

## The Use of Cryogenic Zone Compression for the Measurement of POPs in Human Serum at Attogram Levels by GCxGC/ID-HRMS

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### Introduction

It has been known for some time that thermal modulation can enhance the sensitivity of gas chromatographic measurements by increasing the signal-to-noise ratio.<sup>1</sup> As the most sensitive gas chromatographic detector for measurements of polychlorinated dibenzo-*p*-dioxins (PCDDs) and related persistent organic pollutants (POPs) is the magnetic sector mass spectrometer, it is to be expected that thermal modulation coupled with a magnetic sector mass spectrometer will result in the ultimate attainable sensitivity for measurement of these compounds. In 1996 and 1998, high resolution mass spectrometry (HRMS) was coupled with comprehensive two-dimensional gas chromatography (GCxGC) for the measurement of PCDDs and related compounds in addition to other environmental contaminants.<sup>2,3</sup> These two reports used painted modulators and a rotating heated arm modulator to achieve the comprehensive two-dimensional separation. In the last few years advances have been made in the design of devices for thermal modulation in gas chromatography, in particular the use of cryogenic trapping. At the same time background levels of PCDDs and related pollutants in serum have declined to the point that their measurement has become increasingly difficult, and the sample size required for quantification has become quite large.<sup>4</sup> Reducing the sample size required for these analyses by increasing the sensitivity of the method is thus an area of considerable interest. A gas chromatograph equipped with a cryogenic modulator coupled to a high resolution mass spectrometer results in attogram (ag) detection of PCDDs in standards and serum samples.

### Materials and Methods

#### *Standards*

Standards were from Cambridge Isotope Laboratories (Andover, MA, USA) and were diluted with nonane to the following concentrations (in fg/ $\mu$ L) of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2378D) and 1,2,3,7,8-tetrachlorodibenzo-*p*-dioxin (12378D): 20, 10, 5, 2.5, 1.25, 0.625, 0.313, 0.157.

#### *Sample preparation*

Serum samples were multiple aliquots of 10 g of National Institute of Standards and Technology Standard Reference Material 1589a ( NIST SRM 1589a; PCBs, Pesticides, and Dioxins/Furans in Human Serum, August 9, 2000). The sample preparation method has been described previously.<sup>6</sup> Samples were reconstituted in 20  $\mu$ l of nonane and diluted to the following approximate concentrations (assuming 70% recovery) of 2378D (in fg/ $\mu$ L): 6.5, 1.625, 1.3, 1.1, 0.813, 0.650, 0.540, 0.406, 0.325, 0.203, 0.162.

#### *GC-HRMS analysis*

The gas chromatograph was an Agilent 6890N operated in splitless mode and the mass spectrometer was a MAT95XP (ThermoElectron, Bremen Germany). The column was a DB-5 with dimensions 7 m x 0.1 mm i.d. x 0.10  $\mu$ m film thickness. The modulation device was the loop modulator from Zoex Corporation, which utilizes liquid nitrogen as the cryogen and consists of one cold jet and one hot jet with 2 loops of column passing the jets, effectively creating a quadruple jet or dual-stage system.<sup>5</sup> The modulator was positioned approximately 75 cm from the end of the column. The modulation period for the maximum sensitivity enhancement was either 6 or 9 s and the hot jet pulse time was 800 ms. For resolution of all congeners of a given class, faster modulation cycles were employed.

Mass spectrometry was performed in electron ionization (EI) mode at 10,000 resolution using multiple ion detection (MID), with one MID group per congener class. Cycle time used ranged from 50 to 70 ms (14 to 20Hz). The ion source temperature was 270°, trap current was 0.65 mA and electron energy was 40 eV.

Data analysis and visualization was performed using ThermoElectron XCalibur and GC Image software. Quantification was performed using GC Image.

## Results and Discussion

For sensitivity enhancement by GC with thermal modulation the largest increase is obtained when the entire peak of interest is trapped and remobilized in one event or “slice”. For this reason relatively long modulation times were used. Peak widths under the conditions used were in the 300 to 400 ms range when measured at peak base (200-250 ms at half-height). Figure 1 is a 3D plot of the m/z 322 (321.8936) ion of 2378D resulting from an injection of a standard containing 313 ag.

Due to the very small quantity of sample injected, matrix effects are minimal, even when serum samples are analyzed. For this reason very good signal-to-noise ratios can be obtained. Figure 2 is the m/z 322 trace resulting from an injection of the NIST SRM 1589a human serum extract which is equivalent to approximately 540 ag of 2378 D, assuming a 70% recovery, displaying a signal-to noise ratio of 479 to1, using a  $\pm 4\sigma$  definition. The 3D plots of the m/z 320 and 322 ions from the same injection are presented in Figure 3. Figure 4 is a 3D plot of total ion current (TIC) in which tetra- through octa-chlorinated dioxin congeners can be seen. Cryogenic zone compression coupled with magnetic sector mass spectrometry can be employed to greatly reduce the sample size required for analysis of dioxins and other POPs in human serum.

## References

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Figure 1. Plot showing signal at m/z 322 resulting from an injection of standard containing 313 ag of 2378D.

**Figure 2. Signal-to-noise ratio measurement of the m/z 322 ion resulting from an injection of NIST SRM human serum extract equivalent to approximately 540 ag of 2378D, assuming 70% recovery. Shaded area denotes region used for noise determination.**

**Figure 3 (a) (b)**

**Plots showing signal at m/z 320(a) and 322(b) resulting from an injection of NIST SRM human serum extract equivalent to approximately 540 ag, assuming 70% recovery.**

**Figure 4**

**3D plot showing the total ion current (TIC) resulting from an injection of NIST SRM human serum extract in which 7 dioxin congeners (2 unresolved) and 1 furan congener were monitored.**