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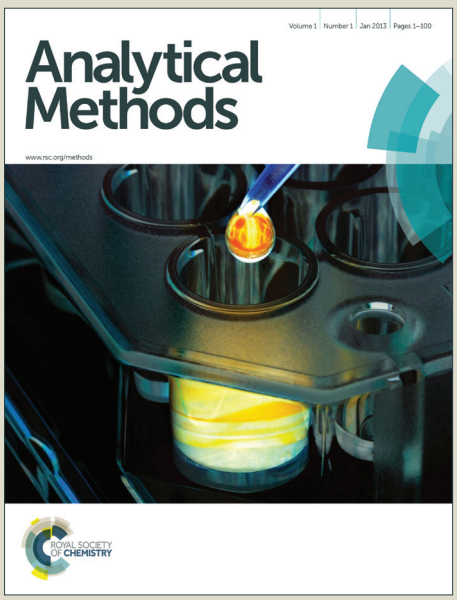

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Comparative Assessment of the Chromatographic Separation of 2,3,7,8-Substituted Polychlorinated Dibenzo-*p*-Dioxins and Polychlorinated Dibenzofurans Using Supercritical Fluid Chromatography and High Resolution Gas Chromatography†

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†Electronic Supplementary Information (ESI) available: Figure S1: Chromatograms illustrating the separation of the PCDF HRGC window definers, PCDD HRGC window definers, and a mixture of 2,3,7,8-substituted PCDD and PCDF congeners using the developed pSFC-MS/MS method. Tables S1 – S4: Components and concentrations of standard solutions. Table S5: Summary of the HRGC/HRMS and pSFC-MS/MS quantification results for a proficiency testing material. See DOI: 10.1039/x0xx00000x

Abstract

The analysis of legacy environmental contaminants, such as polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs), using high resolution gas chromatography (HRGC) is well established and universally accepted. The use of an alternative separation technique, such as packed column supercritical fluid chromatography (pSFC), may be of interest as a fast, green, and cost effective method of analyzing environmental samples. The technique is amenable to a broad range of chemical compounds and could facilitate the simultaneous analysis of multiple compound classes as well as the inclusion of thermally labile compounds in a single targeted analysis. The recent re-emergence of this

technology due to the introduction of more robust and efficient instrumentation may result in an increased acceptance of pSFC analytical techniques in this area. Herein, the first reported analytical separation of PCDDs and PCDFs by pSFC is described and its separation capabilities are compared with established HRGC protocols. Elution profiles of 2,3,7,8-substituted PCDDs and PCDFs were examined and the separation of PCDD/PCDF homologue groups was found to be comparable to those accomplished using HRGC. Similarly, the resolution of tetrachlorodibenzo-*p*-dioxin (TCDD) congeners, as required by current regulatory methods utilizing HRGC, was demonstrated and the separation of possible co-eluting PCDD/PCDF congeners was examined and compared to that achieved using popular HRGC capillary columns. The possibility of concurrent analysis of toxic polychlorinated biphenyls (PCBs) with PCDDs and PCDFs using the developed pSFC method was also investigated. The effective separation of these environmental contaminants obtained using pSFC and subsequent detection utilizing atmospheric pressure photoionization tandem mass spectrometry at environmentally relevant levels demonstrates the promise associated with this technique for the analysis of environmental extracts.

Introduction

The persistent and toxic nature of halogenated compounds that are pervasive in the environment following their release from industrial activities, commercial applications, and/or secondary formation processes has resulted in extensive research into compound specific analytical method development.[1,2] Regulations have been implemented globally to protect both human and environmental health through the monitoring of persistent organic pollutants (POPs)[3-5] and the cessation of manufacturing or formation practices through procedural modifications[6]. Unfortunately, the extraction and testing of environmental samples and foodstuffs destined for human consumption can be time intensive and costly. The application of packed column supercritical fluid chromatography (pSFC), a widely applicable

fast analytical separation technique, to highly regulated POPs, such as polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs), has not yet been investigated. This paper reports the first pSFC method developed for the separation of PCDDs and PCDFs and assesses its separation capabilities against the analytical method, high resolution gas chromatography (HRGC), currently utilized for the analysis of these compounds.

PCDDs and PCDFs have historically been introduced into the environment as unintentional by-products of chemical manufacturing and combustion processes.[7] Sources include the incineration of municipal solid waste and medical waste, chlorine bleaching of paper and pulp, electric arc furnaces, secondary aluminum smelters, sinter plants, and as contaminants in industrial chemicals such as chlorophenols, the phenoxy acid herbicides 2,4-D and 2,4,5-T, and polychlorinated biphenyls (PCBs).[8-12] Non-industrial sources such as vehicle exhaust emissions[13], photochemical synthesis from pentachlorophenol in atmospheric condensed water[14], and combustion of chemically treated wood[15] have also been proposed. The atmospheric transport of these compounds and their ultimate fate after release into the environment have also been extensively studied[16-19] because of their environmental persistence, toxicity, and bioaccumulation potential. Human exposure to PCDDs and PCDFs is believed to occur primarily through consumption of fish, dairy produce, and meat.[20,21] Although the general population is exposed to very low levels, below 4 pg TEQ/kg/day, PCDDs and PCDFs are lipophilic and accumulate primarily in adipose tissue and blood lipids as well as the liver.[22] Health effects are mediated via the arylhydrocarbon receptor (AhR) and toxic responses include dermal toxicity, carcinogenicity, immunotoxicity as well as endocrine, reproductive, and developmental effects.[21-25]

The analysis of PCDDs and PCDFs in environmental samples is typically performed by HRGC coupled with high resolution mass spectrometry (HRMS)[26], but recently, gas chromatography coupled with tandem mass spectrometry (GC-MS/MS) was accepted as a means of confirming compliance with established

regulatory limits of PCDDs, PCDFs, and PCBs in foodstuffs[27]. The elution orders of the 49 PCDD congeners and 87 PCDF congeners substituted with four to eight chlorines on different, commonly used, HRGC stationary phases have been determined and the required GC conditions are well-known.[28-31] However, the development of alternative, more universal separation and detection techniques for the analysis of regulated POPs[32-34] could result in the modification of accepted protocols. The use of packed column supercritical fluid chromatography coupled to a selective and sensitive mass spectrometer capable of atmospheric pressure photoionization (APPI) may be of interest as a fast and cost effective method of analyzing environmental samples. This separation method is amenable to a broad range of chemical compounds and could facilitate the simultaneous analysis of multiple compound classes including thermally labile analytes[35].

