EXPLORING THE CAPABILITY OF LIQUID CHROMATOGRAPHY-ELECTROSPRAY IONIZATION-TANDEM MASS SPECTROMETRY FOR THE IDENTIFICATION OF WASTES CONTAINING SHORT-CHAIN CHLORINATED PARAFFINS

Matsukami H[1], Kajiwara N[1], Kuramochi H[1]

[1]National Institute for Environmental Studies, Tsukuba, Japan, 305-8506, matsukami.hidenori@nies.go.jp

Introduction

Identification of wastes containing short-chain chlorinated paraffins (SCCPs) is the starting point for their effective environmentally sound management[1]. According to the development and dissemination of reliable analytical methods using high-resolution mass spectrometric (HRMS) techniques[2-5], we became to be able to obtain highly accurate and precise data on the concentrations of SCCPs in wastes. However, laboratories and institutions which can carry out the HRMS methods are limited because it requires large expenses to the instruments and their upkeep. Moreover, their high specifications may be more than enough for the identification of wastes containing SCCPs with the Basel Convention provisional low POP content value of 100 or 10,000 mg/kg[1]. So far, an electron capture negative ionization (ECNI)-single quadrupole MS coupled to gas chromatography (GC) system was commonly applied for the determination of SCCPs[6], whereas there are concerns about time-consuming procedures for the extensive clean-up and fractionation to minimize interferences with other halogenated compounds[7]. On the other hand, an electrospray ionization (ESI)-tandem MS (MSMS) coupled to liquid chromatography (LC) system has already been disseminated in many laboratories of the world and the system robustly works to determine pharmaceuticals, pesticides, and environmental contaminants with appropriate data quality. The objectives of this study were to explore the capability of LC–ESI-MSMS for the identification of wastes containing SCCPs. First, we optimized LC–ESI-MSMS measurement parameters for the determination of SCCPs. And then we validated the LC–ESI-MSMS method by comparing the results obtained from the measurements of the mixtures of SCCPs using LC–ESI-MSMS with GC–Orbitrap-HRMS. Finally, we applied the LC–ESI-MSMS method to commercial mixtures of CPs.

Materials and methods

Tetra- to octa-chlorinated decanes, tetra- to nona-chlorinated undecanes, tetra- to deca-chlorinated dodecanes, and tetra- to deca-chlorinated tridecanes were selected as the analytes. Two methods were used for the preparation of calibration standards. In the first method, individual calibration standards of polychlorinated decanes (C10-CS), undecanes (C11-CS), dodecanes (C12-CS), and tridecanes (C13-CS) were respectively prepared from a 1:1:1 mixture of C10 with 44.82%, 55.00%, and 65.02% Cl contents, a 1:1:1 mixture of C11 with 45.50%, 55.20%, and 65.25% Cl contents, a 1:1:1 mixture of C12 with 45.32%, 55.00%, and 65.08% Cl contents, and a 1:1:1 mixture of C13 with 44.90%, 55.03%, and 65.18% Cl contents (Dr. Ehrenstorfer GmbH) in accordance with the optimized method reported by Harada et al (2011)[3]. In the second method, a calibration standard of SCCP mixture (SCCP-CS) were prepared from a 1:1 mixture of SCCPs with 55.5% and 63% Cl contents (Dr. Ehrenstorfer GmbH). The concentrations of carbon and chlorine homologue groups in these calibration solutions were determined on the basis of $[M-2H-3Cl]^+$ ion abundances of carbon and chlorine homologue groups obtained from accurate mass measurements using GC–electron impact (EI)-Orbitrap-HRMS (Table 1). C10-CS, C11-CS, C12-CS, C13-CS, and SCCP-CS were used for the determination of analytes.

A conventional LC–ESI-MSMS system (UPLC H-Class/Xevo TQ-S micro; Waters Corp.) with a column (ZORBAX SB-CN RRHD, 2.1 × 100 mm i.d., 1.8 μm; Agilent Technologies Inc.) was used for the measurement of analytes. The ESI was used in the negative mode for analytes. The capillary voltage was set to 0.75 kV, with source temperature of 110 °C and desolvation temperature of 400 °C. Nitrogen was used as the desolvation and nebulizer gas. Argon was the collision gas. The desolvation gas flow was 1000 L/h and cone gas flow 20 L/h. A water solution containing 5 mM ammonium acetate was used as a mobile phase A and 100% methanol containing 5 mM ammonium acetate was used as a mobile phase B. The following gradient was used: 0 min (60% B), 20 min (99% B), 22 min (99% B), and 22.1 min (60% B). The flow rate of the mobile phase was set to 0.4 mL/min. The LC injection volume was 5 μL.

