Application of Tandem Mass Spectrometry with an Ion Trap Detector to the analysis of PCDD/PCDFs and PCBs.

Guy Hamelin, Charles Brochu, Serge Moore.
Ministère de l'Environnement et de la Faune du Québec (MEF),
850 bl. Vanier, Laval (Québec), H7C 2M7.

Introduction

Organohalogen compounds are widespread contaminants in the environment. PCB, PCDD and PCDF are among these contaminants that appear as complex mixtures and are usually present at very low levels (ppt or ppq). The analytical protocol used for the analysis of these components must be sensitive and very selective, since most of the samples are contaminated by a wide variety of other polychlorinated hydrocarbons.

Considerable interest for these pollutants over the last few decade had led to the development of several sophisticated instruments for the identification and quantification of PCDD/PCDFs and PCBs. Among them, high resolution GC (HRGC) coupled to high resolution MS (HRMS) has been the method of choice since it can afford high sensitivity and high selectivity. Unfortunately, these instruments are very expensive and require highly trained operators. Tandem mass spectrometry (MS/MS) has been proposed as an alternative to HRMS on both hybrid instruments and triple quadrupole systems. However, these instruments are also expensive.

Recently, tandem mass spectrometry on ion trap detector has been introduced has a simple and low cost instrumentation. We have applied this new approach to the analysis of pulp and paper effluent, ambient air, fish tissue and suspended particulates matters. These samples have low levels of targetted compounds and high levels of interfering coextracted components, like pesticides and other chlorinated organic compounds. For example most of the pulp and paper samples analysed in MEF laboratory showed TCDD/F level below 15 pg/L. Even if purification methods are quite effective for PCDD/Fs, EI/MS result suffer from lack of sensitivity and selectivity. In an other way, higher concentration of PCBs (10-100 pg/ul) can be mask by aliphatic hydrocarbon background in the purified PCB fraction. The objective of this study is to evaluate the application of this new technology for the analysis of PCDD/PCDFs and PCBs in environmental samples.

Experimental

GC/MS/MS analyses were performed on a Varian Saturn ion trap GC/MS system upgraded with the Wave-Board MS/MS capabilities. The system is capable of time-programmable isolation of selected parent ions followed by collision-induced dissociation (CID) to characteristic product ions.

Samples were injected from a Varian 8200 AutoSampler to the Septum-Equipped Programmable Injector (SPI) on the Varian 3400 GC. Separation was performed on a J&W DB5-MS column (30 m x 0.25 mm ID x 0.25 μm film thickness). The SPI injector and column ramp were optimized in a prior study[1]. Standards for PCDD/F and PCB were purchased from CIL (Massachusetts, USA). A four point calibration curve was prepared for PCDD/F over the concentration range from 1-200 pg/μl. A second calibration curve covering the range 5-1000 pg/μl was prepared for the three planar PCBs (#77, #126 and #169) and two mono-ortho PCBs (#105 and #118). Extracts of fish, ambient air, sediment and pulp and paper effluent were prepared and analyzed (by VG AutoSpecQ HRMS) according to well established preparation methods[2,3] and standard MEF protocol[4] before analyses were run by ion trap GC/MS/MS.

Description of the MS/MS Scan Function

Ion trap MS/MS is a tandem-in-time process, by contrast to an instrument such as a triple quadrupole system which is tandem-in-space. To accomplish the isolation of parent ions and their subsequent CID and the scanning of the MS/MS spectrum, this technique requires only one mass spectrometer and the different operations are accomplished as a function of time. The Scan Function for MS/MS (Figure 1) shows the applications of different waveforms to the ring (top trace) and endcap electrodes of the ion trap. The four steps involved in the scan function are: 1) Ionization and rough isolation in the parent ion region, 2) Fine isolation of the parent ion, 3) Formation of product ions by CID, and 4) Scanning of the product ion mass spectrum. These four steps are all applied in each analytical scan (the right side of Figure 1). If the Automatic Gain Control (AGC) function is used, then steps 1, 2 and 4 are used to get an accurate count of the number of parent ions created with a fixed ion time and thus to recommend an ionization time to create the optimum target number of ions for each analytical scan. The AGC pre-scan improves the reproducibility of product ion spectra and eliminates the space charge effect which can occur when large numbers of ions are store in the trap.