The use of a supercritical fluid (SCF) as a mobile phase in chromatographic applications affords separation capabilities that differ from both HRGC and high performance liquid chromatography (HPLC). Since SCFs have densities and solvating power similar to that of a liquid and diffusivities and viscosities similar to that of a gas, the use of these fluids as mobile phases results in unique and tunable separations.[36] A supercritical fluid can act as both a substance carrier, similar to mobile phases used in gas chromatography (GC), and a solvent, analogous to the mobile phases used in liquid chromatography (LC).[37] This unique behaviour may result in improvements in chromatography since it allows alteration of the mobile phase by either variation of the physical state of the fluid, through temperature or pressure changes, or by adding organic modifiers (typically alcohols at percentages between 5 -50%) and polar additives (e.g. acids, bases, or salts) at low percent levels.[38,39] The most commonly used SCF in pSFC is carbon dioxide due to the accessibility of its critical temperature (31.3°C) and critical pressure (72.9 atm)[40] using available instrumentation as well as its non-toxic and non-aggressive chemical nature[36]. The application of a fast, cost effective, and green technique such as pSFC for the effective separation of persistent environmental contaminants, specifically PCDDs and PCDFs in this instance,

could result in a widely applicable method with affording separations that complement both HRGC and HPLC.

Materials and Methods

Chemicals

All polychlorinated dibenzo-*p*-dioxin (PCDD), dibenzofuran (PCDF), and biphenyl (PCB) standards were obtained from Wellington Laboratories Inc. (Guelph, ON, Canada) including EPA-1613STOCK (a mixture of 2,3,7,8-substituted PCDDs and PCDFs; concentration range 0.4 – 4.0 µg/ml), EPA-8280CVS (a series of calibration solutions containing native and ^{13}C-labelled PCDDs and PCDFs; concentration range 0.1 ng/µl – 10 ng/µl), 5TCDD (a 2378-TCDD isomer resolution testing mixture; concentration range 0.5 – 1.0 µg/ml), WP-STK (a solution containing PCBs at 2000 ng/ml each component), and individual PCDD and PCDF reference standards (50 µg/ml). Additional information relating to the standard mixtures is provided in the supporting information. Proficiency testing material for polychlorinated dioxins and furans in water by U.S. EPA Method 8280B (PE1102-2ML) was purchased from Sigma Aldrich (Oakville, ON, Canada). HPLC grade methanol, water, and acetonitrile, distilled in glass grade toluene, dichloromethane, nonane, and ethyl acetate, and reagent grade formic acid (88% in water) were purchased from Caledon (Guelph, ON, Canada). LC-MS Chromasolv grade 2-propanol (isopropanol; IPA) and cyclohexane as well as reagent grade ammonium acetate (99.999%), methylcyclohexane, cyclopentane, methylcyclopentane, and fluorobenzene (99%) were purchased from Sigma Aldrich (Oakville, ON, Canada). Food grade carbon dioxide was purchased from Linde Canada Industrial Gases (Guelph, ON, Canada) and research grade helium (99.9999%) was purchased from Praxair Canada Inc. (Mississauga, ON, Canada).

Chromatographic systems and conditions

All HRGC/HRMS analyses were conducted on an Agilent 6890N Gas Chromatograph (Agilent Technologies, Santa Clara, USA) with a direct capillary interface to an Autospec Ultima High Resolution Mass Spectrometer (Waters Corp., Milford, MA, USA). Chromatographic separations were carried out on an Agilent J&W DB5 (60 m x 0.25 mm ID, 0.25 µm film thickness) column in constant flow mode (Helium, 1.0 ml/min). All injections were 1 µl at a temperature of 280°C in splitless injection mode. The mass spectrometer was operated in EI+ selective ion recording mode (SIR) with an optimized electron energy of 40 eV and a mass resolving power of 10,000 or greater. The following temperature program was utilized: initial oven temperature 150°C, hold for 1 minute, ramp at 12°C/minute to 200°C, ramp at 3.0°C/minute to 235°C, hold for 8 minutes, ramp at 8°C/minute to 310°C, hold for 8 minutes.

All pSFC separations were carried out using a Waters Acquity UltraPerformance Convergence Chromatograph (UPC^2) (Waters Corp., Milford, MA, USA) system equipped with an Acquity Photodiode array (PDA) Detector, Acquity Convergence Manager, Acquity UPC^2 Binary Solvent Manager, Isocratic Solvent Manager, Acquity Sample Manager, and an Acquity Column Manager. The UPC^2 was coupled to a Micromass Quattro micro atmospheric pressure ionization (API) Mass Spectrometer (MS) (Waters Corp., Milford, MA, USA) configured in positive-ion atmospheric pressure photoionization (APPI) mode with the following parameters: krypton lamp (eV) = 10.6; repeller voltage (kV) = 1.75; cone voltage (V) = 30.00 – 60.00; cone gas flow (L/Hr) = 50; desolvation gas flow (L/Hr) = 500; desolvation temperature (°C) = 625; source temperature (°C) = 120, collision gas cell pressure (mbar) = 5.0e-3; collision energies (eV) = 30-40. Methanol containing 5% fluorobenzene was used as the MS make-up solvent and was added to the split from the UPC^2 at a flow-rate of 0.075 ml/min. Molecular ion clusters corresponding to $[M]^{+\cdot}$ were generated with these settings for all PCDD,PCDF, and PCB congeners investigated and the major daughter ions observed were $[M\text{-}COCl]^{+\cdot}$ for PCDDs and PCDFs and $[M\text{-}Cl_2]^{+\cdot}$ for PCBs. All MS data were acquired in MRM mode and processed using Waters MassLynx software (mean smoothing was applied to all data; window = 1, N = 1). PDA data were also collected from 200 -350 nm and two absorbance-

compensated UV channels were monitored (240 nm and 310 nm with a compensation reference range of 350-450 nm). Optimal separations were achieved with a Waters UPC^2 Torus 1-AA column (1.7 µm, 3.0 x 100 mm) at 60°C using a methanol (MeOH) modified carbon dioxide (CO_2) mobile phase with the back-pressure maintained at 2500 psi at a flow-rate of 1 ml/min. The gradient program was initially set to 99% CO_2, 1% MeOH and then ramped to 10% MeOH over 10 minutes. This was followed by a second 10 minute ramp to 35% MeOH and a third 7 minute ramp to 40% MeOH before returning to initial conditions in 1 minute. The total run-time was 30 minutes.

pSFC Stationary Phases

The following stationary phases were investigated for the separation of the seventeen PCDDs/PCDFs substituted in the 2,3,7,8-positions: Waters UPC^2 BEH (1.7 µm, 3.0 x 100 mm), Waters UPC^2 HSS C18 SB (1.8 µm, 3.0 x 100 mm), Waters Acquity UPLC BEH C8 (1.7 µm, 2.1 x 100 mm), Agilent Zorbax SB-CN (1.8 µm, 2.1 x 100 mm), Waters Acquity UPLC BEH Phenyl (1.7 µm, 2.1 x 100 mm), Restek Pinnacle DB Biphenyl (3 µm, 4.6 x 150 mm), Cosmosil 5PYE (5 µm, 4.6 x 150 mm), and Waters UPC2 Torus 1-AA (1.7 µm, 3.0 x 100 mm).

