$ exhibit the same exact mass. Consequently, the homologue pattern of the $[M - H]^-$ ions (Figure S1a) could partly come from interfering in-source dechlorination products such as chlorinated alkenes, which can be formed by loss of HCl when CPs are exposed to heat[38]. However, Schinkel et al.[38] showed that after 2 h at 220° C, only 22% hexachlorinated tridecanes had degraded to hexachlorinated tridecenes. It can thus be hypothesised that CPs degradation does not occur within the time spent in the ESI source, even though the probe temperature of the experiment was higher. Moreover, as the intensity of the $[M - H]^-$ ions did not

increase with increasing probe temperature (Figure S4), we estimated that even if the observed $[M - H]^-$ signal was interfered with CP degradation products $[M - HCl + Cl]^-$, this would have only a limited impact on the overall distribution of the $[M - H]^-$, $[M + Cl]^-$, and $[M + C_2H_3O_2]^-$ ions in the source. For a more robust conclusion, it would be necessary to study the degradation phenomenon with constitutionally defined CPs[28]. Thus, the comparison of the intensities of the three adducts showed that $[M + Cl]^-$ ions were the most suitable for all CPs analysis in a single injection, although the intensity of the signal was a bit lower compared to acetate ions for SCCPs. Yet this choice prevents this method from characterising the unsaturated homologues, as it cannot discriminate between chlorine adducts of mono-unsaturated homologues and pseudo-molecular ions of saturated homologues. For CP degradation studies, it would be preferable to use another target ion.

3.2. Chlorine ions enhancement: influence of the modifier

Although the four standards of the *CP standard mixture* were observed when monitoring the $[M + Cl]^-$ ions, the signal was rather weak, especially for LCCPs, which can be explained by the lack of sensitivity of ESI for hydrophobic compounds such as CPs[22]. It was already shown that signal enhancement could be achieved in GC and LC analysis[22–24] for various instrumentations, mainly with DCM addition, although chloroform[22] and more recently bromoform[26] were also reported, to form $[M + Cl]^-$ and $[M + Br]^-$ ions, respectively.

Our selected mobile phase gradient involving a mixture of water and ACN, it was not possible to add DCM directly into the mobile phases as it is not miscible with water. It could have been possible to add the modifier only in the ACN, however DCM levels in the mobile phase would have consequently varied during the elution, not to mention that its interaction with CPs shifts their elution (Figure S2). Hence, we considered a previously reported post-column addition strategy[24].

Unfortunately, no significant trend in CPs signal areas was observed between DCM flow rates of 0, 0.02 and 0.04 $mL.min^{-1}$. We hypothesised that mixing pure DCM with the mobile phase was not favoured under pressure conditions because of the presence of water. In order to favour the mixing, we replaced

pure DCM by a mixture of ACN/DCM (1:1, *v/v*) at a flow rate of 0.08 mL.min^{-1}. Great enhancement was achieved between 0 and 0.08 mL.min^{-1}, with a signal increase from 91% for LCCPs highly chlorinated to 616% for SCCPs lowly chlorinated (Figure 3). It is noteworthy that the LCCPs displayed the lower signal increase, which is probably caused by their higher hydrophobicity. Nevertheless, addition of the ACN/DCM mixture revealed all CPs compared to no addition.

In order to understand the mechanism associated to the observed signal enhancement, it would have been interesting to investigate alternative halogenated modifiers (Table S4). However, although chloroform, dibromomethane, bromoform, and diiodomethane were considered as potential candidates with regard to their suitable melting and boiling points, the bromine and iodine chemicals were not soluble with ACN and/or the mixture of ACN/H_2O. In our mobile phase conditions, only chloroform was thus suitable. Hence, a comparison of the mixtures of ACN/DCM (1:1, *v/v*) and ACN/chloroform (1:1, *v/v*) only was performed (Figure S3). No significant differences in homologue summed intensities were observed, which was in good agreement with Zencak and Oehme findings[22]. Moreover, signals obtained with chloroform were less reproducible than signals obtained with DCM. Since the chloroform was given a lower maximum professional exposure value than DCM by the French National Institute for Research and Security (INRS, 2 ppm[39] and 50 ppm[40], respectively), DCM was selected as modifier for the CPs analysis method.