Multiple Reaction Monitoring (MRM)

The basic Saturn MS/MS software allows the user to construct an Ion Preparation Method (IPM) file for each target compound (e.g. the m/z 322 ion of tetra-dioxins, see Table 1). The Multiple Reaction Monitoring feature of the new Toolkit software package now allows the sequential isolation and CID of up to ten different target ions in a single segment of the chromatogram. The constraint on the applicability of the software is really the acquisition rate required for MRM analyses, which is tied to the width of the GC peak. The PCDD/F analysis requires that four different ions be monitored to quantitate both target compounds and isotopically-labelled internal standards.

Important Considerations for Quality Control

Confirmation criteria used for positive identification of PCDD/F were as follows:
1) Relative retention time within 0.002.
2) Detection of product ions coming from losses of $CO^{35}Cl$ and $CO^{37}Cl$, with (S/N) > 3.
3) Isotopic ratio between product ions within acceptable range +/- 20%
4) The sum of both product ions should be S/N > 10 for quantitation.

After optimization, a good product ions spectrum for TCDF at 1 pg/ul was obtained. Great care must be taken not to distort the isotopic ratio for the product ions. Pattern of product ions varied with the excitation time and voltage. Increasing the voltage tend to displace the major fragment from the $([M+2]\text{-}Cl)^{+\cdot}$ to the specific $([M+2]\text{-}COCl)^{+\cdot}$ loss. Furthermore, increasing the time favored the loss of 2COCl for dioxin. It appears that the best approach to maximize the intensity of COCl loss is to keep the voltage between 3.0 V - 4.0 V and keep the reaction time for CID to 5 ms. The minimum time approach has the big advantage of reducing the entire scan time and thus increases the number of μscan per scan to maintain good quality spectrum at low concentration (below 2 pg/ul).

Results and Discussion

The linearity of calibration plots and the isotopic ratios of product ions were routinely acceptable. Several types of samples were analysed by the ion trap GC/MS/MS. A typical pulp and paper effluent sample is shown in Figure 2. Four TCDF congeners are detected but only two are concentrated enough to pass isotopic ratio. In this sample, 2,3,7,8-TCDF has been found at 3.8 pg/μl with S/N = 75. Figure 3 shows an ambient air sample in which low level of TCDF were found in HRMS. Total TCDF was measured at 6 pg/μl and the major congener was found at 0.8 pg/μl. This chromatogram suggest that sample detection limit is below 1 pg/μl.

Chromatograms of low PCBs concentration in a water sample can be see in Figure 4. Comparison is being made between EI/MS and EI/MS/MS with the same instrument on the same day. It can be seen that EI/MS/MS is quite capable of removing matrix interfering ions from the sample and let appear targetted compounds. Highest T4ClBp congener in that chromatogram is at 50 pg/μl and planar PCB-#77 was found at 4 pg/μl.

Conclusions

EI/MS/MS results on the ion trap confirm the high selectivity and sensitivity for PCDD/Fs and PCBs. Detection limit in samples analyzed are around 0.5 to 1 pg/μl for TCDD/F and between 1 to 5 pg/μl for T4ClBp. Enhanced ionization efficiency should improve the ultimate sensitivity of the method. We are currently evaluating an enhanced hardware feature to accomplish this purpose.

Acknowledgements

We wish to thank especially Dr. Robert D. Brittain from Varian for reviewing the manuscript and the software development team for there marked interest to keep the GC/MS/MS system a simple and efficient technique.

References

1) Brochu, C.; Hamelin, G.; Moore, S.; Varian GC/MS Applications Note, Number 32.
2) Smith, L.M.; Stalling, D.L.; Johnson, J.L.; Anal. Chem., 1984, 56, 1830-1842.
3) Sakai, S.; Hiraoka, M.; Takeda, N.; Chemosphere, 1993, 27, 233-240.
4) Ministère de l'Environnement du Québec, Pâtes et Papiers,MENVIQ,89.04/414-D.F.

