QuEChERS-based extraction and two-dimensional liquid chromatography-high resolution mass spectrometry for the determination of long chain chlorinated paraffins in sediments

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ABSTRACT

In this study, a method was established for the analysis of long-chain chlorinated paraffins (LCCPs) in sediment based on quick, easy, cheap, effective, rugged, and safe (QuEChERS) extraction and two-dimensional liquid chromatography-Orbitrap high resolution mass spectrometry (2DLC–Orbitrap HRMS). Compared with other reported methods, this method greatly reduces sample preparation time (2 h) and solvent consumption. The QuEChERS extraction method presented satisfactory recoveries, 90.5–95.2, 84.7–86.6, and 81.4–83.4% of 5, 50, and 200 ng/g LCCPs with 49% Cl spiked into sediments. Meanwhile, no matrix effects were found in the LCCPs analysis after online purification by the 2DLC system. With the current commercial LCCP standards and a mixture of three chlorinated paraffins (CPs) industrial products, a suspect screening strategy was established and accurate identification of LCCPs (including vLCCPs, which carbon chain length greater than 20) under the plight that the reference standards for vLCCPs are currently unavailable. A total of 21 C_{18-20}-LCCP and 22 vLCCP congeners were identified in sediment samples collected from Dongting Lake, China. The total concentrations of LCCPs in six sediment samples ranged from 1.69 to 18.0 ng/g (median 6.66 ng/g) and was dominated by C_{18} groups (mean, 28.8%), C_{19} groups (mean, 19.1%) and C_{21} groups (mean, 16.9%). Taken together, the successful application of this method to analyze sediment samples shows great potential for the analysis of LCCPs in environmental samples in future studies.

1. Introduction

Chlorinated paraffins (CPs) are extensively distributed in the environment, biota, and humans because of their huge production and use [1–3]. Conventionally, CPs are composed of short chains (SCCPs, C_{10-13}), medium chains (MCCPs, C_{14-17}), and long chains (LCCPs, $C_{\geq 18}$) [4]. LCCPs with carbon chain lengths greater than 20 can be further subdivided into very long-chain CPs (vLCCPs) [5]. With SCCPs listed in Annex A as a new group of persistent organic pollutants (POPs) under the Stockholm Convention in May 2017, CPs have been well known by the public and researchers world-wide because of their environmental, biological, and human risks [1,6]. In contrast to SCCPs and MCCPs, however, LCCPs have attracted less attention because of their environmental persistence [7,8], biomagnification in biota [9], and toxicity [10,11], although higher proportions and higher levels of LCCPs have been reported in lake sediments from China [8], coastal sediments from Sweden [7], and air samples from China [12]. A main obstacle for this situation may originate from the lack of a simple and fast pretreatment method and reliable instrumental analysis for LCCPs.

Generally, Soxhlet extraction and accelerated solvent extraction (ASE) are commonly used extraction methods for LCCPs in solid samples (e.g., soil, sediment, and biota) [13–16]. However, Soxhlet extraction requires a long time to perform and has high solvent consumption, and is therefore unsuitable for time-sensitive conditions or large-amount of samples for analysis. The ASE method requires a shorter extraction time, but still requires the usage of a significant amount of solvents [7,17-19]. Other alternative extrac-

tion procedures, such as ultrasound-assisted extraction [20] and microwave-assisted extraction [8,21], have also been developed by researchers for the quicker extraction of LCCPs. LCCPs extracted by these methods usually require a further purification protocol (e.g., silica gel column, aluminum oxide column) prior to instrument analysis [8,14,18-20], which makes these methods not user-friendly, consumes large amounts of organic solvents, and more importantly, does not conform to green chemistry principles [22].

In 2003, the quick, easy, cheap, effective, rugged, and safe (QuEChERS) extraction method was first proposed by Anastassiades et al. for multi-residue pesticide analysis in various agricultural products, such as fruits and vegetables [23]. This method commonly involves initial solvent partitioning based on the salting-out effect, followed by a dispersive solid-phase extraction (d-SPE) step that comprises of further clean-up using several combinations of porous sorbents to remove matrix interfering substances [24]. The advantage of this extraction method is that a sample concentration step is not required. In addition, the extraction solvent, sample amount, partitioning salts and their relative amounts and proportions, and pH value can be easily adjusted and optimized to improve the effectiveness of this method, given that the target analyte properties and matrix composition vary widely between different studies [25]. This advantage results in high selectivity, sensitivity, and specificity in the analysis of various organic pollutants, including polycyclic aromatic hydrocarbons, POPs, and pharmaceuticals in food and environmental matrices, particularly when the QuEChERS extraction method is combined with tandem mass spectrometry (MS/MS) or high-resolution mass spectrometry (HRMS) detection [24,26,27]. Consequently, the first goal of this study was to develop a rapid method for the extraction and purification of LCCPs based on the QuEChERS approach. Considering that the QuEChERS extraction process has been heavily simplified, a significant amount of co-extractives present in the final extract will inevitably interfere with the instrumental analysis of LCCPs. The next step to consider is how to implement online purification of LCCPs to further remove interfering substances prior to mass spectrometry (MS) analysis. For this purpose, a hydrophilic interaction chromatography (HILIC) column was introduced to establish a two-dimensional liquid chromatography (2DLC) system by a dynamic connection with a C_{18} column. This technique was first proposed by Albert Andrew in 1990 and has gained popularity in areas such as pharmaceutical analysis, bioanalysis, and food chemistry [28–32].