3.3. Optimisation of source parameters for chlorine ions monitoring

Once the nature of the modifier was selected, the ionisation conditions were optimised to allow a sensitive analysis of all CPs. Three main parameters of the HESI source were studied: the optical lenses, the heater temperature and the capillary temperature (Figure S4, SI section 1). The highest signal intensities were obtained for the highest temperature of the heater for all CPs, thus it was set at 350 °C. For both the optical lenses and the capillary temperature, an opposite behaviour could be observed between SCCPs low %Cl and LCCPs High %Cl. Consequently, different parameters could result in varying relative responses between CP groups. This set of parameters should be considered whenever

298 comparing CPs intensity profiles between different methods. The optical lenses and capillary heater
299 were set at consensual values of 70 AU and 275 °C, respectively.
300
301 *3.4. Comparison of ESI and APCI*
302 ESI and APCI sources, involving different ionisation pathways, have been proven to be
303 complementary[41]. Comparison of their ionisation efficiencies can be of great interest. Schinkel and co-
304 workers have compared methods from the literature using either APCI or ESI. However too many
305 parameters were different between methods, which made it difficult to conclude. Thus, we compared
306 the response areas of $[M + Cl]^-$ adduct ions, using the parameters optimised in the present study for
307 ESI, and consensual parameters for APCI, selected from a similar experiment as for ESI (optical lens
308 voltage = 100 AU; probe temperature = 300 °C; capillary temperature = 200 °C) (Figure S6). ESI enabled
309 a higher signal response with relatively low standard deviations (<15%) for all CPs standards, and
310 particularly for SCCPs (5- to 7-fold) compared to APCI results. As the intensity of the SCCPs and LCCPs
311 were similar in APCI, it can be expected that the intensity of MCCPs would reach the same intensity
312 level. On the contrary, ESI ionised better SCCPs than LCCPs, thus it can be expected that MCCPs would
313 reach an intensity level in-between. Overall, higher sensitivity would be reached with ESI
314 independently of the chain length and was thus maintained as ionisation source for the analysis of CPs.
315 However, the parameters of APCI being different from those of ESI, it can be argued that comparison
316 of both instrumentations was limited. Notably, APCI usually demands higher mobile phase flow
317 (1 $mL.min^{-1}$), which could not be achieved due to back-pressure considerations. The ionisation
318 efficiency comparison should still be investigated for better understanding of CPs ionisation processes.
319
320 *3.5. Considerations on mass interferences*
321 Due to the highly complex isotopic profiles of CPs, concerns about interferences between homologues
322 have been previously reported[18,26,42]. For example, $[C_xH_{2x+2-y}Cl_y + Cl]^-$ ions differ of only 0.1591 Da from
323 $[C_{x+5}H_{2(x+5)+2-(y-2)}Cl_{y-2} + Cl]^-$ ions, which are not discriminated with low resolution instruments such as

quadrupoles and thus require chromatographic separation beforehand. Even though new mass analysers were developed to reach high resolutions, the existence of various ions in the source makes it even more complicated as stated by Yuan et al[18].

The high resolution of the Orbitrap system has already proven to discriminate SCCPs and MCCPs homologue groups between them as well as from other halogenated contaminants, in GC-ENCI[5]. However, considering the decrease of the resolution when increasing the mass-to-charge ratio of the target ion, a thorough analysis of the theoretical resolution of CPs with the Orbitrap available in our laboratory was necessary, particularly for LCCP analysis. The *m/z* ratios of the quantifier and qualifiers of the $[M + Cl]^-$ ions for each targeted CPs homologue were compared to the isotopologues of the $[M - H]^-$, $[M + Cl]^-$ and $[M + C_2H_3O_2]^-$ ions. Notably, the resolution necessary to discriminate between a diagnostic ion and its interfering ion was compared to the resolution of the Orbitrap (Table S3, cf. § 2.4). When no filter was applied, 181,347 potential interfering pairs (i.e a diagnostic ion and its interfering ion) were found at nominal resolution, emphasizing the complexity of such mixtures. However, numerous isotopologues are of very low theoretical abundance and would not significantly interfere. With a threshold of 1% of theoretical abundance compared to the base peak for each isotopic cluster, 45,641 interfering pairs were remaining. When a mass analyser exhibiting a resolution of 10 000 FWMH is used, this number decreases to 12,803. Applying the Orbitrap resolution filter, only 2,892 potential interfering pairs remained, which indicated that high resolution instruments are decisive for LCCP analyses.