Analysis of PCDD/PCDF Proficiency Testing Material

The proficiency testing (PT) material (PE1102-2ML) consisted of a solution of PCDD and PCDF congeners of unknown concentrations in methanol for the quantification of the contained analytes in water according to U.S. EPA Method 8280B. The water sample was prepared by adding exactly 1.0 ml of PE1102-2ML to 1 litre of HPLC grade water. The sample was then spiked with ^{13}C-labelled PCDD and PCDF surrogates and extracted with dichloromethane. The resulting extract was cleaned-up using a multi-layer column followed by a carbon column and the final extract was concentrated to 100 µL in

nonane before being analyzed by HRGC/HRMS and pSFC-MS/MS for PCDDs and PCDFs using appropriate calibration standards.

Results and Discussion

Method Development

pSFC method development involved column screening followed by cosolvent, temperature, pressure, and gradient optimizations on promising stationary phases. Many of the columns explored (specifically the bare silica and alkyl bonded stationary phases) exhibited poor retention of the PCDD/PCDF congeners even at very low percentages of polar CO_2 modifiers (typically methanol) and non-polar modifiers (cyclohexane). It was found that stationary phases with varying degrees of aromatic character exhibited the most promising elution profiles. Stationary phases with higher degrees of aromaticity required more polar solvents to effect elution of the analytes within a reasonable timeframe. The availability of the π electrons in the dioxin and furan skeletons, and the presence of polar halogen substituents, makes dispersion interactions and π-π overlap probable mechanisms in the retention of these analytes on aromatic stationary phases.[41] Indeed, the anthracene based Torus 1-AA column with isopropanol and methanol cosolvents exhibited stronger retention than the DB Biphenyl column with an acetonitrile cosolvent which in turn provided superior results to the BEH Phenyl column when a cyclohexane cosolvent was employed. Elution of the PCDD/PCDF congeners from the Cosmosil 5PYE column, which is a pyrenylethyl group bonded stationary phase, could not be accomplished using multiple cosolvents (methanol, acetonitrile, acetonitrile with 5% toluene, and ethyl acetate). It is believed that the planar pyrene ring structure resulted in strong π-π interactions which could not be disrupted with these particular separation conditions.

The Torus 1-AA column was found to provide an elution profile comparable to that accomplished during HRGC separations using a DB-5 column with only minor changes in elution order noted. Effective separation of the homologue windows was observed when isopropanol or methanol was used as the cosolvent (Figure 1), but the overall observed congener resolution (of the hexachlorinated analytes in particular) using the 100 mm pSFC column was inferior to that obtained by HRGC. Other cosolvents were investigated for this stationary phase, but all produced poor separation and/or elution of the higher chlorinated analytes. Assuming that the dominant retention mechanism was π-π interactions due to the observation that increasing the π character of the stationary phase resulted in increased retention of the PCDDs/PCDFs, the methanol cosolvent was supplemented with an ammonium salt additive (5 mM ammonium acetate) in an attempt to disrupt these interactions. Unfortunately, no substantial effect was observed. Acetonitrile was found to be the optimal cosolvent for the biphenyl stationary phase, so the use of an acetonitrile cosolvent doped with 5% toluene was also investigated. It was speculated that the increased solubility of the highly substituted PCDDs and PCDFs in toluene would facilitate the elution of the analytes from the column since larger amounts of the additive/modifier would increase the interaction between the analytes and the mobile phase as the gradient increased. However, at this level, any increased solubility was not sufficient to effect elution. The separation of the PCDDs and PCDFs utilizing an IPA cosolvent doped with 0.5 % formic acid was also attempted. If the retention mechanism was dominated by hydrogen bonding, it was speculated that formic acid in the mobile phase might compete for bonding sites and result in an altered elution profile, but a noticeable effect was not observed.

Methanol was determined to be the most effective cosolvent that was also compatible with the selected ionization process. With an ionization energy of 10.84eV, methanol was not ionized by the krypton lamp of the APPI source and therefore did not contribute to background noise or ionization suppression. However, in order to obtain optimal response for all PCDD and PCDF congeners, the use of a dopant was

required. Both alkyl and aryl dopants were investigated, but it was determined that a dopant with aromatic character was essential for charge transfer to the analytes of interest. It is believed that the methanol cosolvent had a high enough proton affinity to abstract a proton from the radical cations formed during the photoionization of the alkyl dopants investigated (cyclohexane, methylcyclohexane, cyclopentane, and methylcyclopentane). Essentially, the increased levels of the methanol cosolvent in the source at later stages of the gradient elution suppressed the ionization of the higher chlorinated analytes. In the case of aryl dopants (toluene and fluorobenzene), the existing aromaticity of the ring structure made it more difficult for a protic solvent to abstract a proton from the generated radical cation. Fluorobenzene has a higher ionization energy than toluene (9.20 eV versus 8.83 eV) and was found to provide higher sensitivity of the hepta- and octa-chlorinated dibenzofurans. Therefore, the temperature, pressure, and gradient were optimized using undoped methanol as the cosolvent, the Torus 1-AA stationary phase, and a make-up solvent of methanol with 5% fluorobenzene. The increased system back-pressure associated with the use of the selected cosolvent and column was advantageous since it allowed for a higher automated back-pressure regulator (ABPR) setting to be utilized. The addition of a cosolvent to supercritical CO_2 results in an increase in the critical temperature and pressure associated with the system. This is especially evident when the gradient reaches higher percentages of cosolvent and can result in separations being conducted when the binary solvent system is not in a sub- or super-critical state. In this case, varying the ABPR setting resulted in changes in retention of all of the PCDD/PCDF congeners indicating that the system was being maintained in at least a sub-critical state throughout the gradient elution.