In recent years, ultra-high-performance liquid chromatography (UHPLC) coupled with Orbitrap HRMS has been applied to LCCPs analysis because it has higher selectivity and sensitivity (up to 450,000 full width at half maximum (FWHM) at m/z 200) and resolves the interference from SCCPs, MCCPs, and long-chain chlorinated olefins (LCCOs) [20,33]. The author of this study also developed an analytical procedure for LCCPs using Orbitrap HRMS in an ammonium-acetate-enhanced system [16]. However, the available reference standards for qualitative analysis of LCCPs currently mainly contain $C_{18–20}$-LCCP congeners, which makes the identification of potential vLCCPs unavailable, especially large LCCP-like substances that coexist in environmental samples, which share a close retention time to vLCCPs, and with similar primary mass spectrometry (MS^1) and isotopic patterns [16,20,33]. Integrated target, suspect, and characteristic fragment-dependent screening is a recently developed instrumental strategy for the identification of known and unknown chemicals in complex mixtures based on HRMS, as well as accurate mass and isotope patterns [34–37]. For instance, Zhang et al. identified three target citric acid esters (CAEs) and six novel CAEs in 50 indoor dust samples using LC-Orbitrap-HRMS, based on their full mass spectral information in both MS^1 and MS^2 patterns and the chemical inventory of PubChem [34]. Wang et al. conducted a comprehensive identification of aryl organophosphate

triesters (OPTEs) and discovered 11 novel aryl OPTEs in North China house dust using an HRMS-based method directed by characteristic aryl phosphate fragments [36]. Therefore, the second goal of this work is to establish an alternative instrument analysis procedure that can accurately identify LCCPs, including vLCCPs, using UHPLC-Orbitrap-HRMS based on the available $C_{18–20}$-LCCP standards and industrial products. Finally, as a case study, comprehensive identification and semi-quantitative analysis of LCCPs and vLCCPs in six sediment samples from Dongting Lake, China, was conducted to verify the newly established procedure.

2. Experimental

2.1. Chemicals

Two LCCP standards (36 and 49% Cl) were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). D_{18}-labeled α-hexbromocyclododecane (HBCD) was obtained from Cambridge Isotope Laboratories (Andover, MA, USA). All HPLC-grade solvents used for sample extraction, cleanup procedures, and instrumental analysis were purchased from Merck (Darmstadt, Germany). Three commercial clean-up kits (P/N 5982-0029, containing 1200 mg $MgSO_4$, 400 mg C18EC, 400 mg PSA, and 45 mg GCB; P/N 5982-1010, no available information on its composition because of patent protection, and P/N 5982-5156, containing 150 mg PSA, 150 mg C18EC, and 900 mg $MgSO_4$), NaCl, and $MgSO_4$ were purchased from Agilent Technologies (Canada). Three commercial CP products were obtained from a manufacturer in China.

2.2. Sample preparation

Six sediment samples (SD_1–SD_6) were collected from Dongting Lake, China. The lyophilized sediment samples (5 g) were each spiked with 10 ng D_{18}-labeled α-HBCD was mixed with 5 mL of water in a 50 mL Teflu centrifuge tube and vortexed for 1 min. Then, 15 mL of MeCN, 1 g of NaCl, and 4 g of Mg_2SO_4 were added to the centrifuge tubes and vortexed for 1 min, and the samples were centrifuged at 4000 rpm for 5 min. The supernatant (12 mL of the supernatant was collected and transferred to a commercial kit tube. No 5982–0029), vortexed for 1 min, and centrifuged at 4000 rpm for 5 min. The supernatant was collected and the residues were re-extracted twice with 5 mL of MeCN. The obtained supernatants were pooled and concentrated to 1.5 mL using a rotary evaporator and filtered using a 0.22 μm nylon membrane prior to instrumental analysis.

2.3. Online purification of LCCPs by 2DLC system

The 2DLC system was established for LCCPs online purification based on the dynamic connection of a HILIC column (Poreshell 120 Hilic column, 4.6 × 150 mm, 2.7 μm, Agilent Technologies) and a C_{18} column (XDB C_{18} column, 4.6 × 50 mm, 1.8 μm, Agilent Technologies) (Fig. S1). Briefly, 5 μL of each sample was first separated by a HILIC column and then entered into a C_{18} column, leading to the polar extract and LCCPs remaining on the HILIC column and C_{18} column, respectively (Fig. S1A). The two columns were then separated by valve A, and the polar matrix was removed from the HILIC column by pump A, whereas LCCPs was eluted from the C_{18} column by pump B (Fig. S1B and S1C). To reduce instrument contamination, only the eluent containing the LCCPs was allowed to enter the MS system by switching valve B (Fig. S1C, retention time (RT), 10–17 min), and the remaining eluent was transferred to a waste bottle (Fig. S1A and S1B, RT, 0–10 min and 17–26 min). The mobile phase of both pumps consisted of water with 3 mM CH_3COONH_4, and 0.01% acetic acid (A), methanol (B), and acetonitrile (C) at a flow rate of 0.4 mL/min, and the specific composi-

Table 1
The linear range, limit of detection (LOD) and limit of quantification (LOQ) of the established 2DLC–Orbitrap HRMS method.

Standards	Instrument	Linear range ng/mL	LOD ng/mL	LOQ ng/mL	Reference
LCCPs 36%Cl	2DLC-Orbitrap HRMS	5–2000	2.10	7.00	This study
	LC-Orbitrap HRMS	40–10,000	16.0	53.3	[16]
	LC-qTOF HRMS	20–5000	15	-	[39]
	LC-qTOF HRMS	-	60	-	[40]
LCCPs 49%Cl	2DLC-Orbitrap HRMS	2–500	0.41	1.38	This study
	LC-Orbitrap HRMS	40–10,000	7.9	26.3	[16]
	LC-qTOF HRMS	20–5000	10	-	[39]
	LC-qTOF HRMS	-	10	-	[40]

[a]not reported.

tion of the mobile phases of pumps A and B are listed in Table S1. To ensure sufficient response of LCCPs and retention of polar substances on the HILIC column, a portion of water/MeOH/MeCN (5/30/65) was chosen as the initial mobile phase [30,32,38]. As shown in Fig. S2, lower intensity of total ion chromatography were obtained using the developed extraction and purification strategy in this study. This indicated that it has a better removal efficiency for matrix compare to previous method [16].