Among those 2,892 remaining interferences, 713 arose from the contribution of ^{13}C isotopologues in the $[M + Cl]^-$ isotopic profile. This is thus not an external interference as it arises from the same molecule and will always exhibit the same behaviour. The other 2,179 potential interfering pairs were divided in 3 types of $[M - H]^-$ interferences and 2 types of $[M + C_2H_3O_2]^-$ interferences (Table S5). Three types of interferences were occurring with ions with a theoretical abundance below 8% of the base peak, which was considered as of low significance and will not be discussed in this paper. The fourth interference occurs between the $[C_xCl_y + Cl]^-$ and $[C_xCl_{y+1} - H]^-$ ions with a mass difference of 0.0186 due

to the $^{35}Cl/^{37}Cl$ mass defect. However, the Orbitrap mass analyser was able to discriminate this mass difference for a *m/z* up to 1000, thus only LCCPs ($C_{\geq 24}$) with very high chlorination degree (≥ 17 chlorines) would be impacted. The fifth interference occurred between the $[C_xCl_y + Cl]^-$ and $[C_{x-2}Cl_y + C_2H_3O_2]^-$ ions with a *m/z* difference of 0.0073 due to the $^{35}Cl/^{37}Cl$ mass defect and the ^{16}O contribution. This smaller *m/z* difference was discriminated for SCCPs and lowly chlorinated MCCPs but LCCPs would be strongly impacted. However, the formation of acetate ions is not favoured in the chlorine-enhanced conditions of our method. We hypothesise that if some acetate ions are formed, they will be in relatively low amounts compare to the chlorine ions.

Although the Orbitrap is highly performant, it does not overcome all the interferences potentially occurring in such complex mixtures as LCCPs. Nevertheless, the remaining interferences can be eliminated with optimised ionisation conditions as discussed above.

Besides interferences between CPs, potential interferences with other halogenated contaminants such as toxaphenes, PCBs and other POPs were previously reported[42,43]. Thus, standards of octachlorobornane, TBBPA, ^{13}C-HBCDD and a mixture of chlorinated POPs including lindane, endosulfan sulfate, chlordecone and nonachlor were analysed in our optimized conditions and potential interferences with CPs were investigated (Table S6). For almost all considered contaminants, the necessary resolution was below 32,000, which is achieved by the used mass analyser (R=140,000@200, meaning R=51,121@1500). However, two contaminants could require resolutions >140,000. The first one is endosulfan sulfate, which forms an $[M - H]^-$ adduct that could interfere with the C_8Cl_{10} CP homologue. The second one is HBCDD, which forms three major ions: $[M - H]^-$; $[M + Cl]^-$; and $[2M + Cl]^-$. The two most intense isotopologues of the $[M - H]^-$ isotopic profile of ^{12}C-HBCDD could interfere with the $C_{11}Cl_{13}$ CP homologue (R>200,000).

To separate these contaminants, chromatographic separation is necessary. In the developed method, CPs are eluted from 2.5 ($C_{10}Cl_5$) to 14.5 minutes ($C_{34}Cl_4$). Broad homologues peaks (2-3 minutes large) lead to the co-elution of SCCPs and MCCPs which then rely on the high resolution mass spectrometer to be discriminated. However, a trend of elution according to the chain length and chlorine degree was

observed: SCCPs Low %Cl (2.5-4.5 min) < SCCPs High%Cl (4-6.5 min) < MCCPs (4.5-6 min) << LCCPs low %Cl (7-14 min) = LCCPs High %Cl (7-10 min) (Figure S7). It is worthy to note that the peaks are enlarged with longer chains, which can be explained by more positional isomers. Similarly, when the homologues are highly chlorinated, the number of potential isomers decreases, leading to narrower peaks (LCCPs high %Cl). With these chromatographic characteristics, the endosulfan sulfate was eluted at 1.7-2.2 minutes which is earlier than any CP. Similarly, the retention time window of the $C_{11}Cl_{13}$ CP was [5.2-6.6] min whereas the retention times of α-, β- and γ-HBCDD were [3.3-3.6], [3.6-4.0] and [4.3-4.8] min, respectively (Figure S8). Thus, in both cases, the liquid chromatography enabled the separation of contaminants that would interfere with CPs. The combination of both high resolution and chromatographic separation improved the selectivity of the method.