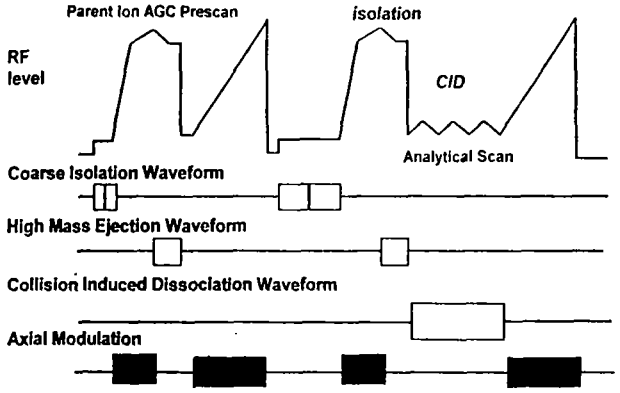


Figure 1. The Scan Function for MS/MS showing the AGC prescan based on parent ion isolation and the analytical scan including CID. The RF level is applied to the central ring electrode; other waveforms are applied to endcap electrodes.

Ions used for Multiple Reaction Monitoring and theoritical isotopic ratio for product ions.

# Acq. Seg.	PCDD/F's	Parent Ions (m/z)	Prod. Ions	Theoritical Isotopic Ratio Value	Range (+/- 20%)
1	TCDF	305.90 (M+2)	241/243	0.33	0.26<value<0.40
	13C-TCDF	317.94 (M+2)	252/254	0.33	0.26<value<0.40
	TCDD	321.89 (M+2)	257/259	0.33	0.26<value<0.40
	13C-TCDD	333.93 (M+2)	268/270	0.33	0.26<value<0.40
2	PCDF	339.86 (M+2)	275/277	0.25	0.20<value<0.30
	13C-PCDF	351.90 (M+2)	286/288	0.25	0.20<value<0.30
	PCDD	355.85 (M+2)	291/293	0.25	0.20<value<0.30
	13C-PCDD	367.89 (M+2)	302/304	0.25	0.20<value<0.30
3	H6CDF	373.82 (M+2)	309/311	0.20	0.16<value<0.24
	13C-H6CDF	385.86 (M+2)	320/322	0.20	0.16<value<0.24
	H6CDD	389.82 (M+2)	325/327	0.20	0.16<value<0.24
	13C-H6CDD	401.86 (M+2)	336/338	0.20	0.16<value<0.24
4	H7CDF	409.78 (M+4)	345/347	0.40	0.32<value<0.48
	13C-H7CDF	421.78 (M+4)	356/358	0.40	0.32<value<0.48
	H7CDD	425.77 (M+4)	361/363	0.40	0.32<value<0.48
	13C-H7CDD	437.81 (M+4)	372/374	0.40	0.32<value<0.48
5	OCDF	443.74 (M+4)	379/381	0.33	0.26<value<0.40
	OCDD	459.73 (M+4)	395/397	0.33	0.26<value<0.40
	13C-OCDD	471.78 (M+4)	406/408	0.33	0.26<value<0.40
# Acq. Seg.	**PCB's**	**Parent Ions (m/z)**	**Prod. Ions**	**Theoritical Isotopic Ratio Value**	**Range (+/- 20%)**
1	T4ClBp #77	291.92 (M+2)	220/222	1.00	0.80<value<1.20
	13C-T4ClBp#77	303.96 (M+2)	232/234	1.00	0.80<value<1.20
2	P5ClBp #126	325.88 (M+2)	254/256	0.67	0.54<value<0.80
	13C-P5ClBp#126	337.92 (M+2)	266/268	0.67	0.54<value<0.80
3	H6ClBp#169	359.84 (M+2)	288/290	0.50	0.40<value<0.60
	13C-H6ClBp#169	371.88 (M+2)	300/302	0.50	0.40<value<0.60

Table 1: Parent Ions masses, product ions masses and acceptable range for isotopic ratio.