2.4. Determination of LCCPs by Orbitrap HRMS

After purification using the 2DLC system, LCCPs were detected using an Orbitrap HRMS (Thermo Fisher Scientific, USA) operating in electrospray ionization (ESI) negative mode. The parameters of the MS^1 spectra (range, m/z 400–1500 at an MS resolution of 120,000) were based on our previous study [16]. Briefly, the ion transfer tube temperature was set at 175 °C, the vaporizer temperature was set at 300 °C, the spray voltage was 3900 V, the radio frequency (RF) lens level was 45 V, the flow rates of the auxiliary and sheath gas were 5 and 25 arbitrary units, respectively, and the MS with the automated gain control (AGC) target was set at 2.0×10^5. The collision-induced dissociation (CID) mode with 10, 20, and 30% collision energy was chosen to obtain the MS^2 spectra of LCCPs in the target MS^2 (tMS^2) scan, and the MS resolution of the tMS^2 scan was set to 30,000.

The limit of detection (LOD) and quantification (LOQ) of the 2D LC-Orbitrap HRMS method were calculated based on 3×SD/S and 10×SD/S, respectively, where SD is the standard deviation of the response of the seven replicate injections at a lower concentration (5 ng/mL for LCCPs 36%Cl and 2 mg/L for LCCPs 49%Cl) and S is the slope of the linear range curve [16]. Compared to previously method, lower LOD (0.41–2.10 ng/mL) and LOQ (1.38–7.00 ng/mL) were achieved (Table 1).

2.5. Screening procedure for LCCPs

The workflow of LCCPs' identification in the sediment samples is described in Fig. 1. Briefly, we firstly obtained the fragment mode of the C_{18-20}-LCCP standards by CID mode in tMS^2 scan mode. Due to the lack of vLCCP standards, a mixture of three CP industrial products (including one CP42 sample (∼42% chlorine content), one CP52 sample (∼52% chlorine content), and one CP70 sample (∼70% chlorine content)) was selected for screening vLCCPs based on the similar fragment mode of C_{18-20}-LCCPs in standards, and a total of 174 vLCCPs congeners were identified. Combined with the C_{18-20}-LCCP congeners present in industrial products and LCCP standards, a total of 202 LCCP congeners were obtained (Fig. S3). When the acetate adducts of $C_nH_{2n+2-m}Cl_m$ ($n = 18$–40, $m = 4$–30) in sediment samples were extracted using Xcalibur software with their MS^1 accurate mass, if the extracted

peaks are also contained in Fig. S3 with $\Delta RT < 0.1$ min, they were identified as LCCP congeners in the sediments. As for the extracted peaks are not on the list of identified LCCPs, we will use the CID mode in the tMS^2 mode for further identification. All data processing of the LCCP chromatography peaks was accomplished using the Xcalibur software (version 3.0), whose mass error was set at 5 ppm for MS^1 and 10 ppm for MS^2.

2.6. Matrix effects

Depending on the pretreatment procedures and column separation capabilities, matrix effects are likely to occur in LC-MS, especially in ESI mode, thereby affecting the detection of target compounds. In this study, 5, 50, and 200 ng/g of LCCPs were spiked into the sediment and MeCN to observe the matrix effects, and the specific calculation was as follows [41]:

% Matrix Effects (ME) = (Peak area $_{spiked\ in\ sediments}$ - Peak area $_{spiked\ in\ MeCN}$)/Peak area $_{spiked\ in\ MeCN}$ ×100 (1) where the Peak areas $_{spiked\ in\ sediments}$ and Peak area $_{spiked\ in\ MeCN}$ indicate the average peak areas of LCCPs spiked into sediments and MeCN, respectively.% ME value between −20% and 20% indicates no matrix effects, while% ME < −20% and% ME > 20% indicate ion suppression and enhancement, respectively.