Three mixtures of SCCPs (SCCP-52%, -56%, and -63%), a mixture of medium-chain CPs (MCCP-52%) (Dr. Ehrenstorfer GmbH), and six mixtures of SCCPs and MCCPs with various ratios of their concentrations (CP-MIX-A to -F) were used for the method validation (Table 2). Nine commercial CPs (Chlorafin 40, Chlorowax 500C, CPW-100, Diable 700X, Unichlor 40-90, Unichlor 502-50, Unichlor 70AX, Paroil 170-8, and Paroil 179-HV), which were purchased from Accustandard, Inc., two commercial CPs (Paraffin chlorinated 40% and 70% Cl contents), which were purchased from FUJIFILM Wako Pure Chemical Corp., and two commercial CPs (T-SCCP and T-MCCP), which were specially synthesized for our earlier study[8] and then the concentrations of carbon and chlorine homologue groups were determined at that time, were used for the method application.

Table 1: Concentration ratios (%) of carbon and chlorine homologue groups determined in C10-CS, C11-CS, C12-CS, C13-CS, and SCCP-CS using GC–EI-Orbitrap-HRMS.

		C10-CS (%) 1:1:1 mixture of C10 with 45%, 55%, and 65% Cl contents	C11-CS (%) 1:1:1 mixture of C11 with 45%, 55%, and 65% Cl contents	C12-CS (%) 1:1:1 mixture of C12 with 45%, 55%, and 65% Cl contents	C13-CS (%) 1:1:1 mixture of C13 with 45%, 55%, and 65% Cl contents	SCCP-CS (%) 1:1 mixture of SCCP with 55%, and 63% Cl contents
C10	Cl3	2.8	0	0	0	0
	Cl4	18	0	0	0	0.93
	Cl5	29	0	0	0	2.2
	Cl6	23	0	0	0	2.5
	Cl7	18	0	0	0	1.7
	Cl8	8.0	0	0	0	0.56
	Cl9	1.5	0	0	0	0.087
	Cl10	0.047	0	0	0	0.0012
C11	Cl3	0	3.9	0	0	0
	Cl4	0	17	0	0	2.6
	Cl5	0	25	0	0	8.4
	Cl6	0	18	0	0	9.5
	Cl7	0	14	0	0	8.4
	Cl8	0	14	0	0	5.4
	Cl9	0	7.1	0	0	1.5
	Cl10	0	1.3	0	0	0.14
	Cl11	0	0.015	0	0	0.00080
C12	Cl3	0	0	2.6	0	0
	Cl4	0	0	12	0	1.4
	Cl5	0	0	21	0	5.7
	Cl6	0	0	19	0	7.7
	Cl7	0	0	12	0	7.1
	Cl8	0	0	13	0	7.1
	Cl9	0	0	13	0	3.7
	Cl10	0	0	5.3	0	0.70
	Cl11	0	0	0.70	0	0.045
C13	Cl3	0	0	0	1.8	0
	Cl4	0	0	0	10	0.39
	Cl5	0	0	0	19	2.0
	Cl6	0	0	0	18	3.4
	Cl7	0	0	0	15	4.7
	Cl8	0	0	0	15	6.0
	Cl9	0	0	0	14	4.4
	Cl10	0	0	0	6.4	1.5
	Cl11	0	0	0	0.92	0.16
ΣC10		100	0	0	0	8.0
ΣC11		0	100	0	0	36
ΣC12		0	0	100	0	33
ΣC13		0	0	0	100	23
SCCP		100	100	100	100	100

Table 2: Concentrations (ng/mL) of SCCP and MCCP mixtures s used for the method validation.