3.6. Application of the optimised method to vegetable oils from French market

The optimised method was applied to olive, hazelnut, and linen oils from French markets. Olive and hazelnut oils exhibited profiles dominated by MCCPs, although hazelnut oil profile contained SCCPs and LCCPs as well (Figure 4a and 4b). This is in accordance with recent studies showing a shift from SCCPs to MCCPs occurrence in environmental samples[38,39]. Linen oil featured a more complex profile, from SCCPs lowly chlorinated to LCCPs highly chlorinated. Interestingly, homologue groups presenting more chlorine than carbon atoms were detected for C_{13} and C_{14} chain lengths, although they were not expected according to the already described synthesis mechanism as mentioned earlier. These homologues could be the result of synthesis performed in extreme conditions (high temperature, very long reaction time, high concentration of chlorine).

Finally, a profile similar to the CPW-100 standard (Dr. Ehrenstorfer) was observed in linen oil (Figure 1, Figure 4c), at a level similar to MCCPs (81 $ng.g^{-1}$ lw). This profile is particularly highly chlorinated, the most intense homologues containing as many chlorines as carbons. LCCPs have already been detected in sewage sludge[20,34], fish and seafood[23], and human blood[24]. However, to our knowledge, it is the first

time that such a highly chlorinated profile is reported in food samples, which may result from the optimisation of the method that was dedicated towards longer and higher chlorinated CPs.

A semi-quantification CPs was attempted using the technical mixtures used for method development as well as I-42. The MCCP technical mixture (I-42), SCCPs high %Cl and LCCPs high %Cl were very similar to the profiles observed in the samples (Table S7), which allowed quantifying this type of CPs. The traces of SCCPs low %Cl found in the oils most likely came from a MCCP technical mixture with SCCP impurities, hence could not be totally matched to the quantification mixture. Nevertheless, MCCPs were estimated at 76-267 $ng.g^{-1}$ lw (Table S8), which was several times higher than SCCPs that were neither detected or quantified below 79 $ng.g^{-1}$ lw. SCCPs were investigated in vegetable oils from China and were found to vary from a few to thousands of $ng.g^{-1}$ [44]. In our work, we showed that SCCPs represent only part of the CP contamination. Most importantly, LCCPs high %Cl were estimated at 81 $ng.g^{-1}$ lw, which is in the same range than SCCPs and MCCPs. Although it has been mentioned that the toxicity of CPs may decrease with increasing chain length[45], it was also shown that highly chlorinated CP technical mixtures (70% Cl) had neurobehavioral effects on zebrafish[45] . The toxicity of LCCPs is still controversial and should not be underestimated, as stated by the European Food Safety Authority recently[47] Thus, it enhances the need for as much comprehensive analytical methods as possible for the sake of CPs human exposure assessment.

4. Conclusions and perspectives

To date, there is still no consensus on CPs analysis due to their complex composition and associated physicochemical properties. Analytical methods reported until now face interferences, poor or varying ionisation depending on homologue/isomer pattern. In order to achieve efficient analysis of such compounds and harmonize the approaches, it is crucial to widen and share the knowledge related to all the factors which come into play, from instrumentations to quantification procedures[48]. Our work focused on a critical step of their analysis related to ionisation prior to MS monitoring, thus complementing the recent study of Zheng et al.[49] which discussed the dependence of the ionisation

efficiency on the selected modifier and monitored adducts for CPs up to C_{20}. In the present study, we considered a more global range of CPs from C_{10} to C_{36} and from Cl_4 to Cl_{30} for the longest chains, thus highlighting the opposite behaviours between SCCPs and LCCPs and the limitation of intensity profiles comparison.