3. Results and discussion

3.1. Establishment of QuEChERS extraction method and its performance

3.1.1. Selection of the sample amount and extraction solvent

Considering the convenience of the QuEChERS extraction method, we introduced this method for LCCPs analysis of sediment samples in this study. Initially, 10 g of the sample and 10 mL of MeCN were used to extract LCCPs in sediments as the original QuEChERS method proposed [23]. However, only a small volume of supernatant could be taken out after centrifugation, which is similar to the result found by Asensio-Ramos et al. (2010) when extracting 10 g of soil with 10 mL of MeCN [42], which may be ascribed to the strong hydrophobicity of LCCPs [43]. Then, 5 g of sediment and 15 mL of MeCN were used to obtain sufficient supernatant, and better recoveries were obtained, with an average value of 97.8 ± 5.1% (Fig. S4A). Besides MeCN, other organic solvents, such as dichloromethane (DCM) and acetone (ACE), have also been used as extraction solvents for the QuEChERS method [44,45]. Nevertheless, poor recoveries of LCCPs extracted by pure DCM or ACE were found in our preliminary experiment, and the most likely reason may be that these solvents cannot easily penetrate the water base to extract LCCPs from sediments [24]. Instead, a mixture of MeCN/ACE (v/v, 2:1, 15 mL) and MeCN/DCM (v/v, 2:1, 15 mL) was tested to recover LCCPs in the sediment. The results
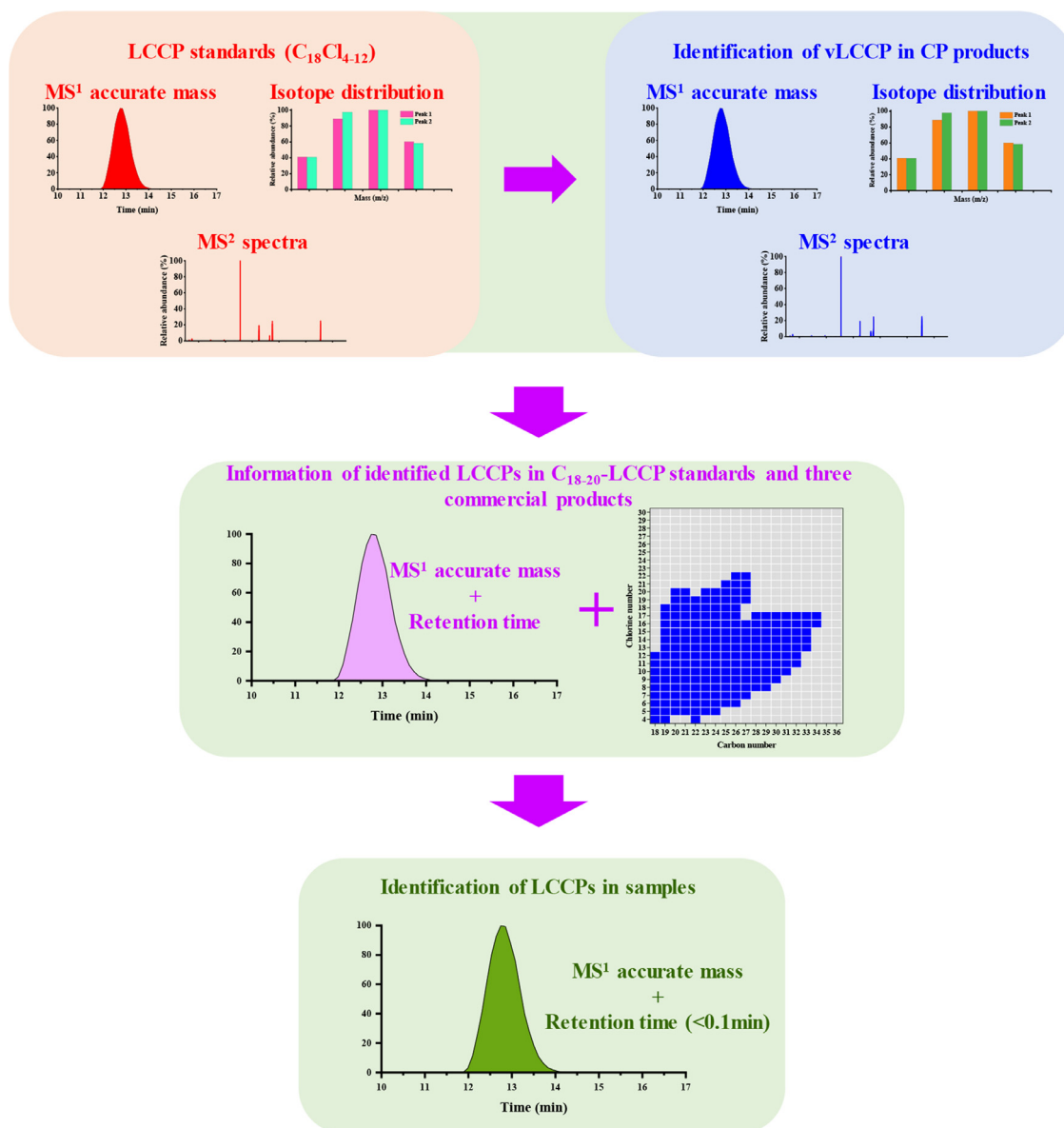
Fig. 1. Workflow of the identification of LCCPs in sediment samples.

showed that MeCN/ACE (v/v, 2:1) and MeCN/DCM presented a similar extraction ability for LCCPs as pure MeCN (Fig. S4A). Based on green chemistry principles, 5 g of sediment and 15 mL of MeCN were selected for LCCPs extraction in this study.

3.1.2. Optimization of clean-up procedures

After extraction, three commercial clean-up kits (P/N 5982-0029, P/N 5982-1010, and P/N 5982-5156 from Agilent Technologies) for QuEChERS extraction were chosen to further purify the extracts containing LCCPs in the present study. To maintain the stabilization of recoveries, the supernatant was collected and the residues were re-extracted twice with 5 mL MeCN when the extracts were purified using clean-up kits. As shown in Fig. S4B, kit 5982-0029 demonstrated the best recoveries (85.6 ± 1.3%), whereas the recoveries of kit 5982-5156 and kit 5982-1010 were 64.2 ± 2.4% and 31.2 ± 5.7%, respectively. As shown in Fig. S5, the good recovery observed for kit 5982-0029 might be attributed to its removal efficiency of the sample matrix (e.g., pigment removed by GCB), resulting in the lowest intensity of total ion chromatography [24]. As a universal kit, kit 5982-0029 has been applied to several biota samples such as eggs, birds, and wheat flour [26,41,46]. The remaining two were developed for fat-rich samples (kit 5982-5156) and lipid-rich samples (kit 5982-1010), both of which were present in low amounts in the sediment [47,48]. Thus, it is understandable that low recoveries of LCCPs were obtained using these two kits because of their poor matrix removal from the sediments. Therefore, kit 5982-0029 was selected for LCCPs purification from the sediments.

