Time controlled Cryogenic Zone Compression (t-CZC) GC-HRMS – a Novel Tool for Target Compound Analysis at Ultra Trace Levels?

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Overview
Purpose: The purpose of time controlled cryogenic zone compression (t-CZC) is to increase sensitivity and lower instrument detection limits for selected analytes.

Methods: A single time controlled cryo-jet from a GCxGC modulator was used to trap and refocus selected analytes at the end of an analytical column shortly before the detector.

Results: The combination of time controlled cryogenic zone compression (t-CZC) with the Thermo Scientific™ DFS™ high resolution mass spectrometer resulted in strongly increased peak height and signal to noise of selected analytes. This enabled the detection of selected target analytes down to the sub-femtogram level. It is a promising tool for dried blood spot (DBS) analysis.

Introduction
The analysis of dioxins and furans with low limits of detection (LODs) is often challenging and requires highest selectivity and sensitivity in GC-MS analysis. Combined with increased sample size preparation techniques these low detection limits can be achieved routinely for many sample types, such as feed or food and environmental samples. However, small sample sizes with low residue levels present a unique analytical challenge, such as bio-monitoring of environmental contaminants in infant dried blood spots (DBS) with sample volumes as small as 20-100 µL. Current routine analysis techniques do not achieve the required instrument sensitivity. However, large archives of newborn dried blood spot samples exist in hospitals globally and their study would be of highest toxicological relevance.

In this study a novel GC signal enhancement tool was investigated: time controlled cryogenic zone compression (t-CZC). In CZC analytes eluting from the first column dimension are trapped completely in one event maximizing the signal enhancement effect as known from GCxGC. In GCxGC the constant modulation of all 1st dimension peaks results in several 2nd dimension chromatograms maximizing chromatographic separation power.

FIGURE 1. Cryogenic signal enhancement in t-CZC (left) versus GCxGC (right)

Methods
Samples
1/10 diluted EPA 1613 CSL standard; special prepared ag level standards: TF-LL CS 1-6 Wellington Laboratories; pooled human blood sample (ca. 10 fg/µL, 2378-TCDD)

Gas Chromatography and t-CZC
A Thermo Scientific Trace™ GCxGC was used for all experiments, equipped with a cryogenic GC modulator device (Figure 2) using liquid CO₂ as cryogen. Only one of the jets was used controlled via so-called ‘time events’, which form part of the GC instrument method. Jet on/off times were determined from preceding experiments where standard analyte retention times and 2nd dimension column retention times needed to be determined. Thermo Scientific Trace TRSims 30 and 60 m GC columns were used for all experiments.

FIGURE 2. CO₂ Modulator (located inside the GC oven)

Results
Combination of t-CZC and standard GC analysis
Due to the simplicity and flexibility of this approach the combination of t-CZC and standard GC analysis is easily achievable even within one analytical run. In Figure 4, three out of 16 analytes are cryo-focused within a single GC run.

For all t-CZC target analytes for which signal enhancement is required, the CO₂ modulator jet must be activated shortly before the peak reaches the position of the jet (Figure 3a). Then the jet is turned off and the cryo-focussed chromatographic band is re-injected onto the column behind the jet (Figure 3b). The 2nd dimension chromatography is fast, due to the short length of the 2nd dimension column part and the high oven temperature. This results in very high and narrow GC peaks.

Sensitivity increase
The CZC signal enhancement effect can be seen unambiguously in these examples. Figure 5 shows the increase in peak height and the gain in signal to noise for 2378-TCDD in a pooled human blood sample.

FIGURE 5. Relative sensitivity increase using t-CZC when analysing 2378-TCDD in a human blood sample; 5a: conventional GC analysis, 5b: t-CZC experiment

Calibration curves
Using low concentration standards (LL-TF CS2-C56 Wellington Laboratories) a five point calibration curve was determined injecting 4 µL per measurement. The lowest calibration point is 200 attogram per microliter.

FIGURE 6. 2378-TCDD 4µl, injections of 200 pg/µL to 5 fg/µL.

Conclusion
Time controlled cryogenic zone compression (t-CZC) was developed as a tool to increase GC sensitivity for selected target analytes. In combination with magnetic sector high resolution MS this approach allows for ultra trace level analysis of selected PCPs.

- Atto gram concentrations of selected analytes like 5a: 2378-TCDD can be detected.
- Sample volumes can be reduced; with further developments microtome sample volumes, like in blood spot analysis, could become feasible
- t-CZC is a general GC sensitivity enhancement tool
- Due to its flexibility t-CZC can be used in combination with standard GC chromatography even within one and the same analysis run

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