Comparison of the elution profiles of PCDDs/PCDFs by HRGC and pSFC

The successful application of an analytical technique for the separation of PCDDs and PCDFs in environmental samples is very dependent on the elution profile and resolution that can be achieved.

The importance of accurate determinations of specific positional isomers with indistinguishable mass spectra makes the ability to separate isomers of complex mixtures of critical importance, especially when calculating the dioxin toxicity equivalence (TEQ) of a sample. A significant amount of scientific research has been conducted to understand the mechanisms of PCDD and PCDF formation during thermal processes in order to interpret observed homologue patterns.[42-46] Formation processes are generally classified as either condensation of precursors (e.g. chlorophenols) or de novo synthesis (i.e. formation from carbon residues followed by chlorination).[47] The conditions under which these environmental contaminants are formed can influence the resulting homologue profile which can, in turn, facilitate source identification in some instances if adequate isomeric resolution can be achieved.[48] Since PCDDs and PCDFs are produced unintentionally from a variety of processes, the prevalent isomers in samples can be variable, but the accurate quantification of the most toxic isomers is always a requirement.

Utilizing the developed pSFC method, separation of the PCDD and PCDF congener groups was achieved with some noticeable differences in the separation of the individual 2,3,7,8-substituted PCDDs and PCDFs compared to that observed in HRGC using a DB-5 capillary column. The most prominent difference in the elution profiles is that the PCDDs elute from the column faster than the PCDFs (see Figure 1). Indeed, the first notable difference relates to the relative elution order of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2378-TCDD) and 2,3,7,8-tetrachlorodibenzofuran (2378-TCDF). In pSFC, using the developed method, 2378-TCDD elutes before 2378-TCDF. This is opposite to what is observed in HRGC analysis of these compounds. Changes in the relative elution order of other PCDD and PCDF isomers are also observed (see Figure 1), but it is interesting to note that only a few differences in the HRGC and pSFC elution order within the PCDD and PCDF compound classes occur using the selected columns. In terms of the elution of the PCDFs, changes to the elution order observed for the 2,3,7,8-substituted isomers from HRGC to pSFC are 1) 123478-HxCDF and 123678-HxCDF and 2) 234678-HxCDF

and 123789-HxCDF. For PCDDs, the only observed change in elution order is between 123478-HxCDD and 123678-HxCDD. Another notable difference between the HRGC and pSFC separations of these compounds is the increased separation of the octachlorinated dibenzo-*p*-dioxin (OCDD) and dibenzofuran (OCDF) using the pSFC technique.

When comparing the run times of HRGC analyses of PCDDs and PCDFs to the developed pSFC method, the requirements of EPA Method 1613 (and other regulatory methods) must be taken into account. EPA method 1613 requires that the absolute retention of $^{13}C_{12}$-1234-TCDD during HRGC analysis exceed 25 minutes on a DB-5 column and 15 minutes on a DB-225 column.[26] Satisfying the conditions of this regulatory method results in a typical run time of 45 minutes on the DB-5 stationary phase and up to 60 minutes on a DB-225 column. The total run time of the pSFC method is 30 minutes, but the first tetrachlorodibenzo-*p*-dioxin is eluted in approximately 5 minutes.

Resolution of Non-2,3,7,8 substituted PCDD/PCDF congeners from 2,3,7,8-substituted congeners

The most toxic PCDD and PCDF congeners are substituted in the 2,3,7, and 8 positions of the dioxin and furan skeleton and are the most important congeners targeted for identification and quantification in environmental and biological samples.[28] Toxicity equivalence factors (TEFs) have been developed to express the potency of PCDDs and PCDFs in a complex mixture as a single value, the dioxin toxicity equivalence (TEQ) concentration, which is calculated relative to 2378-TCDD.[25] The 17 PCDD and PCDF congeners substituted in the 2,3,7,8 positions are those used in TEQ calculations for regulatory reporting. One of the main challenges associated with HRGC analysis of polychlorinated dibenzo-*p*-dioxins and dibenzofurans is the separation of 2,3,7,8-substituted PCDD/PCDFs from non-2,3,7,8-substituted congeners so that the reported TEQ value is not artificially inflated. This is often accomplished on HRGC using multiple capillary columns of different polarity and requires at least two runs of the sample being investigated which can be a time consuming process.

Separation of a selection of possible co-eluting non-2,3,7,8 substituted congeners was accomplished using the described pSFC method and compared to the co-elutions observed on commonly used HRGC capillary columns, specifically DB-5, DB-225, and SP-2331 (see Table 1) to ensure adequate resolution using the described method. Of the known tetrachlorodibenzofuran isomers that co-elute with 2378-TCDF on a DB-5 HRGC capillary column, 1279-TCDF, 2348-TCDF, 2347-TCDF and 2346-TCDF were investigated (see Table 1, Figure 2). Of the TCDF isomers investigated, only 2347- and 2348-TCDF did not fully resolve from 2378-TCDF, which is a promising result. Other co-elutions that were observed include 234678-HxCDF with 123489-HxCDF (Figure 2)and 12378-PeCDF with 12348-PeCDF (Table 1). In order to verify that other isomers, which are resolved by HRGC analysis, do not co-elute with 2,3,7,8-substituted congeners using the developed pSFC method, additional PCDD and PCDF standards would have to be tested or a sample containing all of the possible PCDD/PCDF congeners, such as in a fly ash extract, would have to be analyzed against any available standards.

Resolution of 2,3,7,8-substituted HxCDD/HxCDF congeners

Achieving adequate resolution for the 2,3,7,8-substitued PCDD/PCDF congeners is also important for accurate quantification during a targeted analysis. Analysts have to demonstrate the linearity of their calibration curves and proper integration of individual isomers is required. For both HRGC and pSFC, the partial resolution of the 2,3,7,8-substituted isomers is most pertinent for the hexachlorinated PCDD/PCDF congeners. When comparing these analytical techniques, it is evident that both the 2,3,7,8-substituted HxCDF and HxCDD congeners are better separated by HRGC. The height of the valley between 123678-HxCDF and 123478-HxCDF by HRGC is 13%, but this increases to 68% using pSFC. Similarly, HRGC affords baseline separation of 123789-HxCDF and 234678-HxCDF, but a valley of 24% was measured between these two isomers using pSFC. Also, the height of the valley between 123678-HxCDD and 123478-HxCDD is 24% by HRGC and 22% by pSFC using the developed method. The use of a

longer pSFC column or different stationary phase may provide better resolution, but this requires further investigation.