3.1.3. Method performance

Extraction recovery and matrix effects were determined to evaluate the performance of the QuEChERS extraction procedure established in this study. As shown in Fig. S4C, three levels of LCCPs 49% Cl (5, 50, and 200 ng/g) were spiked into sediments, and their corresponding recoveries were 90.5–95.2% (mean, 93.0%), 84.7–86.6% (mean, 85.6%) and 81.4–83.4% (mean, 82.4%), respectively. Simultaneously, D_{18}-labeled α-HBCD, which acts as a recovery standard, was also spiked into the sediments, and the recoveries ranged from 81.3 to 101% (mean, 90.5%). These results indicated that the established QuEChERS extraction method meets the analytical require-
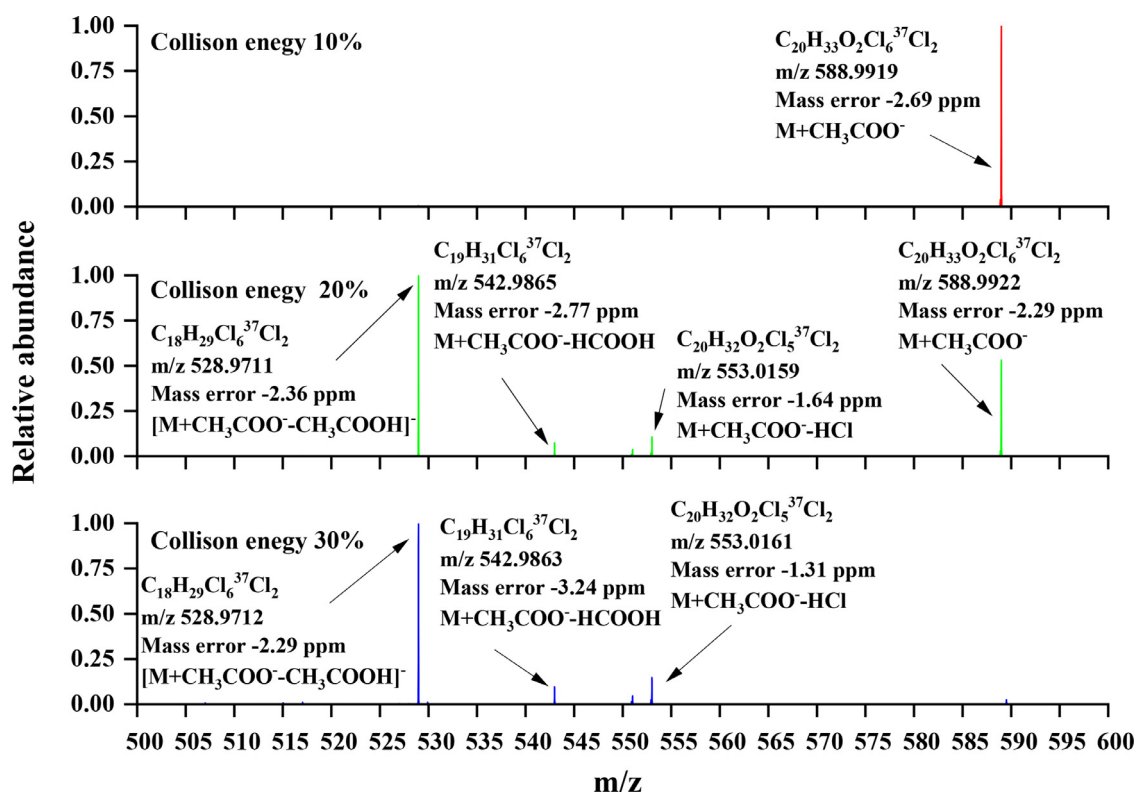
Fig. 2. The fragments of LCCP congener $C_{18}H_{30}Cl_8$ under different collision energies of CID mode.

ments of LCCPs in sediments. The matrix effects in this study were evaluated using 5, 50, and 200 ng/g of LCCPs 49% Cl spiked into the sediments and MeCN. The values of% ME were 0.9–2.9%, 11.0–12.9% and 16.0–18.0% for 5, 50, and 200 ng/g of LCCPs 49% Cl, respectively, suggesting that the current established method showed no matrix effects for LCCPs analysis.

3.2. Identification procedure for LCCP homologues

3.2.1. Characterization of fragmentation patterns of C_{18-20}-LCCP standards

To obtain the fragmentation patterns of the C_{18-20}-LCCP standards, the profile of LCCPs 49% Cl was first determined in full scan mode. As shown in Fig. S6, a total of 19 C_{18-20}-LCCPs were observed in the LCCPs 49%Cl, which is consistent with the results reported in our previous work [16]. To obtain their MS^2 spectra, we initially used the higher-energy collisional dissociation (HCD) mode to scan with three collision energies (10, 15, and 20%) because HCD can easily pulverize the target ions. However, the expected target ions, for example, the precursor of $C_{18}H_{30}Cl_8$, m/z 588.9922 ([M+CH$_3$COO$^-$], $C_{20}H_{33}O_2Cl_6{}^{37}Cl_2$), were not observed in the MS^2 spectra for all three HCD collision energies. Conversely, only acetate ions (m/z 59.0135) remained in the MS^2 spectra (Fig. S7). These results indicate that the HCD mode is unsuitable for the identification of LCCPs. The CID mode with three collision energies (10, 20, and 30%) was further selected to generate the MS^2 spectra of the LCCPs. As illustrated in Fig. 2, the precursor of $C_{18}H_{30}Cl_8$ ([M+CH$_3$COO$^-$], $C_{20}H_{33}O_2Cl_6{}^{37}Cl_2$, m/z 588.9922, −2.29 ppm) mainly generated three ions at m/z 528.9711, 542.9865, and 553.0159. These ions were assigned to the corresponding formulas of $C_{18}H_{29}Cl_6{}^{37}Cl_2$ (−2.36 ppm), $C_{19}H_{31}Cl_6{}^{37}Cl_2$ (−2.77 ppm), and $C_{20}H_{32}O_2Cl_5{}^{37}Cl_2$ (−1.64 ppm) using the Xcalibur software. We further reasoned that these ions were generated from $C_{20}H_{33}O_2Cl_6{}^{37}Cl_2$ via the neutral loss of acetic acid (CH$_3$COOH), formic acid (HCOOH), and hydrogen chloride (HCl), re-

spectively. These results suggest that the CID mode is more suitable for MS^2 spectra scanning of LCCPs characteristic fragment ions, which might be further used for the identification of vLCCPs.