Overall, the chlorine-enhanced RPLC-ESI-Orbitrap method optimised in the present study enables the identification of SCCPs, MCCPs and LCCPs in one single injection, at relatively comparable response although SCCPs are slightly favoured. The in-house software enables the semi-automatic integration of 573 homologue groups (C_8-C_{36}, Cl_4-Cl_{30}) in a few minutes. Moreover, we demonstrated that the resolution of the Orbitrap mass analyser allows for the discrimination of all homologue groups without significant interference contribution when appropriate ionisation conditions are used, but should be used after chromatographic separation of halogenated contaminants from CPs.

The optimised method was applied to commercial vegetable oils (n=3), which were found to be contaminated with SCCPs, MCCPs and LCCPs with a majority of MCCPs. Most importantly, linen oil was found to be contaminated with as much highly chlorinated LCCPs as MCCPs and SCCPs. This emphasises that more attention should be paid to unusual CPs. It is thus crucial to pursue effort on comprehensive studies to expand the CP dietary exposure assessment.

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Conflict of interest

The authors declare no financial competing interest.

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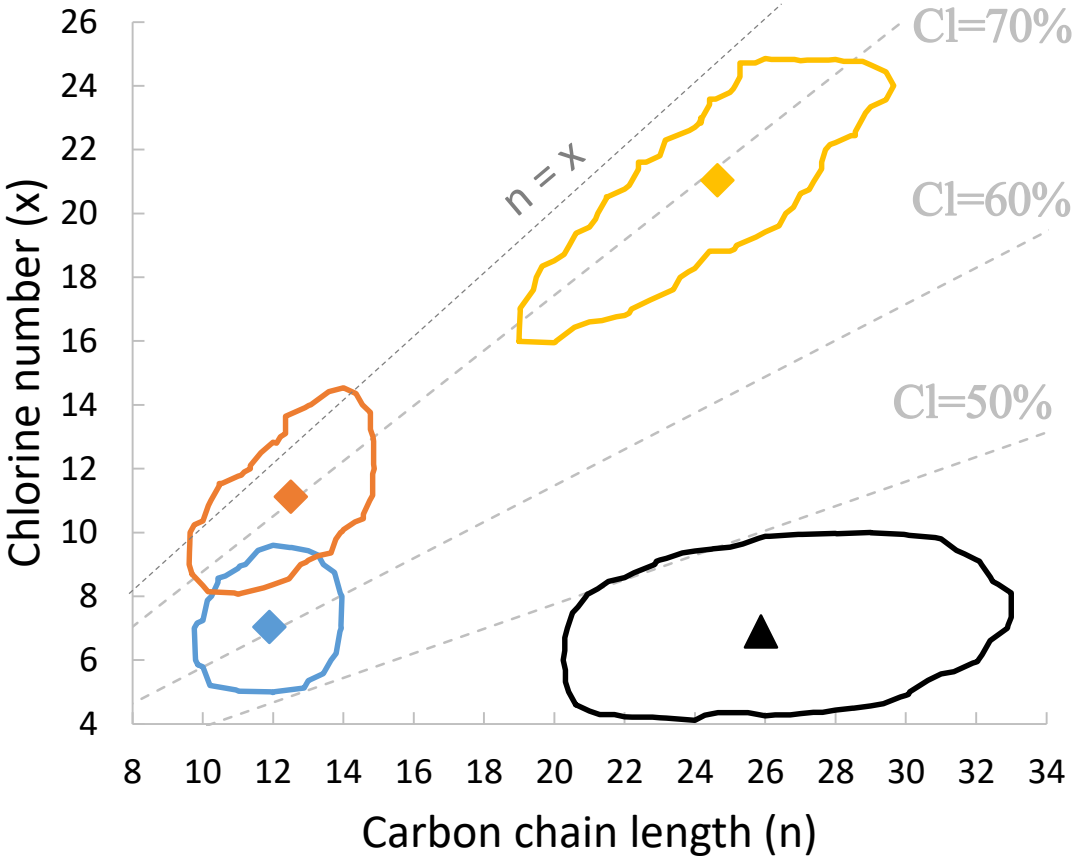
Figure captions