Tetrachlorodibenzo-*p*-dioxin (TCDD) Resolution

According to EPA method 1613, during HRGC/HRMS analysis of PCDD/PCDFs, a window defining/column performance mixture must be analyzed to verify that the 2378-TCDD is separated from the nearest eluting congener with a valley of no more than 25%. If the valley criterion cannot be met, corrections to the HRGC column or system must be made and the column performance mixture has to be reanalysed.[26] In order to verify that the required TCDD resolution could be achieved using the developed method, a column performance mixture (5TCDD) was analyzed along with individual standards to verify resolution and elution order. It was found that 2378-TCDD was separated from its nearest eluting congener (1234-TCDD) with a 14% valley further confirming the potential of this method (Figure 3). Another interesting finding was the partial separation of 1237-TCDD and 1238-TCDD using the pSFC method. These congeners co-elute on the DB-5 stationary phase, but are also partially separated on DB-225 and SP-2331 during HRGC analysis of the same mixture.

Window Definers

U.S. EPA Method 1613 also states that the retention time windows of each homologue group must be set using a mixture which contains the first and last eluting PCDD and PCDF from each homologue group.[26] Since the elution order of the PCDDs/PCDFs does not appear to deviate substantially between HRGC analysis on a DB-5 capillary column and the developed pSFC method, a mixture of the known HRGC window definers was run to establish possible homologue windows (see Figure S1). The resulting separation of the homologue groups is quite impressive, but first and last eluting isomers need to be confirmed when additional congeners are analyzed.

Separation of PCDD/PCDFs from toxic Polychlorinated Biphenyls (PCBs)

Toxicity has been associated with PCB congeners that are coplanar (coplanar PCBs) or contain only one chlorine atom in the positions ortho (mono-ortho PCBs) to the C-C bond in the biphenyl skeleton.[25,49] For this reason, PCBs are often analyzed concurrently with PCDDs and PCDFs. Using HRGC/HRMS, there is significant overlap between the PCDD/PCDF and PCB windows. To examine the elution of mono-ortho and coplanar PCBs using the developed pSFC method, a solution containing the most toxic PCB congeners (WP-STK) was analyzed and it was found that good separation could be achieved before the elution of 2378-TCDD (see Figure 4). The only complete co-elution is between PCBs 77 and 105, but since they belong to different homologue groups, tetrachlorinated and pentachlorinated respectively, they are separable using mass spectrometry. The increased retention of the coplanar PCBs on the Torus 1-AA column compared to the mono-ortho PCBs is notable. The difference in retention of these congeners on the aromatic stationary phase can likely be attributed to the decreased π-π interactions with increasing out-of-plane orientation.

Applicability of the Developed Method to Environmental Samples

In order to demonstrate the applicability of the developed pSFC method to environmentally relevant concentration levels, a calibration curve designed to be used with U.S. EPA Method 8280B[50], a low resolution mass spectrometric method for the analysis of PCDDs and PCDFs in water, soil, fly ash, and other matrices, was run to ensure that acceptable linearity could be achieved for all components. The method requires the percent relative standard deviation (%RSD) to be below 20% for each individual component and this was accomplished using the developed method when the data was collected in MRM mode (see Table 2). Once linearity was established, the extract of the PE1102 proficiency testing sample was analyzed by both HRGC/HRMS and pSFC-MS/MS and the percent differences between the

measured and reported results were found to be comparable with the average percent difference for the HRMS data being 9.9% and that for the pSFC-MS/MS data being 21.0% (tabulated results are provided in the supporting information). It should be noted that in order for the same extract to be run on both the high resolution and low resolution detectors, its concentration was at the high-end of the HRGC/HRMS calibration and the low-end of the pSFC-MS/MS calibration and internal standards were only used in the generation of the HRGC/HRMS data since the spiked levels were too low to be detected using pSFC-MS/MS. The retention times of all of the PCDD and PCDF components were also found to be reproducible with an average standard deviation of 0.04 min over the course of the calibration.

Conclusions

High resolution gas chromatography is an established separation method utilized in environmental monitoring. Although PCDDs and PCDFs can be quantitatively analyzed by this technique, pSFC could provide an analytical tool complementary to HRGC that increases an analyst's ability to tackle difficult analytical problems and/or screen environmental samples. The development of pSFC methods for environmental contaminants is important since it has the potential to enable the simultaneous analysis of GC amenable compound classes with thermally labile analytes. Indeed, the separation of homologue groups, TCDD resolution, and separation of possible co-eluting congeners achieved with the developed pSFC method makes further work in this area justifiable.

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References

1 W. Xu, X. Wang and Z. Cai, *Analytica Chimica Acta*, 2013, **790**, 1-13.

2 S. P. J. van Leeuwen and J. de Boer, *Journal of Chromatography A*, 2008, **1186**, 161-182.

3 D. H. Cleverly, R. M. Morrison, B. L. Riddle and R. G. Kellam, *Chemosphere*, 1989, **18**, 1143-1153.

4 C. Bogdal, C. E. Muller, A. M. Buser, Z. Y. Wang, M. Scheringer, A. C. Gerecke, P. Schmid, M. Zennegg, M. MacLeod and K. Hungerbuhler, *Environmental Science & Technology*, 2014, **48**, 482-490.

5 J. Reinmann, R. Weber and R. Haag, *Science China-Chemistry*, 2010, **53**, 1017-1024.

6 A. Buekens and H. Huang, *Journal of Hazardous Materials*, 1998, **62**, 1-33.

7 D. M. Wagrowski and R. A. Hites, *Environmental Science & Technology*, 2000, **34**, 2952-2958.

8 H. R. Buser, H. P. Bosshardt and C. Rappe, *Chemosphere*, 1978, **7**, 165-172.

9 M. Schuhmacher and J. L. Domingo, *Environment International*, 2006, **32**, 397-404.

10 W. S. Lee, G. P. Chang-Chien, L. C. Wang, W. J. Lee, P. J. Tsai, K. Y. Wu and C. Lin, *Environmental Science & Technology*, 2004, **38**, 4937-4944.

11 T. Chen, J. H. Yan, S. Y. Lu, X. D. Li, Y. L. Gu, H. F. Dai, M. J. Ni and K. F. Cen, *Journal of Hazardous Materials*, 2008, **150**, 510-514.

