APPLICATION OF GC-HRTOFMS FOR ENVIRONMENTAL ANALYSIS

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Abstract
Applications of gas chromatography/high resolution time-of-flight mass spectrometry (GC-HRTOFMS) for environmental analysis were investigated. HR-TOFMS had successfully high sensitivity such as sub-picogram with high resolution full-scan information and confident exact mass measurement within around 5ppm.
Additionally it is possible to investigate for the identification of unknown interferences in routine Dioxin analysis. Semi quantification data for POPs analysis by isotope dilution method with GC-HRTOFMS evaluated also for the comparison of conventional GC-HRMS SIM data in biological sample.

Introduction
Current methodology for Dioxin, PCB, POPs and brominated flame retardant analysis utilizes HRGC-HRMS at 10,000 resolution to obtain the highest sensitivity, selectivity and specificity after extensive clean-up as samples are often complex matrices containing many potential interferences at much higher levels. Even with the use of such a method, some interferences can still be present. Identification of these interferences can yield valuable information on their suspected sources and/or indicate possible alternative clean-up strategies. When the presence of potential interferences are suspected, an alternative mode GC-HRMS-SIM, mass peak profile monitoring(MP)[1,2,3] can be employed to furnish valuable data. The accurate mass and isotope ratios enable the confirmation of the target compound identities and tentative identification of the interferences in combination with scanning analysis data.

TOFMS has been used successfully to acquire much faster full-scan information, while maintaining high sensitivity compared to conventional MS. There are some report on comprehensive two-dimensional GCxGC-TOFMS for environmental samples[4,5,6]. But these report utilizes low resolution TOFMS.
Recently GC-high resolution TOFMS (GC-HRTOFMS) are commercially available by Waters/Micromass, which instrument has advantage not only rapid spectral acquisition rates but also accurate mass spectra in wide range with high full scan sensitivity. It is expected that by GC-HRTOFMS ensure full characterization and profiling in a whole range of sample types with sensitive, accurate exact mass GC-MS analysis to determine the elemental compositions for complex mixtures in environmental samples. These HRTOFMS are applied for analytical chemistry field including synthetics confirmation and QC checks in the fine chemical and pharmaceutical industries, food, flavor and fragrance analysis, metabolomic/metabonomic studies, multi-residue screening in fruit and vegetables, unknown contaminant monitoring, material emissions monitoring and natural products research.
In this paper, we focused on the application and possibility of GC-HRTOFMS for environmental analysis especially for organohalogen compounds[7].

Materials and Methods
Samples applied and evaluated for GC-HRTOFMS were Dioxin or POPs standard calibration solution mixture, Dioxin contaminated soil sample, which exhibit interferences in GC-HRMS SIM analysis and biological sample for POPs analysis.
Dioxin standard calibration solution mixture is custom made for our laboratories by Wellington laboratories.
Soil sample fortified ^{13}C clean-up spike for Dioxins analysis were soxhlet-extracted by toluene and purified followed by multilayer silica, alumina and carbon clean up according to Japanese official method by Ministry of Environment..
POPs standard calibration solution mixture is made by ourselves and ^{13}C-POPs are supplied from Cambridge Isotope laboratories. Biological samples fortified ^{13}C clean-up spike for POPs analysis were soxhlet-extracted with dichloromethane (DCM) for 6 hours after homogenization and dehydration, and 20% of the crude extracts were purified by florisil column chromatography. Each clean-up sample were concentrated to <0.1 mL and

measured by GC-HRMS SIM and GC-HRTOFMS. Detailed analytical methodology for POPs was described elsewhere[8].

GC-HRTOFMS measurement was performed on a GCT Premier™ (Waters/ Micromass® Micromass) which offers the high resolution accurate mass spectra with full scan high sensitivity and rapid data acquisition rates. Spectral acquisition rates: 2-20 scans/sec. MS resolution: 7,000 FWHM(Full Width Half Maximum), which better than any other instrument in its class with internal lock mass correction.

