TIME CONTROLLED CRYOGENIC ZONE COMPRESSION (T-CZC) GC-HRMS – A NOVEL TOOL FOR TARGETTED ANALYSIS AT ULTRA TRACE LEVELS

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Introduction

The analysis of dioxins and furans with low limits of detection (LODs) is often challenging and requires GC-MS capabilities of enhanced selectivity and the lowest attainable sensitivity. Even with GC-HRMS, low LODs can often not be met with instrumental performance alone, especially when residue levels are very low within a sample. Thankfully, low limits of detection can regularly be achieved with the use of increased sample size and high volume sample introduction techniques for most sample types, such as food.

However, when sample sizes are small, and residue levels are low, a unique analytical challenge is presented, such as with infant dried blood spot analysis - typical sample sizes can be as small as 20-100 µL only [1], the residue levels are very low, there is no opportunity to scale up sample size and there is no advantage in using high volume sample introduction techniques. Typically this challenge would preclude the analysis of dioxins and furans in such samples. However, large archives of dried blood spot samples exist in hospitals globally. These are routinely sampled from children at birth in many countries. These samples present an unprecedented sample resource for epidemiological and toxicological studies of population background exposure to dioxins and furans, providing these significant analytical challenges can be overcome.

In this study a novel GC signal enhancement tool – time controlled cryogenic zone compression (CZC) – coupled to high resolution GC-MS was investigated for lowest level Dioxin analysis in human serum samples for application areas as described above.
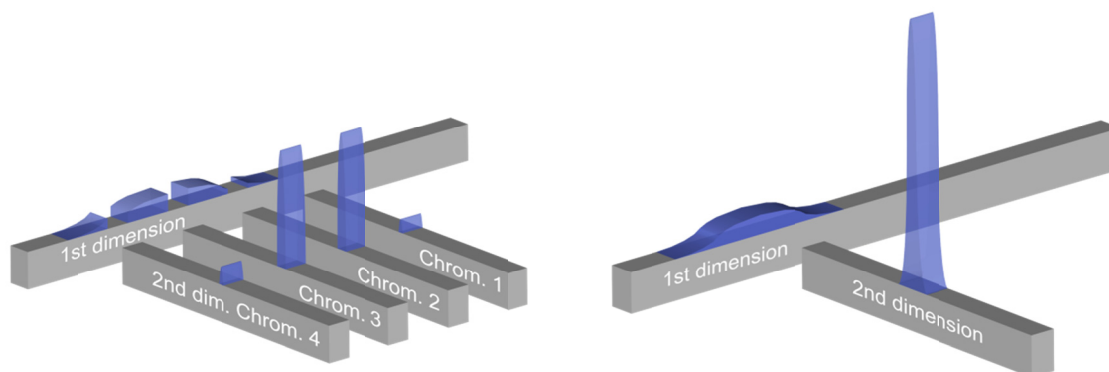

Figure 1. Cryogenic signal enhancement in GCxGC versus CZC.

Cryogenic Zone Compression (CZC) in combination with high resolution mass spectrometry was first described by Patterson et al. [2]. In contrast to the established technique of GCxGC; CZC analytes eluting from the first column dimension are trapped completely using a standard GCxGC cryogenic modulator. Consequently only one single 2nd dimension peak is obtained; the first dimension peak is not modulated into several 2nd dimension peaks. Accordingly the signal enhancement effect as known from GCxGC is maximized in CZC (Figure 1). Patterson et al. achieved this effect by using single short columns attached to a cryogenic GCxGC modulator combined with long modulation times.Their method was refined here into ´time controlled´ CZC, which

basically consists in timed controlled switching of a single cryogenic modulator jet. In this way CZC is simplified and flexibility is strongly increased, which among other advantages allows for standard Dioxin GC columns to be used with this technique.T-CZC is akin to a carefully timed cryogenic heart-cut of the entire peak of interest.

Materials and methods
All experiments were carried out on a Thermo Scientific™ DFS™high resolution GC/MS coupled to a Thermo Scientific Trace™ GCxGCunit equipped with a Thermo Scientific TriPlus™ auto-sampler. The GCxGC was equipped with a cryogenic GC modulator deviceusing liquid CO_2 as cooling medium. A standard split/splitless injector was employed in splitless mode for all injections.

CZC experiments were carried out using single standard columns; Thermo Scientific TR-Dioxin 5MS 30 m and 60 m, I.D 0.25 mm, 0.1 μm film. The modulator was placed between 1-4 m from the MS end of the analytical column.