	SCCP			MCCP	CP-MIX					
	52%	56%	63%	52%	A	B	C	D	E	F
SCCPs with 51.5% Cl	10000	0	0	0	100	0	0	10000	0	0
SCCPs with 55.5% Cl	0	10000	0	0	0	100	0	0	10000	0
SCCPs with 63% Cl	0	0	10000	0	0	0	100	0	0	10000
MCCPs with 52% Cl	0	0	0	10000	10000	10000	10000	10000	10000	10000

Results and discussion:

The precursor ions of target homologue groups respectively gave stable acetate adduct molecule ions using a mobile phase that contained ammonium acetate. As the characteristics of ESI for SCCPs, it is noteworthy that the set values of source temperature and capillary voltage were important factors for their enhanced ionization. The product ions of tetra- and penta-chlorinated homologue groups were observed at m/z 59 (acetate ion) corresponding to the losses of molecule ions, whereas those of hexa- to deca-chlorinated homologue groups were observed at their molecule ions corresponding to the losses of an acetate ion. The application of a SB-CN column with a mobile phase composed of water and methanol containing 5 mM ammonium acetate in a gradient mode separated the carbon and chlorine homologue groups of SCCPs. All analytes were eluted completely from the SB-CN column at retention times of 2.9−7.0 min.

The calibration curves of analytes in C10-CS, C11-CS, C12-CS, C13-CS, and SCCP-CS were made in the total concentration range of 500–10,000 ng/mL, showing good linearity (R > 0.99). The instrumental detection limits of analytes were in the range of 0.5–50 ng/mL, which were calculated using the signal-to-noise ratio of multiple-reaction-monitoring (MRM) chromatograms obtained from LC–ESI-MSMS measurements of the calibration solutions. The higher response factor was observed for the analytes with the higher number of chlorine atoms.

To validate LC–ESI-MSMS method, the concentrations of analytes in the mixtures of SCCP-52%, -56%, and -63% obtained using GC–Orbitrap-HRMS and LC–ESI-MSMS were compared. Same individual calibration standards (C10-CS, C11-CS, C12-CS, and C13-CS) were used for GC–Orbitrap-HRMS and LC–ESI-MSMS measurements. As a result, the concentrations of analytes obtained by LC–ESI-MSMS method were significantly correlated with those by GC–Orbitrap-HRMS method (correlation coefficient = 0.98, $P < 0.001$, $n = 62$) (Fig. 1A). Most of the relative standard deviations (RSDs) from the results obtained by the methods using GC–Orbitrap-HRMS and LC–ESI-MSMS were within 30% (Fig. 1B). The results obtained by comparing with GC–Orbitrap-HRMS method suggest that LC–ESI-MSMS method might be useful for the determination of SCCPs.

In addition to the comparison of instrumentations, the concentrations of analytes in the mixtures of SCCP-52%, -56%, and -63% calculated using individual calibration standards and SCCP-CS were compared to validate the determination using SCCP-CS. The concentrations of analytes calculated using SCCP-CS were significantly correlated with those using individual calibration standards (correlation coefficient = 0.99, $P < 0.001$, $n = 62$) (Fig. 2A). And then most of the RSDs from the results calculated using individual calibration standards and SCCP-CS were within 30% (Fig. 2B). The results obtained by comparing with individual calibration standards suggest that SCCP-CS might be useful for the determination of SCCPs. Furthermore, the method using SCCP-CS was expected to be more practical than that using individual calibration standards because the expense of SCCP-CS is considerably lower than that of individual calibration standards.
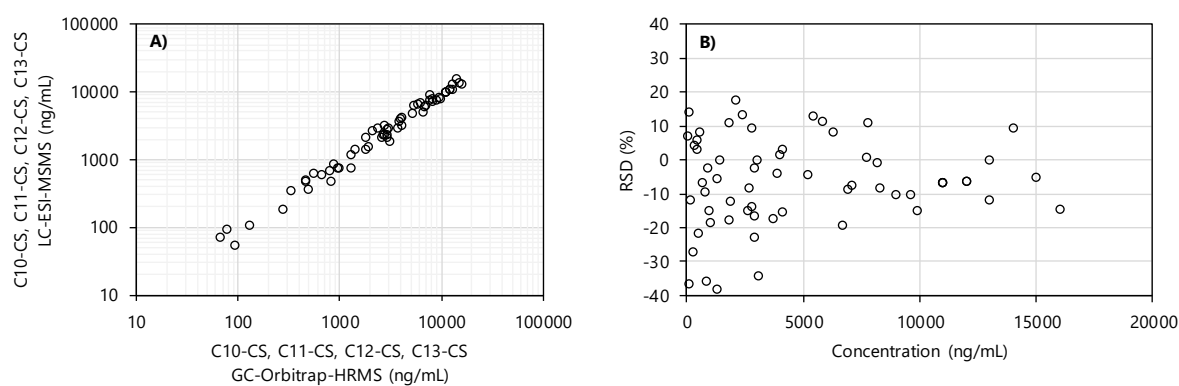