3.2.2. Suspect screening vLCCPs in commercial products

Because the carbon chain length and the number of substituted chlorine atoms of LCCPs can be up to 40 and 27, respectively, and currently commercially available LCCP standards (LCCPs 36% Cl and 49% Cl) mainly contain C_{18-20}-LCCPs [16], we chose a mixture of three CP industrial products (one CP42 sample, one CP52 sample, and one CP70 sample) for the screening of vLCCPs. The accurate mass of the $C_nH_{2n+2-m}Cl_m$ precursor (n = 21–40, m = 4–30, [M+CH$_3$COO$^-$]) was initially used to identify the suspected vLCCPs in full scan mode. For the suspected C_{21-25}-LCCPs, only one peak was observed in the extracted chromatograms. For example, the chromatograms of the acetate adducts of $C_{21}H_{36}Cl_8$ ($C_{23}H_{39}O_2Cl_6{}^{37}Cl_2$, m/z 631.0383, −3.41 ppm), $C_{22}H_{38}Cl_8$ ($C_{24}H_{41}O_2Cl_6{}^{37}Cl_2$, m/z 645.0540, −3.22 ppm), $C_{23}H_{40}Cl_8$ ($C_{25}H_{43}O_2Cl_6{}^{37}Cl_2$, m/z 659.0710, −1.19 ppm), and $C_{24}H_{42}Cl_8$ ($C_{26}H_{45}O_2Cl_6{}^{37}Cl_2$, m/z 671.0895, −1.53 ppm) are shown in Fig. 3. After fragmentation by the CID mode in tMS^2 scan mode, the most abundant fragment ions of these peaks were m/z 571.0177, 585.0339, 599.0490, and 611.0679, respectively, which were further assigned to the formulas $C_{21}H_{35}O_2Cl_6{}^{37}Cl_2$ (−2.82 ppm), $C_{22}H_{37}O_2Cl_6{}^{37}Cl_2$ (−1.85 ppm), $C_{23}H_{39}O_2Cl_6{}^{37}Cl_2$ (−2.72 ppm), and $C_{24}H_{41}O_2Cl_6{}^{37}Cl_2$ (−2.24 ppm). This implied that these fragments were generated by the acetate adduct of the suspected C_{21-25}-LCCPs via the neutral loss of acetic acid. In addition, the neutral loss of HCOOH and HCl from the precursor of the suspect C_{21-25}-LCCPs under the CID mode was also observed. This fragment pathway of suspected C_{21-25}-LCCPs was similar to that of the C_{18-20}-LCCPs in LCCP 49% Cl, indicating that they are longer chain homologues of C_{18-20}-LCCPs.

Notably, two peaks (e.g., $C_{29}H_{51}O_2Cl_6{}^{37}Cl_2$, m/z 715.1320, −3.27 ppm, Fig. 4A) were observed when extracting $C_{>25}$-vLCCPs
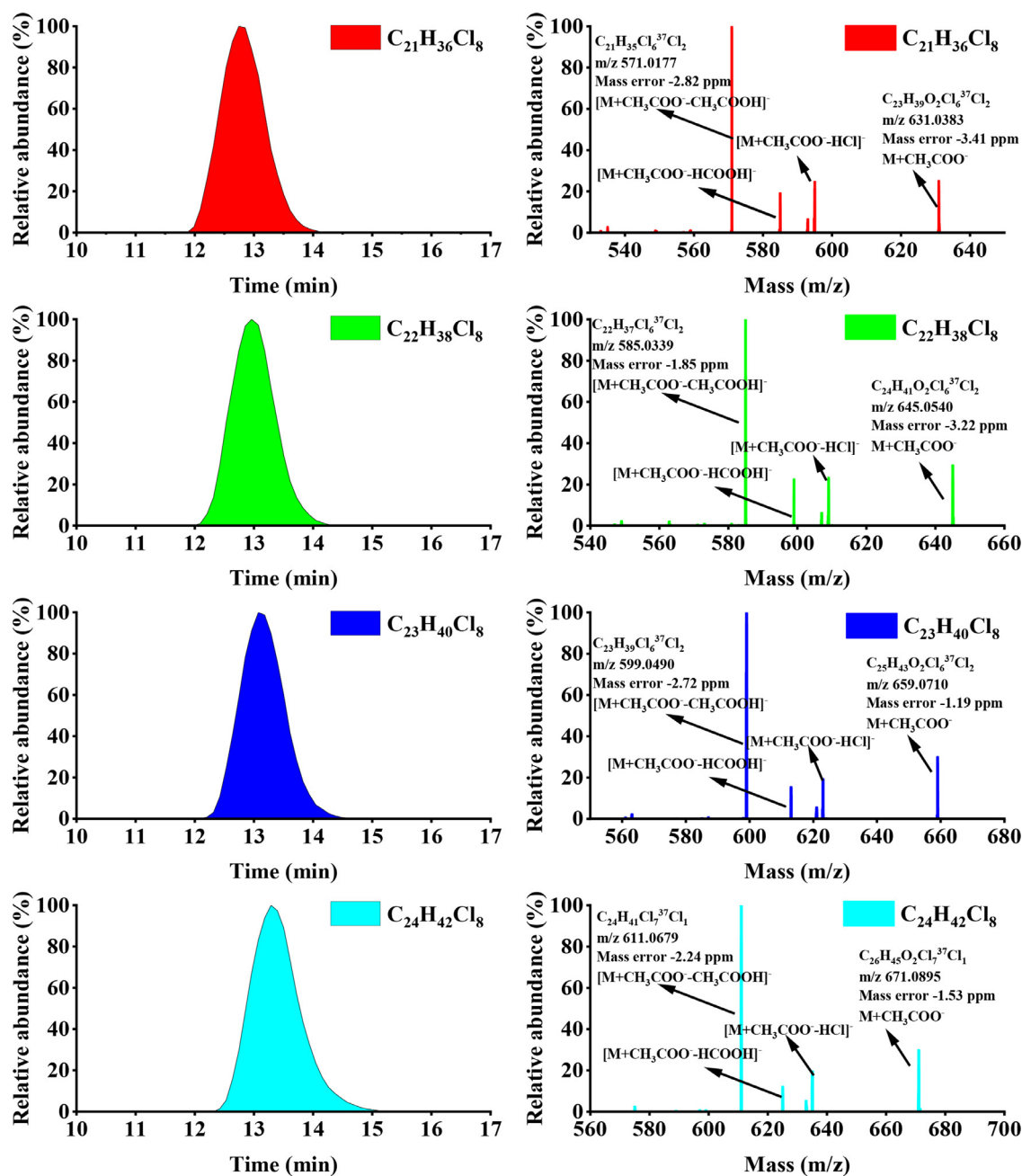
Fig. 3. Chromatograms of vLCCPs extract by MS^1 accurate mass and their corresponding MS^2 spectra in the mixture of commercial CP products.