Results and discussion
Time controlled CZC for target analyte refocusing allows combining standard analysis and CZC within one analysis sequence and even within the same measurement (Figure 2).
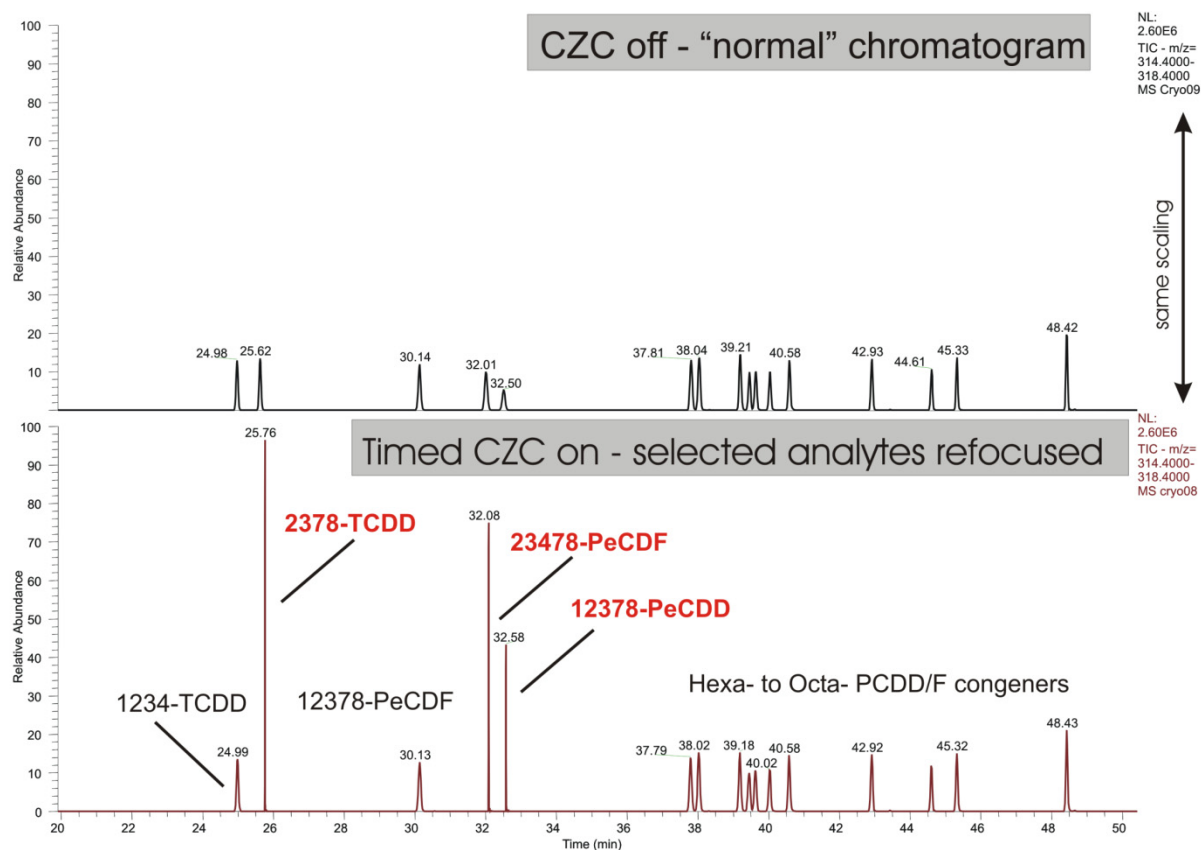

Figure 2.Upper mass trace - standard dioxin/furan chromatogram; lower mass trace: CZC chromatogram with 3 CZC cryo-focused target analytes; same scaling for both traces

CZC parameters where investigated and optimized in order to achieve maximum sensitivity.Column dimensions and GC parameters define final peak width resulting from the CZC experiment. Whereas - for given settings - the 2nd dimension column length proved to be the decisive factor for the CZC peak width, the 1st dimension

column length is decisive for the cryo jet activation timing (jet activation time and jet activation length). The broader the first dimension peak is the longer the jet activation time must be in order to capture the entire peak.

One of the challenges was to adapt the sector field HRMS detection to the very narrow GC peaks produced by CZC. Whereas HRMS is highly selective and sensitive, it does not easily allow for very rapid detection frequencies. Nevertheless optimized SIM settings with selected target masses allowed to use frequencies of 10 – 15 Hz, which for GC peaks with baseline width of 600-700 ms allowed to sample between 5-8 data points.

In all experiments the CZC signal enhancement effect could be seen unambiguously with peak heights increasing inversely in function of the peak width, e.g. 2378-TCDD with standard analysis conditions producing peaks of 9 – 10 sec baseline peak width versus 600-700 msand more than 10 fold increased peak height in CZC. Signal intensity increase observed with CZC was almost the same for solvent standard analysis and matrix sample analysis.