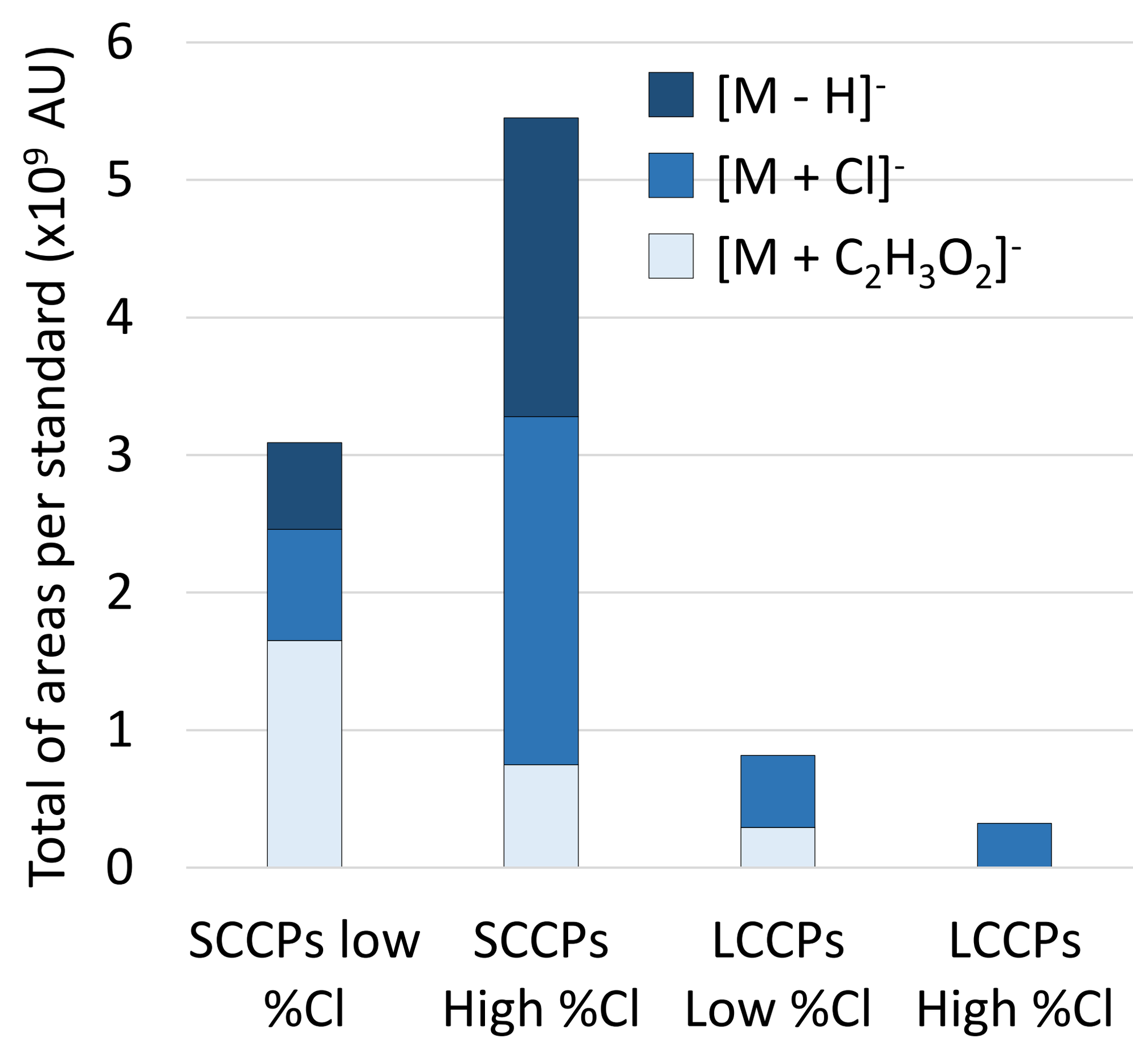
Figure 1 Distribution of the four technical mixtures of the *CP Standard Mixture* with regard to CP homologue groups. ■: Chlorowax™ 500C ($C_{10\text{-}13}$, $Cl_{5\text{-}10}$)= *SCCPs low %Cl*; ●: Paroil™ 179-HV ($C_{10\text{-}15}$, $Cl_{9\text{-}15}$) = *SCCPs high %Cl*; ▲: Unichlor™ 540-90 ($C_{20\text{-}33}$, $Cl_{5\text{-}11}$) = *LCCPs low %Cl;* ◆: CPW-100 ($C_{18\text{-}14}$, $Cl_{14\text{-}29}$) = *LCCPs high %Cl*.