from commercial products with their accurate MS^1 mass, which were challenging to resolve because of their identical isotope distribution (Fig. 4B). Their MS^2 spectra were then scanned in CID mode and are shown in Fig. 4C and 4D. Peak 1 was hardly fragmented under the CID mode, resulting in almost no fragments observed. The MS^2 spectrum of peak 2 mainly contained a peak at m/z 655.1113, which matched the formula of $C_{27}H_{47}Cl_6{}^{37}Cl_2$ (−3.03 ppm), indicating that it was formed by the neutral loss of acetic acid from peak 2. We also found fragments of peak 2 produced by the neutral loss of HCl (m/z 679.1557, $C_{29}H_{50}O_2Cl_5{}^{37}Cl_2$, −2.91 ppm) and HCOOH ($m/z$ 669.1228, $C_{28}H_{49}O_2Cl_6{}^{37}Cl_2$, −3.13 ppm) in the CID mode. Hence, peak 2 was identified as the acetate adduct of vLCCP congener $C_{27}H_{48}Cl_8$ because it had a similar MS^2 fragment pathway to that of C_{18-20}-LCCPs in LCCPs 49% Cl. This screening strategy comprehended accurate MS^1 mass extraction, isotopic distribution, and

MS^2 fragment pathway of suspicious peaks to determine whether they were vLCCPs. A similar strategy has also facilitated the screening of unknown congeners of emerging pollutants (e.g., OPEs and CAEs) [34–37]. In the end a total of 174 vLCCPs congeners were finally identified in the mixture of three commercial products. Combined with the C_{18-20}-LCCPs presented in industrial products and LCCP standards (36% Cl and 49% Cl), 202 LCCP congeners were obtained, as illustrated in Fig. S3.

3.3. Method application in sediment samples

3.3.1. Full screening of LCCPs in sediment samples

Six sediment samples collected from Dongting Lake, China, were extracted using the QuEChERS procedure established in this study, and comprehensively screened for the presence of LCCPs by 2DLC–Orbitrap HRMS. In the first step, we screened all po-
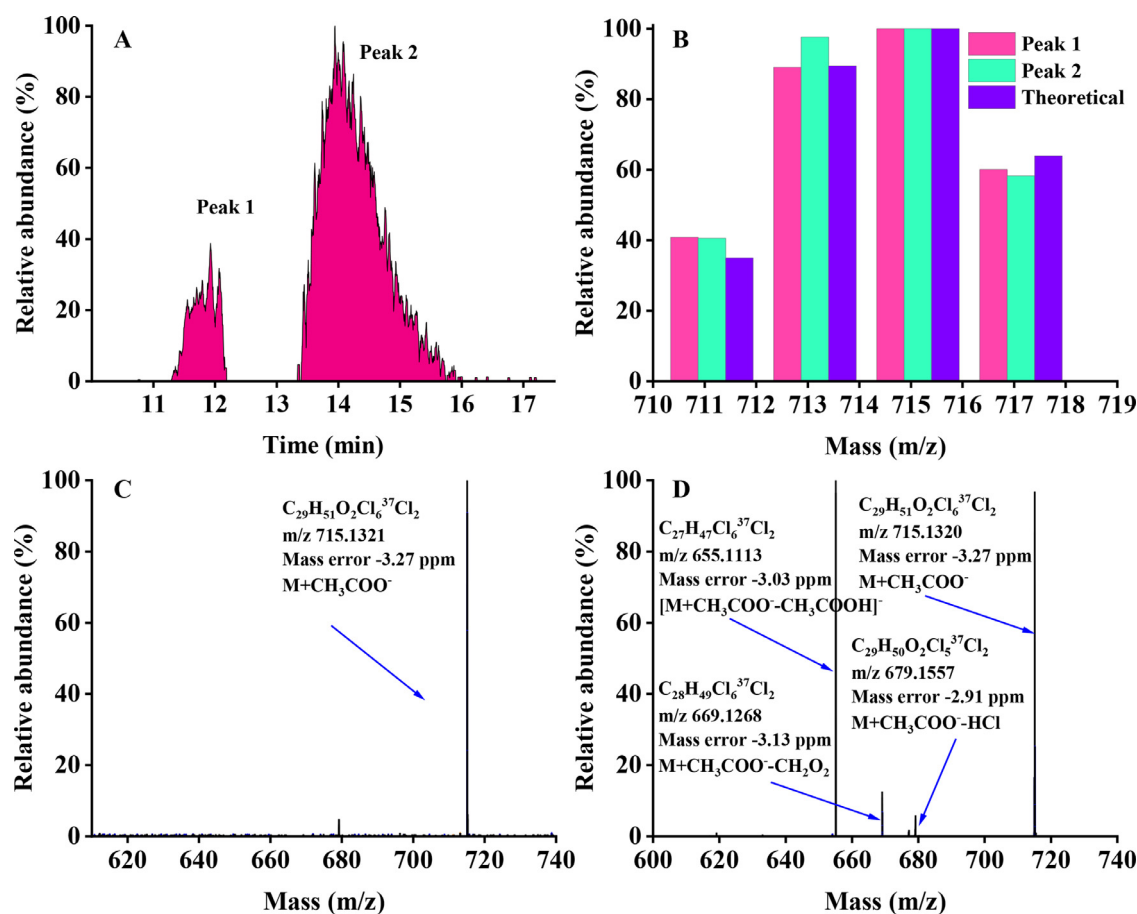
Fig. 4. The information of formula $C_{29}H_{51}O_2Cl_6{}^{37}Cl_2$ in the mixture of commercial CP products. (A) chromatogram of formula $C_{29}H_{51}O_2Cl_6{}^{37}Cl_2$ extracted by accurate MS^1 mass, (B) measured isotope distribution of peak 1 and peak 2, and the theoretical isotope distribution of $C_{29}H_{51}O_2Cl_6{}^{37}Cl_2$ (C) MS^2 spectra of peak 1 (unknown peak), and (D) MS^2 spectra of peak 2 (acetate adduct of $C_{27}H_{48}Cl_8$).