The CZC signal enhancement effect can be seen unambiguously in all examples. Figure 3 shows the increase in peak height and the gain in Signal to noise on TCDD in a pooled blood sample using the t-CZC.
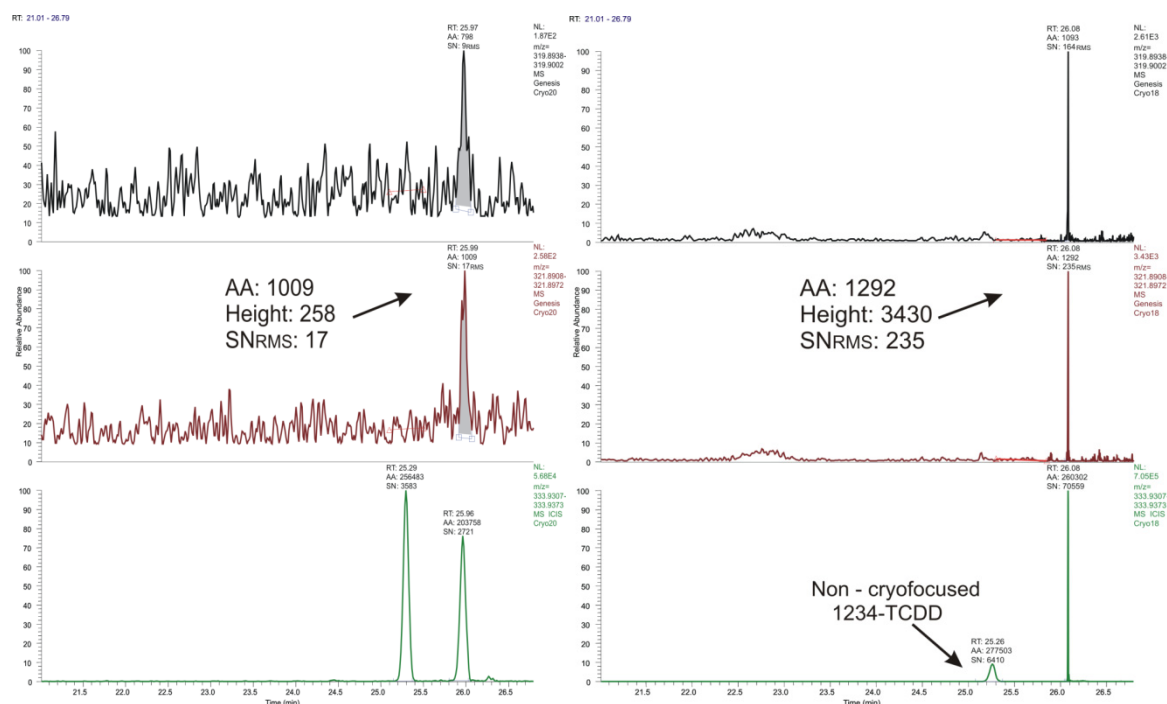

Figure 3: **Sensitivity increase for 2378-TCDD with CZC in low level pooled blood sample (ca. 10 fg/µL native TCDD); left - standard analysis; right - CZC experiment**

A number of real serum samples with different target analyte concentrations was analysed and compared to standard analysis conditions.

Using special low level standards (LL-TF CS2-CS6 Wellington Laboratories) a 5 point calibration curve was determined injecting 4 µL per measurement. The lowest calibration point is **200 attogram per microlitre**; equivalent to 800 attogram injected on column (Figure 4.).
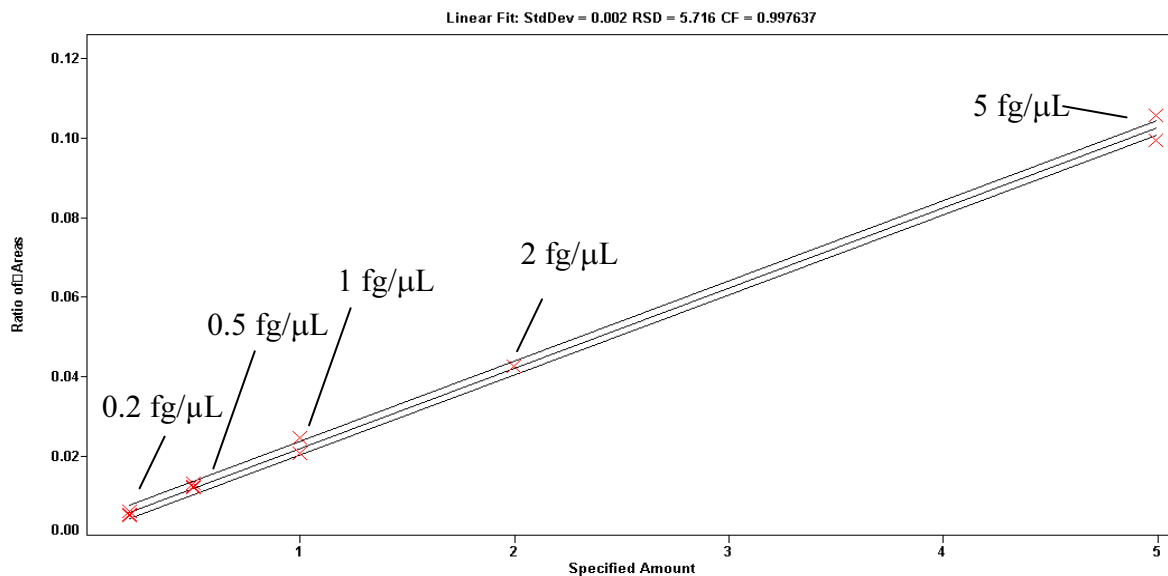
Figure 4. Triplicate five point calibration curve of TCDD (4μL injected) using t-CZC.

References
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2. Patterson DG, Welch SM, Turner WE, Sjödin A, Focant JF; (2011) J. of Chromatography A, 1218, 3274-3281