GC column: DB-5MS (30 m x 0.25 mm, 0.25 µm, J&W), DB-17HT (30 m x 0.32 mm, 0.15 µm, J&W)

GC column temperature: 120°C (1 min)-(20°C/min)-220°C (0 min)-(3°C/min)-280°C (0 min) -(5°C/min)-300°C (10 min) for DB-5MS, 130°C (1 min)-(20°C/min)-200°C (0 min)-(3°C/min)-250°C (0 min) -(5°C/min)-300°C (10 min) for DB-17HT, 120°C (1 min)-(20°C/min)-160°C (0 min)-(3°C/min)-220°C (0 min) -(10°C/min)-300°C (10 min) for DB-17HT (POPs), carrier gas: He (1.0 mL/min)

injector temperature: 120 or 130°C-(100°C/min)-300°C, with on-column injection.

injection volume: 2.0 µL, interface temperature: 300°C, source temperature: 300°C,

Results and Discussion

Figure 1 shows the mass chromatograms of TeCDDs/DFs (within ±25mDa) in Dioxin calibration solution mixture by GC-HRTOFMS. It shows enough sensitivity and selectivity for 2pg of native TeCDDs/DFs, but interference from $^{13}C_{12}$-TeCDF to native TeCDD could be seen when expanded to within ±50mDa in each mass.

Figure 2 shows accurate mass full spectra of 1379-TeCDD (2pg) observed by GC-HRTOFMS. To our knowledge, this is a first repot on the accurate mass full spectra detection of 2pg of TeCDD. These deviations of parent ions are within 0.3 to 1.0 mDa compared with the theoretical accurate masses. These results indicate that GC-HRTOFMS has enough sensitivity and selectivity and confident exact mass measurement for ultra trace analysis and in the confirmation of the identity and concentration of known compounds.

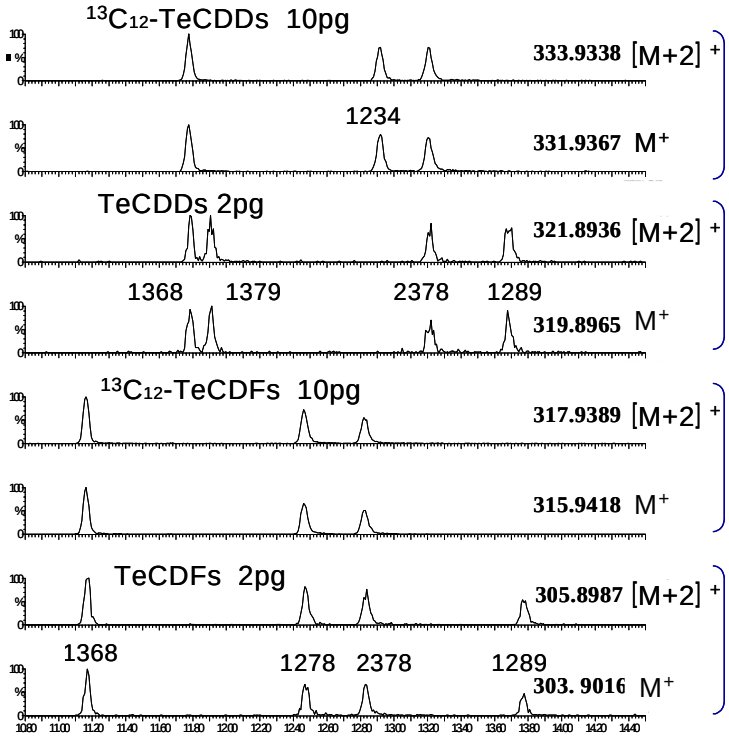

Figure 1. Mass Chromatograms of TCDDs/DFs (within ±25mDa) in Dioxin calibration solution mixture by GC-HRTOFMS.
GC column: DB-5MS 30m