Table 2
The determination of LCCPs in sediment samples.

Samples	Chlorinecontent (%)	Concentrations (ng/g)		
		C_{18-20}-LCCPs	vLCCPs	ΣLCCPs
SD$_1$	51.9	0.75	0.93	1.69
SD$_2$	53.0	1.19	0.61	1.80
SD$_3$	53.4	6.07	4.08	10.1
SD$_4$	51.5	2.06	1.81	3.87
SD$_5$	52.9	13.2	4.87	18.0
SD$_6$	52.9	4.95	4.51	9.46
Median	52.9	3.50	2.94	6.66

tential C_{18-20}-LCCPs in the sediment samples following the above-mentioned screening strategy. Peaks present in the sediment samples that matched those observed in C_{18-20}-LCCPs standards and commercial CP products (ΔRT < 0.1 min) were identified as C_{18-20}-LCCP congeners. Ultimately, 21 C_{18-20}-LCCP congeners were identified in six sediment samples (Table S2). The specific number of identified C_{18-20}-LCCPs ranged from 9(SD$_1$)–20(SD$_2$). Their carbon chain lengths and chlorine numbers varied from 18 to 20 and 5–12, respectively. Among them, congeners $C_{18}Cl_{7-10}$ and $C_{19}Cl_{6-10}$ were found in all samples, while congeners $C_{20}Cl_{8-10}$ existed in five of the six samples. In the second step, we screened the vLCCPs in the sediment samples based on the same strategy. Extracting peaks present in the sediment samples matching those found in the commercial CP products (ΔRT < 0.1 min) were identified as vLCCP congeners. As listed in Table S2, 22 vLCCP congeners were identified in six sediment samples. The number of identified vLCCPs varied from 9(SD$_2$)–20(SD$_3$). Overall, these identified vLCCPs have carbon

chain lengths of 21–24 and chlorine numbers of 5–12. Congeners $C_{21}Cl_{7-10}$ and $C_{22-23}Cl_{8-10}$ were detected in all samples, whereas congeners $C_{23}Cl_{10}$ and $C_{24}Cl_{9-10}$ were observed in five of the six samples. All identified vLCCPs in the sediments were present in commercial CP products (Fig. S3), indicating that the established identification procedure will facilitate the identification of vLCCPs in sediment samples.

3.3.2. Profiles and concentrations of LCCPs in sediment samples

Based on the above screening results, the LCCP profiles in Dongting Lake sediments were dominated by C_{18} groups (mean, 28.8%), followed by C_{19} groups (mean, 19.1%) and C_{21} groups (mean, 16.9%) (Fig. S8). The chlorine group was dominated by Cl_9, followed by Cl_8, which together accounted for 61.6%. This result is in line with the results found in sediments from nine lakes in China [8] and from the Yangtze River Estuary in China [16]. The total LCCPs concentrations in sediments are listed in Table 2, which

varied from 1.69 to 18.0 ng/g (median, 6.66 ng/g). These results were lower than those found in sediments from nine Chinese lakes (median, 130 ng/g) [8], but were comparable to those found in river sediments in East China (median, 8.4 ng/g) [16], and in marine sediments from Sweden (median, 8.7 ng/g) [7].

4. Conclusions

This study describes an original, environment-friendly, and robust analytical method for the analysis of LCCPs based on QuEChERS extraction and 2DLC–HRMS. Compared with other reported methods, the low cost of the solvent required for the extraction of LCCPs and the greatly shortened sample pretreatment time (2 h) make this method ideal for large-scale sample preparation. Moreover, the suspect screening strategy established in this work, which includes accurate MS^1 mass extraction, isotopic distribution, and MS^2 fragment pathway of suspicious peaks, makes it reliable for the accurate identification of vLCCPs under the plight that reference standards for vLCCPs are currently unavailable. Finally, the successful application of this method to sediment samples show great potential for the analysis of LCCPs in environmental samples in future studies.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Yang Wu: Methodology, Formal analysis, Data curation, Writing – original draft, Funding acquisition. **Shutao Gao:** Conceptualization, Writing – review & editing, Funding acquisition. **Juntao Cui:** Data curation, Visualization. **Biao Zhang:** Investigation, Formal analysis. **Zhanjun Zhu:** Investigation, Resources. **Qian Song:** Data curation, Visualization. **Xiangying Zeng:** Methodology, Project administration. **Yi Liang:** Methodology, Data curation. **Zhiqiang Yu:** Project administration, Writing – review & editing.

Data Availability

Data will be made available on request.

Acknowledgments

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2022.463585.

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