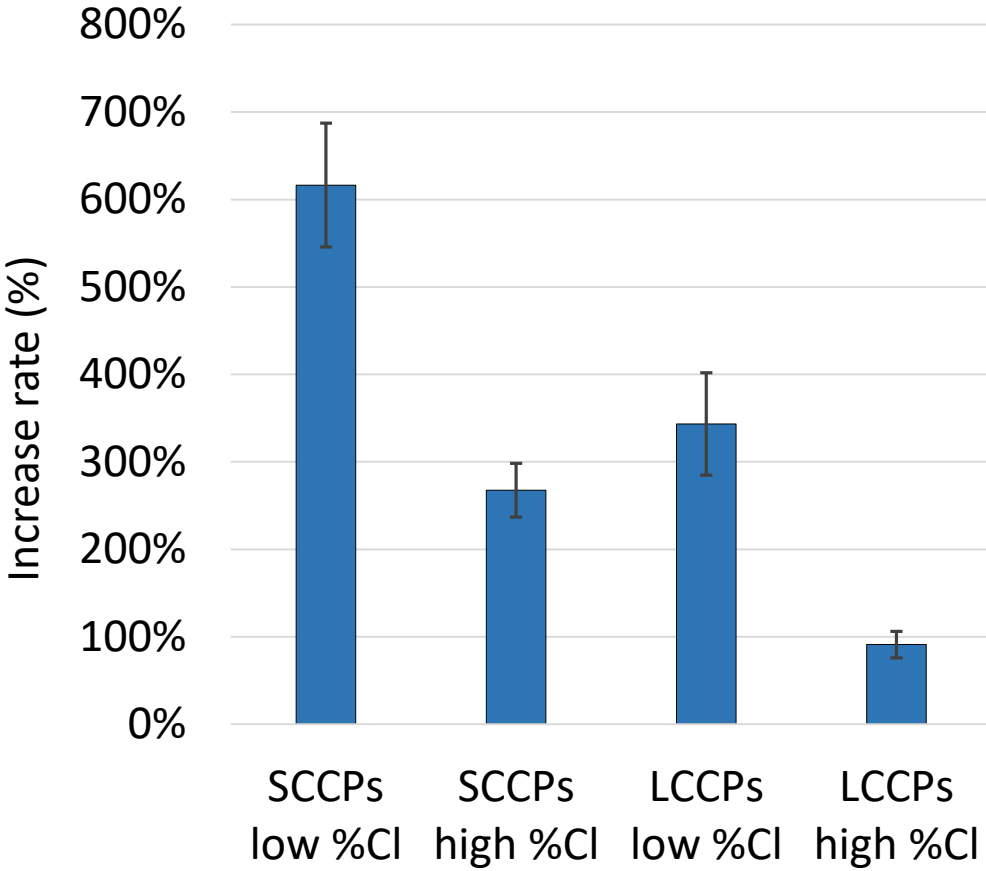
Figure 2. Cumulated adduct ion signal areas obtained for each standard in ESI(-), without modifier.