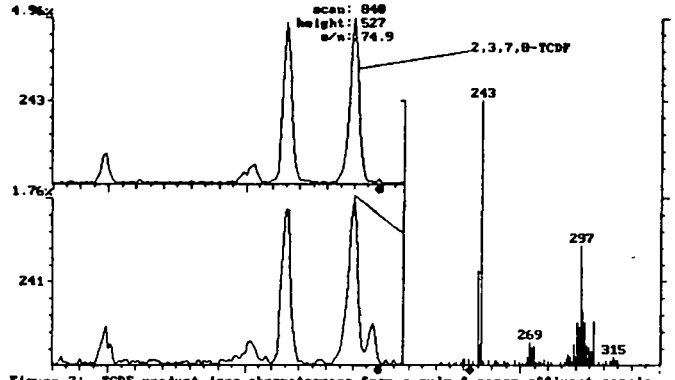
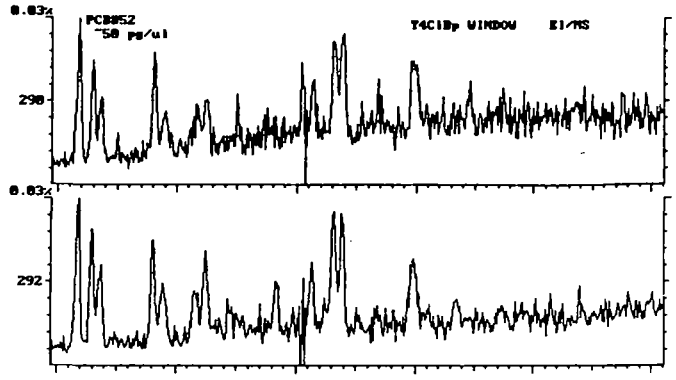


Figure 2: TCDF product ions chromatograms from a pulp & paper effluent sample with the 2 major congeners near 4 pg/ul.



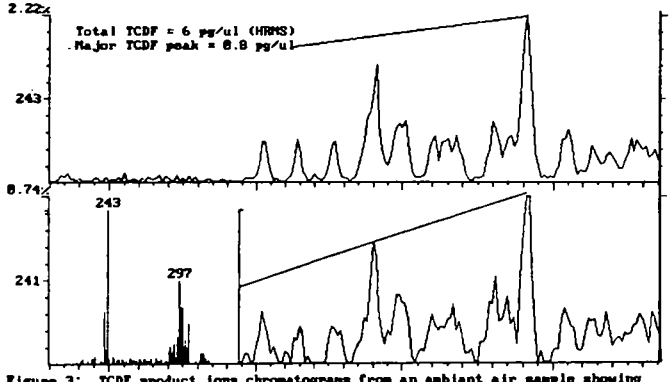


Figure 3: TCDF product ions chromatograms from an ambient air sample showing quantification limit below 1 pg/ul.

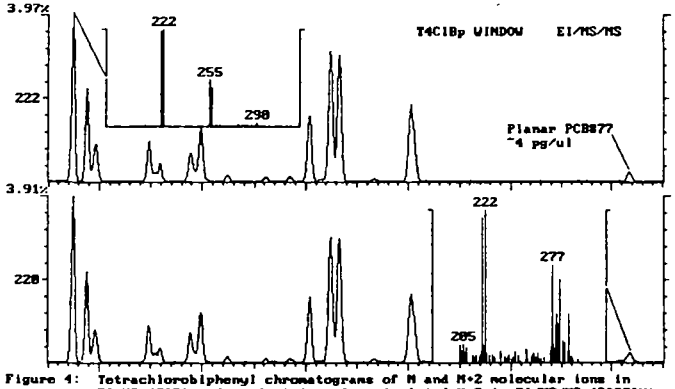


Figure 4: Tetrachlorobiphenyl chromatograms of M and M+2 molecular ions in EI/MS (TOP) and product ions from isolated M+2 in EI/MS/MS (BOTTOM).