12 K. Srogi, *Environmental Chemistry Letters*, 2008, **6**, 1-28.

13 K. Ballschmiter, H. Buchert, R. Niemczyk, A. Munder and M. Swerev, *Chemosphere*, 1986, **15**, 901-915.

14 J. I. Baker and R. A. Hites, *Environmental Science & Technology*, 2000, **34**, 2879-2886.

15 N. W. Tame, B. Z. Dlugogorski and E. M. Kennedy, *Progress in Energy and Combustion Science*, 2007, **33**, 384-408.

16 R. Lohmann and K. C. Jones, *Science of the Total Environment*, 1998, **219**, 53-81.

17 K. Lohman and C. Seigneur, *Chemosphere*, 2001, **45**, 161-171.

18 R. Weber, C. Gaus, M. Tysklind, P. Johnston, M. Forter, H. Hollert, E. Heinisch, I. Holoubek, M. Lloyd-Smith, S. Masunaga, P. Moccarelli, D. Santillo, N. Seike, R. Symons, J. P. M. Torres, M. Verta, G. Varbelow, J. Vijgen, A. Watson, P. Costner, J. Woelz, P. Wycisk and M. Zennegg, *Environmental Science and Pollution Research*, 2008, **15**, 363-393.

19 K. C. Jones and A. P. Sewart, *Critical Reviews in Environmental Science and Technology*, 1997, **27**, 1-86.

20 G. Charnley and R. D. Kimbrough, *Food and Chemical Toxicology*, 2006, **44**, 601-615.

21 S. A. Skene, I. C. Dewhurst and M. Greenberg, *Human Toxicology*, 1989, **8**, 173-203.

22 K. Arisawa, H. Takeda and H. Mikasa, *The journal of medical investigation : JMI*, 2005, **52**, 10-21.

23 J. C. Larsen, *Molecular Nutrition & Food Research*, 2006, **50**, 885-896.

24 J. P. Vandenheuvel and G. Lucier, *Environmental Health Perspectives*, 1993, **100**, 189-200.

25 U. G. Ahlborg, A. Brouwer, M. A. Fingerhut, J. L. Jacobson, S. W. Jacobson, S. W. Kennedy, A. A. F. Kettrup, J. H. Koeman, H. Poiger, C. Rappe, S. H. Safe, R. F. Seegal, J. Tuomisto and M. Vandenberg, *European Journal of Pharmacology-Environmental Toxicology and Pharmacology Section*, 1992, **228**, 179-199.

26 United States Environmental Protection Agency. (1994). *Method 1613, Revision B: Tetra- through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS*.

27 Official Journal of the European Union. (2014). *Commission Regulation (EU) No 589/2014: Laying down methods of sampling and analysis for the control of levels of dioxins, dioxin-like PCBs and non-dioxin-like PCBs in certain foodstuffs and repealing Regulation (EU) No 252/2013* (L164).

28 J. J. Ryan, H. B. S. Conacher, L. G. Panopio, B. P. Y. Lau, J. A. Hardy and Y. Masuda, *Journal of Chromatography*, 1991, **541**, 131-183.

29 V. N. Fishman, G. D. Martin and M. Wilken, *Chemosphere*, 2011, **84**, 913-922.

30 L. Do, P. Liljelind, J. Zhang and P. Haglund, *Journal of Chromatography A*, 2013, **1311**, 157-169.

31 S. B. Singh and G. Kulshrestha, *Journal of Chromatography A*, 1997, **774**, 97-109.

32 E. J. Reiner, *Mass Spectrometry Reviews*, 2010, **29**, 526-559.

33 J. F. Focant, G. Eppe, M. L. Scippo, A. C. Massart, C. Pirard, G. Maghuin-Rogister and E. De Pauw, *Journal of Chromatography A*, 2005, **1086**, 45-60.

34 E. S. Chernetsova, A. I. Revelsky, I. A. Revelsky, I. A. Mikhasenko and T. G. Sobolevsky, *Mass Spectrometry Reviews*, 2002, **21**, 373-387.

35 L. Mullin, J. A. Burgess, I. E. Jogsten, D. Geng, A. Aubin and B. van Bavel, *Analytical Methods*, 2015, **7**, 2950-2958.

36 C. G. Azevedo da Silva and C. H. Collins, *Quimica Nova*, 2014, **37**, 1047-U1210.

37 L. T. Taylor, *Journal of Supercritical Fluids*, 2009, **47**, 566-573.

38 Y. Wu, *Journal of Liquid Chromatography & Related Technologies*, 2004, **27**, 1203-1236.

39 C. West and E. Lesellier, *Journal of Chromatography A*, 2013, **1302**, 152-162.

40 V. Janda, *Critical Reviews in Analytical Chemistry*, 1999, **29**, 87-89.

41 C. West and E. Lesellier, *Journal of Chromatography A*, 2005, **1099**, 175-184.

42 P. H. Taylor and D. Lenoir, *Science of the Total Environment*, 2001, **269**, 1-24.

43 K. Everaert and J. Baeyens, *Chemosphere*, 2002, **46**, 439-448.

44 P. W. Cains, L. J. McCausland, A. R. Fernandes and P. Dyke, *Environmental Science & Technology*, 1997, **31**, 776-785.

45 M. Altarawneh, B. Z. Dlugogorski, E. M. Kennedy and J. C. Mackie, *Progress in Energy and Combustion Science*, 2009, **35**, 245-274.

46 L. C. Wang, W. J. Lee, W. S. Lee, G. P. Chang-Chien and P. J. Tsai, *Science of the Total Environment*, 2003, **302**, 185-198.

47 J. Y. Ryu, K. C. Choi and J. A. Mulholland, *Chemosphere*, 2006, **65**, 1526-1536.

48 A. Buekens, E. Cornelis, H. Huang and T. Dewettinck, *Chemosphere*, 2000, **40**, 1021-1024.

49 O. N. Zabelina, V. I. Saloutin and O. N. Chupakhin, *Journal of Analytical Chemistry*, 2010, **65**, 1098-1108.

50 United States Environmental Protection Agency. (2007). *Method 8280B: Polychlorinated Dibenzo-p-dioxins (PCDDs) and Polychlorinated Dibenzofurans (PCDFs) by High-Resolution Gas Chromatography/Low-Resolution Mass Spectrometry (HRGC/LRMS), Revision 2*.