Fig. 1: (A) Relationship between the concentrations of analytes in the mixtures of SCCP-52%, -56%, and -63% obtained by GC–Orbitrap-HRMS and LC–ESI-MSMS, and (B) RSDs from the results obtained by GC–Orbitrap-HRMS and LC–ESI-MSMS.
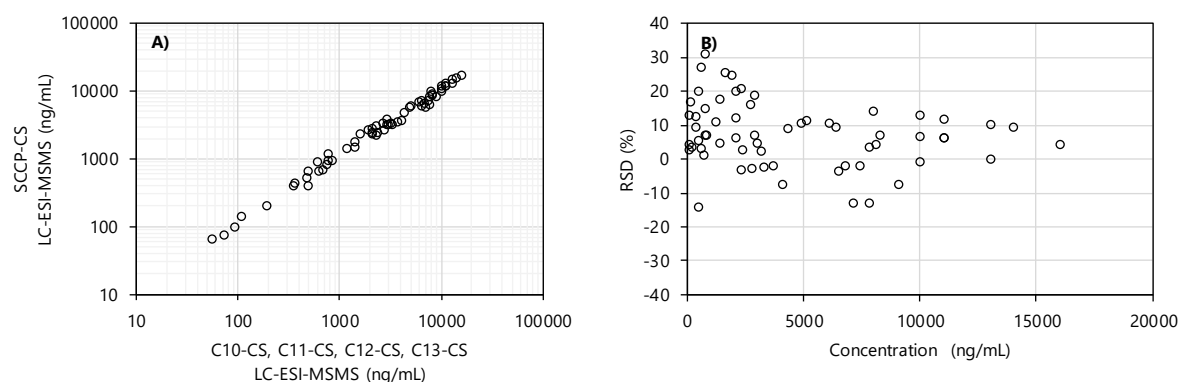

Fig. 2: (A) Relationship between the concentrations of analytes in the mixtures of SCCP-52%, -56%, and -63% obtained by individual calibration standards and SCCP-CS, and (B) RSDs of the results obtained by individual calibration standards and SCCP-CS.

Fig. 3 shows the concentration profiles of carbon and chlorine homologue groups of SCCPs determined in CP-MIX-A to -F using the LC–ESI-MSMS with SCCP-CS method. Although the concentrations of SCCPs in CP-MIX-A to -C were two orders of magnitude lower than those of MCCPs, SCCPs were successfully measured without significant interferences from MCCPs. The good agreement of the profiles between the nominal and observed concentrations suggests that the LC–ESI-MSMS with SCCP-CS method might be able to determine SCCPs contained as impurities of commercial MCCPs accurately.

Finally, we applied the LC–ESI-MSMS with SCCP-CS method to the analysis of commercial CPs. The LC–ESI-MSMS measurements showed suitable retention with good separation and peak shapes for the analytes in the