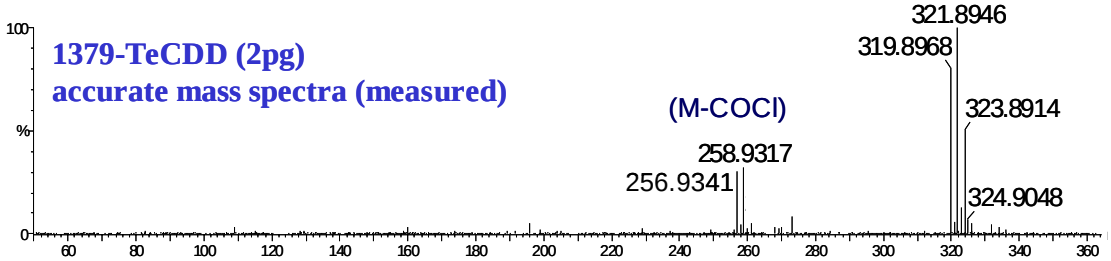

Figure 2. Accurate mass spectra of 1379-TeCDD (2pg) observed by GC-HRTOFMS.
These deviations of parent ions are within 0.3 to 1.0 mDa compared with theoretical accurate masses.

Additionally it is possible to investigate for the identification of unknown interferences in routine Dioxin analysis. Figure 3 shows mass chromatograms for $^{13}C_{12}$-TeCDD (m/z: 333.9338 ±0.025Da) and $^{13}C_{12}$-PeCDD (m/z: 367.8948 ±0.025Da) by GC-HRTOFMS in a contaminated soil sample after extensive clean up[9].
Black colored peaks are recognized as interferences, which were seen by GC-HRMS-SIM also. These interferences eluted later retention windows corresponding to same chlorine degree of PCDDs on medium polar DB-17HT GC column. On the other hand these interferences elute same retention windows on polar cyanopropyl phase (SP2331). The final solution was made after extensive clean up such as acid/base silica, alumina and carbon clean up, which suggest these interferences might has same polarity and same planar structure as same as Dioxins.
Although the observed accurate mass spectra of interferences were very close to $^{13}C_{12}$-TeCDD(-4.0mDa) and $^{13}C_{12}$-PeCDD (-2.7mDa), the elemental composition determination for the structural elucidation of unknown compounds suggested that these interferences were mostly close to the accurate mass of $C_{14}H_8OCl_4$ and $C_{14}H_7OCl_5$. Table 1 shows the summary of interferences to $^{13}C_{12}$-TeCDD/PeCDD, which observed accurate mass and theoretical accurate mass of $C_{14}H_8OCl_4$ and $C_{14}H_7OCl_5$. These deviations were from -1.8mDa to 2.2mDa, and tentatively identified as dimethyl- or ethyl-TeCDF/PeCDF (Figure4). We identified methyl-PCDF in this solution also. The order of these alkylated PCDFs in this contaminated soil sample were PCDFs > methyl-PCDFs > dimethyl-PCDFs.