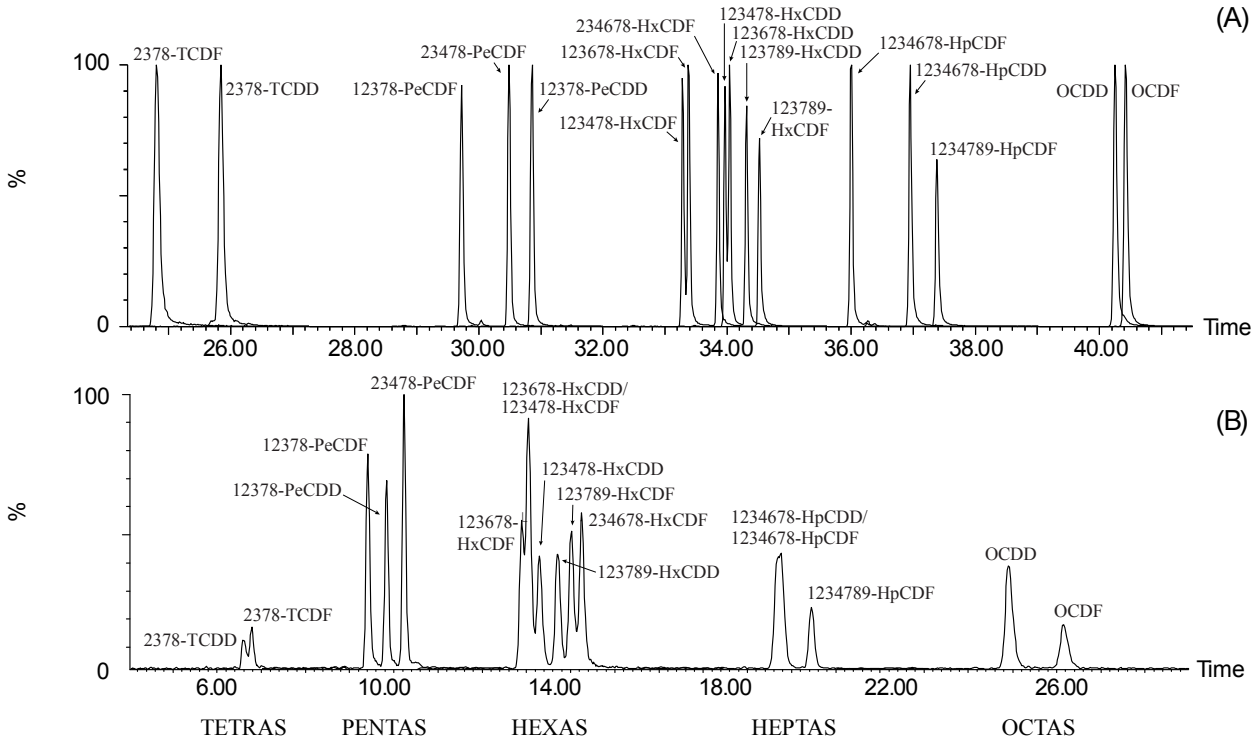

Figure 1: Comparison of the elution profiles of 2,3,7,8-substituted PCDDs and PCDFs by (A) HRGC/HRMS on a DB-5 column and (B) pSFC-MS/MS on a Torus 1-AA column.

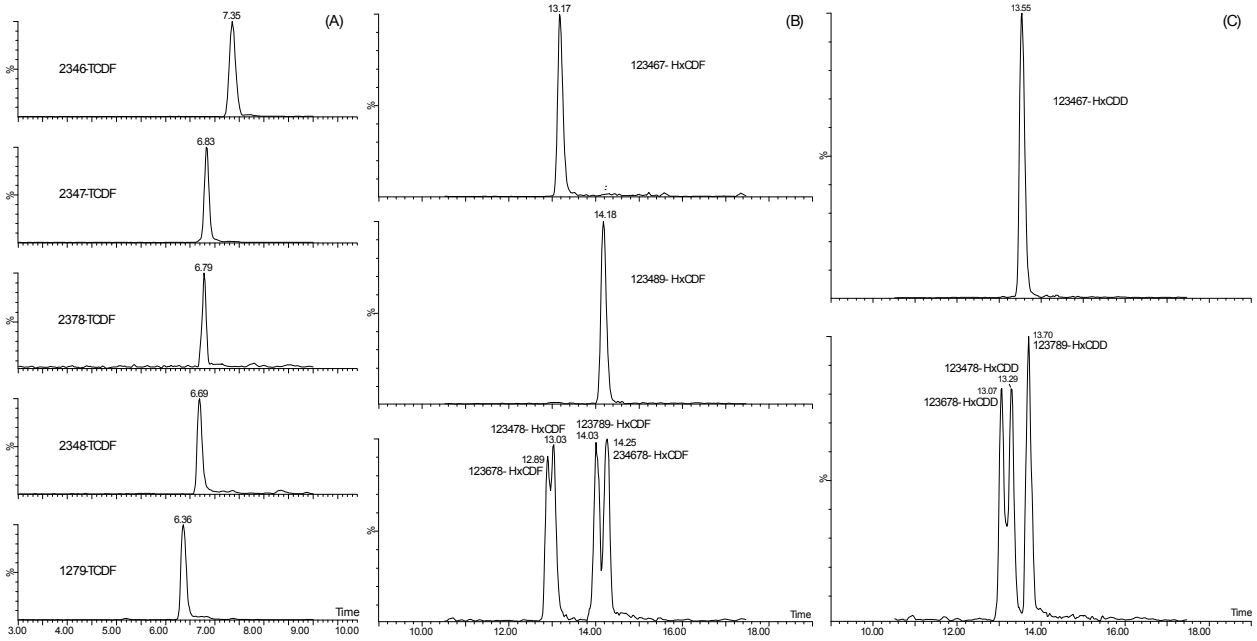

Figure 2: Separation of possible co-eluting (A) tetrachlorinated dibenzofurans, (B) hexachlorinated dibenzofurans and (C) hexachlorinated dibenzo-*p*-dioxins using the developed pSFC-MS/MS method.

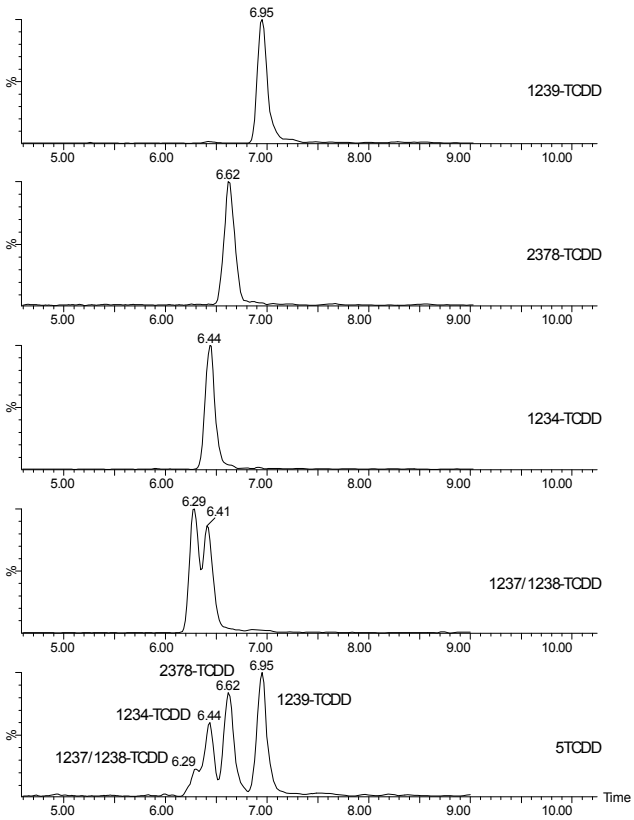

Figure 3: Chromatograms illustrating 2378-TCDD resolution from closely eluting congeners and elution order determinations (valley between 2378-TCDD and 1234-TCDD is 14%).