commercial CPs. Of the 13 commercial CPs, Chlorowax 500C, Diable 700X, Paroil 179-HV, Unichlor 502-50, and T-SCCP were identified to contain SCCPs with the concentrations higher than the Basel Convention provisional low POP content value of 100 or 10,000 mg/kg[1] (Table 3). These results obtained in this study suggest that the LC–ESI-MSMS with SCCP-CS method might be able to identify wastes containing SCCPs appropriately. Additional studies are currently in progress to confirm the capability of the LC–ESI-MSMS with SCCP-CS method for the practical identification of wastes containing SCCPs.
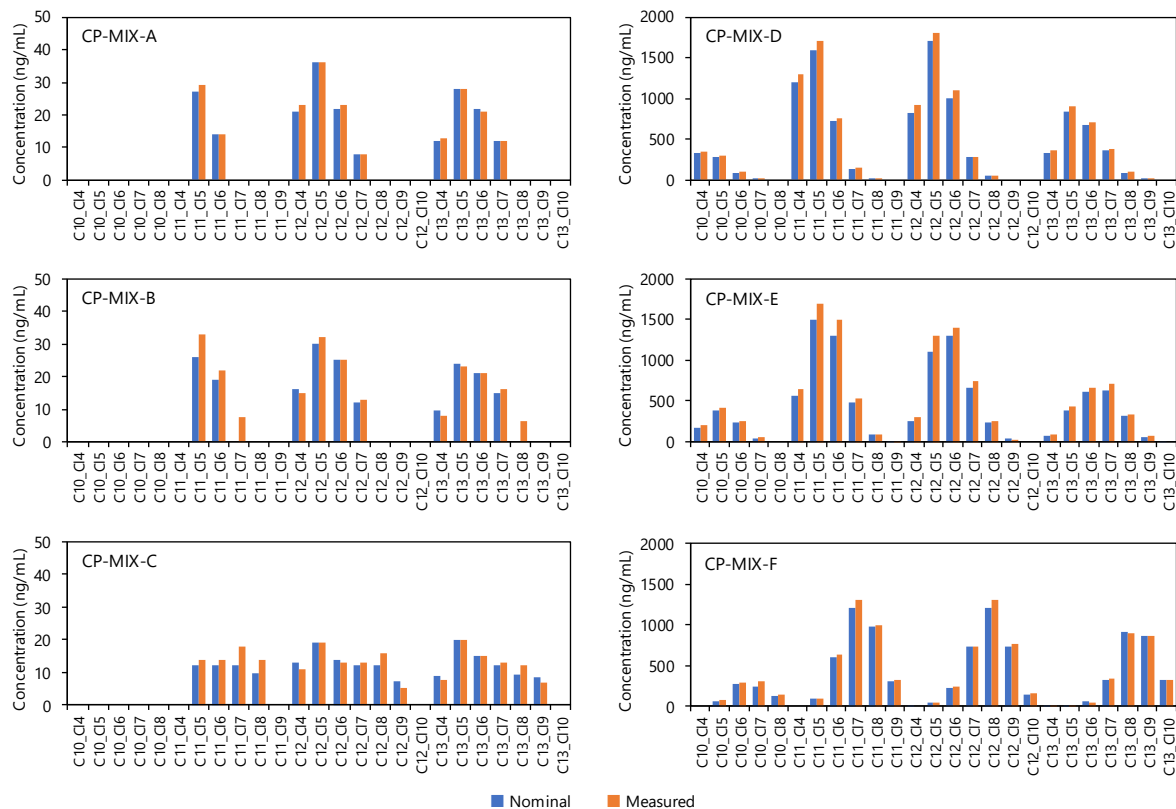

Fig. 3: Concentration profiles of carbon and chlorine homologue groups of SCCPs determined in CP-MIX-A to -F by LC–ESI-MSMS with SCCP-CS method.

Table 3: Concentrations (%) of SCCPs in commercial CPs

	Chlorafin 40	Chlorowax 500C	CPW-100	Diable 700X	Paroil 170-8	Paroil 179-HV	Unichlor 40-90	Unichlor 502-50	Unichlor 70AX	CP 40% Cl	CP 70% Cl	T-SCCP	T-MCCP
ΣC10_Cl4-8	<0.5	12	<0.5	110	<0.5	1.8	<0.5	21	<0.5	<0.5	<0.5	0.057	<0.5
ΣC11_Cl4-9	<0.2	43	<0.2	2.5	<0.2	5.0	<0.2	46	<0.2	<0.2	<0.2	100	<0.2
ΣC12_Cl4-10	<0.1	50	<0.1	0.20	<0.1	6.3	<0.1	37	<0.1	<0.1	<0.1	0.056	<0.1
ΣC13_Cl4-10	<0.1	11	<0.1	0.22	<0.1	1.7	<0.1	4.5	<0.1	<0.1	<0.1	0	<0.1
SCCPs	<0.5	120	<0.5	110	<0.5	15	<0.5	110	<0.5	<0.5	<0.5	100	<0.5
%Cl	-	59	-	66	-	68	-	54	-	-	-	64	-

References:
1. UNEP (2019). Technical guidelines. UNEP/CHW.14/7/Add.1.
2. Tomy GT, Stern GA, Muir DCG, et al. (1997) Anal. Chem. 69, 2762–2771.
3. Harada KH, Takasuga T, Hitomi T, et al. (2011) Environ. Sci. Technol. 45, 7019–7027.
4. Bogdal C, Alsberg T, Diefenbacher PS, et al. (2015) Anal. Chem. 87, 2852–2860.
5. Krätschmer K, Cojocariu C, Schächtele A, et al. (2018) J. Chromatogr. A 1539, 53–61.
6. Sverko E, Tomy GT, Marvin CH, et al. (2012) Environ. Sci. Technol. 46 4697–4698.
7. Reth M, Oehme M. (2004) Anal. Bioanal. Chem. 378 1741–1747.
8. Matsukami H, Kajiwara N. (2019) Chemosphere 230 164–172.