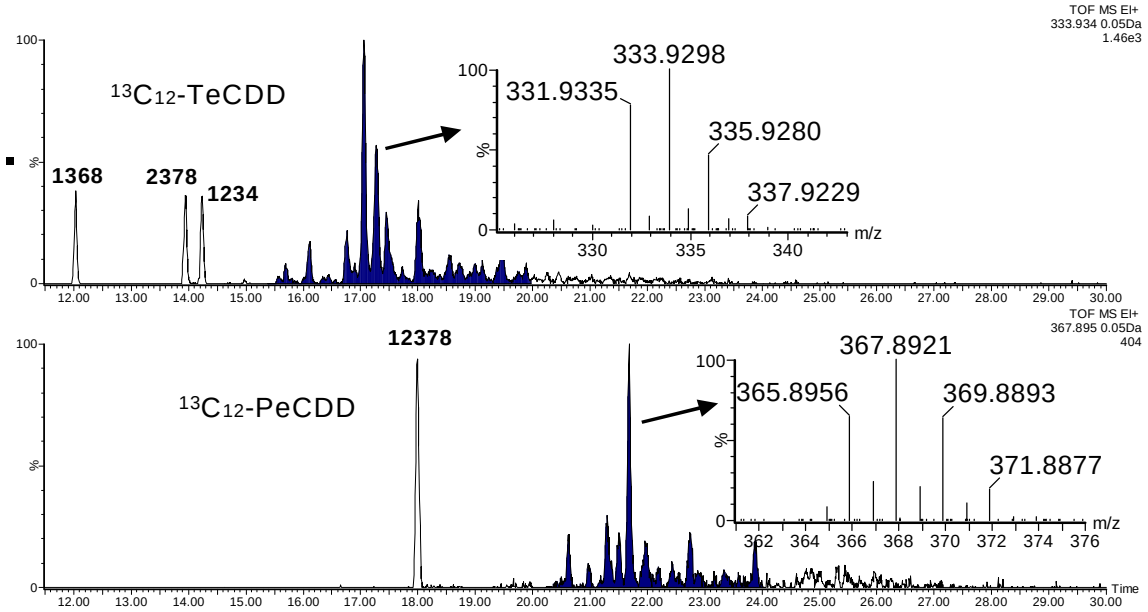

Figure 3. Mass Chromatograms for $^{13}C_{12}$-TeCDD (m/z: 333.9338 ±0.025Da) and $^{13}C_{12}$-PeCDD (m/z: 367.8948 ±0.025Da) by GC-HRTOFMS in a contaminated soil sample after extensive clean up[9].
Black colored peaks are investigated as interferences. GC column: DB-17HT 30m×0.32mm, 0.15 μm

Table 1. Accurate mass (measured and theoretical) for interferences of $^{13}C_{12}$-TeCDD and $^{13}C_{12}$-PeCDD by GC-HRTOFMS in a contaminated soil sample.

$C_{14}H_8OCl_4$ as potential interferences to $^{13}C_{12}$-TeCDD			$C_{14}H_7OCl_5$ as potential interferences to $^{13}C_{12}$-PeCDD		
accurate mass		difference	accurate mass		difference
measured	theoretical	mDa	measured	theoretical	mDa
331.9335	331.9329	0.6	365.8956	365.8940	1.6
333.9298	333.9301	-0.3	367.8921	367.8911	1.0
335.9280	335.9273	0.7	369.8893	369.8882	1.1
337.9229	337.9247	-1.8	371.8877	371.8855	2.2

Additionally semi quantification by isotope dilution method for POPs analysis with GC-HRTOFMS were applied and evaluated by comparison of conventional GC-HRMS SIM data in calibration solution and in biological sample. The calibration solutions are 5 point which ranged from 2, 10, 40, 200 and 800 pg/µL for native and 20 pg/µL for ^{13}C labeled internal standard. Each solution was injected 3 times at 2 µL. The ions evaluated for TOFMS were same as GC-HRMS SIM. High sensitive POPs such as HCB, DDE, DDT were saturated at 800 pg/µL, on the other hand, low sensitive POPs such as Dieldrin, Endrin, Oxychlordane were difficult to detect at 10 pg/µL. In case of much higher level of native compound, it can be seen that native fragment ions interfere to ^{13}C labeled ions or shifting accurate mass of ^{13}C labeled ions. The relative standard deviations of RRF (Relative Response Factor) were within 10 % if used selected concentration range and suitable ions. From the comparison of tentative semi quantified POPs data in biological samples with GC-HRTOFMS resulted in 85 % to 139 % to the conventional GC-HRMS SIM data.

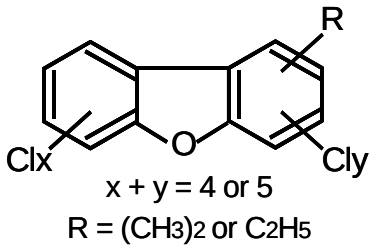

Figure 4. Structure of dimethyl- or ethyl-TeCDF/PeCDF tentatively identified as interferences to $^{13}C_{12}$-TeCDD/PeCDD in a contaminated soil sample.

These results suggest that GC-HRTOFMS is useful instrument for full characterization and profiling of components in a whole range of sample types in addition to selectivity and specificity, increased sensitivity, and improved usability and data interpretation for the highest confidence in our results as well as semi quantification.

References

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