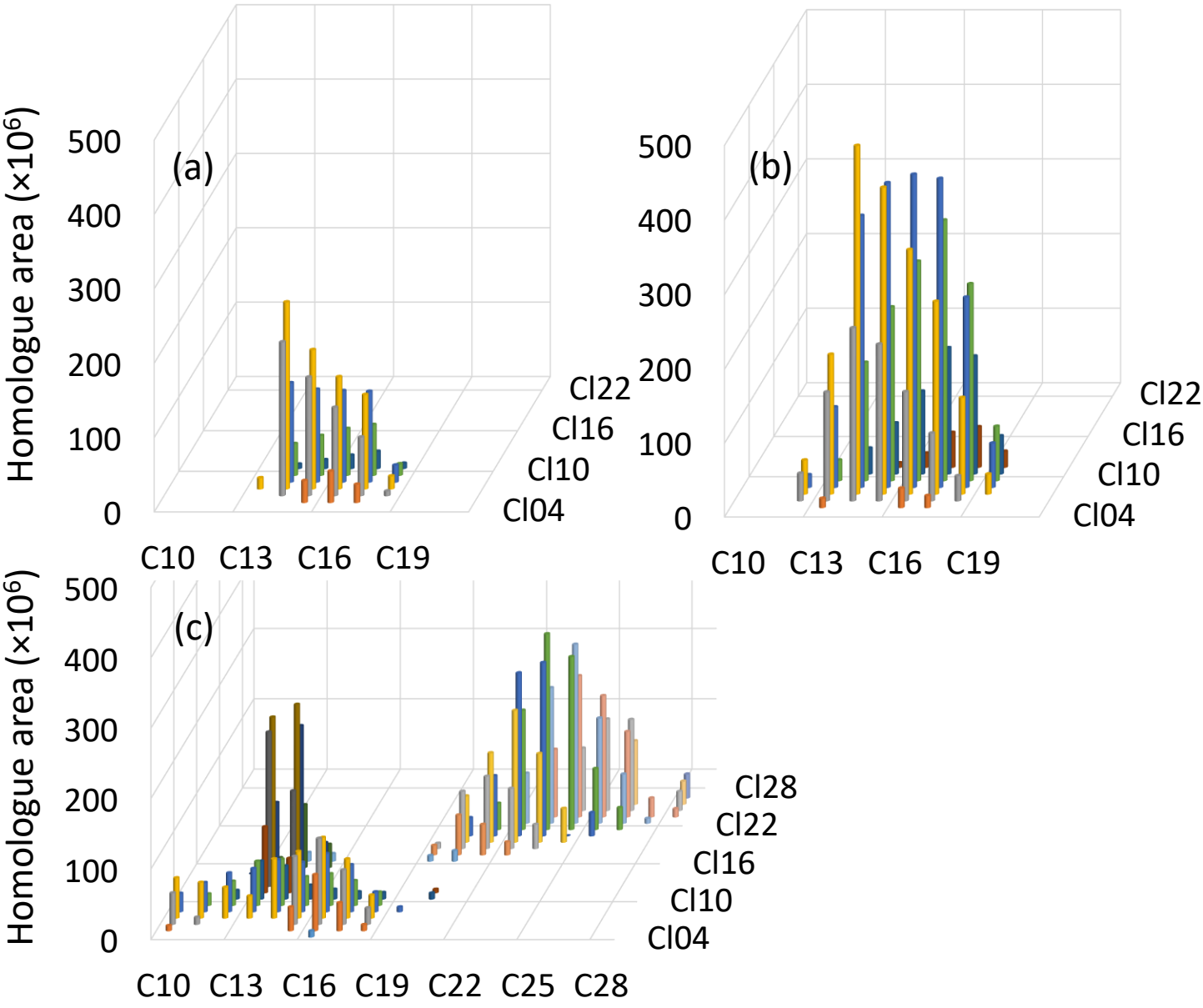
Figure 3. Percentage of signal increase (n=3) when a mixture of ACN/DCM (1:1, *v/v*) is added into the mobile phase post-column.

Figure 4. CP homologue profile obtained for (a) olive, (b) hazelnut, and (c) linen oil samples from the French market, based on optimised method and after procedural blank subtraction.


Cl=70%
Cl=60%
Cl=50%
n = x
Chlorine number (x)
Carbon chain length (n)
26
24
22
20
18
16
14
12
10
8
6
4
8
10
12
14
16
18
20
22
24
26
28
30
32
34


Total of areas per standard (x10^9 AU)
0
1
2
3
4
5
6
[M - H]$^-$
[M + Cl]$^-$
[M + $C_2H_3O_2$]$^-$
SCCPs low %Cl
SCCPs High %Cl
LCCPs Low %Cl
LCCPs High %Cl


800%
700%
600%
500%
400%
300%
200%
100%
0%
Increase rate (%)
SCCPs low %Cl
SCCPs high %Cl
LCCPs low %Cl
LCCPs high %Cl


(a)
(b)
(c)
Homologue area (×10⁶)
500
400
300
200
100
0
Cl04
Cl10
Cl16
Cl22
Cl28
C10
C13
C16
C19
C22
C25
C28