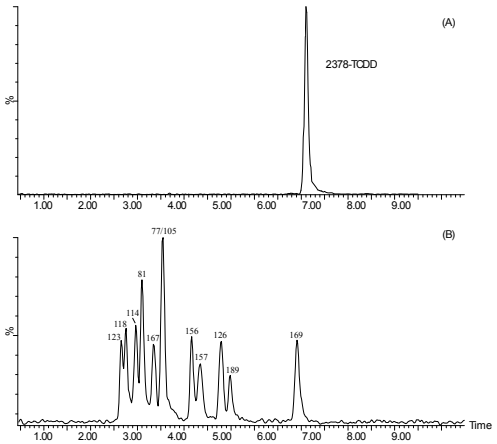

Figure 4: Chromatograms illustrating the elution order and resolution of mono-ortho and coplanar PCBs (B; labeled with IUPAC number) in comparison to the 2,3,7,8-tetrachlorinated dibenzo-*p*-dioxin (A).

Table 1: A comparison of possible non-2,3,7,8-substituted PCDD/PCDF co-eluters on commonly used HRGC columns[28] and a selected pSFC column under optimized separation conditions.

2,3,7,8-Substituted PCDD/PCDF	HRGC: DB-5 (1° column)	HRGC: DB-225 (2° column)	HRGC: SP-2331 (3° column)	pSFC: Torus 1-AA[b]
2378-TCDF	2347-TCDF 2348-TCDF 1249-TCDF 1279-TCDF 2346-TCDF	no co-eluters[c]	2348-TCDF[a]	2347-TCDF 2348-TCDF[a]
23478-PeCDF	12489-PeCDF 12679-PeCDF 12369-PeCDF	no co-eluters	no co-eluters	
12378-PeCDF	12348-PeCDF[a]	13469-PeCDF	12348-PeCDF	12348-PeCDF
123478-HxCDF	123467-HxCDF	no co-eluters	123479-HxCDF	
123678-HxCDF	no co-eluters	124689-HxCDF 123479-HxCDF	no co-eluters	
123789-HxCDF	123489-HxCDF[a]	no co-eluters	no co-eluters	
234678-HxCDF	no co-eluters	123489-HxCDF	no co-eluters	123489-HxCDF[a]
123789-HxCDD	123467-HxCDD	no co-eluters	no co-eluters	

[a]Partially resolved

[b]Congeners investigated: 2348-TCDF, 2347-TCDF, 2346-TCDF, 1279-TCDF, 12348-PeCDF, 123467-HxCDF, 123489-HxCDF, and 123467-HxCDD

[c]In-house data

Table 2: A summary of the calibration data obtained when U.S. EPA 8280 method calibration solutions (CS1 to CS5) were analyzed using the developed pSFC-MS/MS method.

Concentration Range (ng/µl)	Compound	Mean	SD	%RSD	RRF1	RRF2	RRF3	RRF4	RRF5
0.1 - 2.0	2378-TCDF	1.32	0.10	7.52	1.23	1.40	1.36	1.21	1.43
0.1 - 2.0	12378-PeCDF	1.64	0.26	15.83	1.46	1.83	1.43	1.99	1.47
0.5	23478-PeCDF	--	--	--	--	--	--	--	--
1.25	123478-HxCDF	--	--	--	--	--	--	--	--
0.25 - 5.0	123678-HxCDF	1.32	0.11	8.07	1.31	1.24	1.30	1.51	1.26
1.25	234678-HxCDF	--	--	--	--	--	--	--	--
1.25	123789-HxCDF	--	--	--	--	--	--	--	--
0.25 - 5.0	1234678-HpCDF	1.10	0.08	7.02	1.08	1.11	1.04	1.22	1.02

1.25	1234789-HpCDF	--	--	--	--	--	--	--	--
0.5 - 10.0	OCDF	0.40	0.04	8.94	0.37	0.35	0.43	0.41	0.42
0.1 - 2.0	2378-TCDD	1.29	0.09	7.00	1.31	1.25	1.34	1.15	1.38
0.1 - 2.0	12378-PeCDD	1.29	0.07	5.70	1.19	1.37	1.33	1.32	1.24
1.25	123478-HxCDD	--	--	--	--	--	--	--	--
0.25 - 5.0	123678-HxCDD	1.20	0.10	8.58	1.38	1.11	1.19	1.17	1.14
1.25	123789-HxCDD	--	--	--	--	--	--	--	--
0.25 - 5.0	1234678-HpCDD	1.00	0.07	7.30	1.08	1.00	0.92	1.06	0.94
0.5 - 10.0	OCDD	1.07	0.14	12.87	1.13	0.98	1.26	0.91	1.05
0.5	$^{13}C_{12}$-2378-TCDF	1.29	0.16	12.59	1.25	1.10	1.51	1.39	1.20
1.0	$^{13}C_{12}$-1234678-HpCDF	1.24	0.19	15.15	1.10	1.16	1.55	1.11	1.28
0.5	$^{13}C_{12}$-2378-TCDD	1.19	0.07	5.97	1.20	1.10	1.29	1.16	1.18
0.5	$^{13}C_{12}$-123678-HxCDD	1.00	0.05	5.08	0.96	0.95	0.97	1.05	1.06
1.0	$^{13}C_{12}$-OCDD	0.94	0.05	5.53	0.91	0.91	1.00	1.00	0.90
0.5	$^{13}C_{12}$-1234-TCDD	1.00	0.00	0.00	1.00	1.00	1.00	1.00	1.00
0.5	$^{13}C_{12}$-123789-HxCDD	1.00	0.00	0.00	1.00	1.00	1.00	1.00	1.00

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