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Time Controlled Cryogenic Zone Compression (T-CZC): A Novel Gas Chromatographic Tool for Increasing Sensitivity

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The analysis of semi-volatile trace contaminants represent a unique challenge to the analytical chemist. Modern techniques of gas chromatography and mass spectrometry have reduced instrumental limits of detection from the nanogram range in the 1970s, using packed chromatography columns and quadrupole mass spectrometry; to the low femtogram range during the twenty-first century, using capillary columns coupled to high resolution or tandem quadrupole mass spectrometers [1].

Some compounds which require such low levels of detection are chlorinated dioxins and furans; a group of structurally similar halo-organic compounds, which are of great interest because some of these congeners are extremely toxic, persistent and bio-accumulative [2].

For such compounds low limits of detection can now be achieved in many sample types with the combination of sensitive mass spectral detection and careful chromatography. Generally, when even lower limits of detection are required, the analytical chemist has the possibility of combining increasingly selective sample preparation together with significantly increased sample size.

However, when sample sizes are small, and residue levels are low, a unique analytical challenge is presented, such as with dried blood spot (DBS) analysis. Typical DBS sample sizes can be small, only 50-150 µL [3]. Residue levels are very low, especially for lipophillic compounds like dioxins, and there is no opportunity to scale up sample size to achieve low limits of detection. Usually 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 [3]. 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.

Cryogenic peak modulation is a well established technique used for comprehensive GCxGC applications since 1991 [4]. In GCxGC a cryogenic modulator continuously and rapidly traps and releases the eluent from a first dimension column onto another short second dimension column in a very narrow band. The combination of two different column phases for the first and second dimension results in a substantial increase of chromatographic separation power. This is due to combined chromatographic selectivity, cryogenic peak focusing and fast second dimension chromatography. Each first dimension chromatographic peak is modulated and several second dimension chromatograms are obtained (Figure 1); dedicated software tools allow to construct two-dimensional chromatograms for data evaluation. This

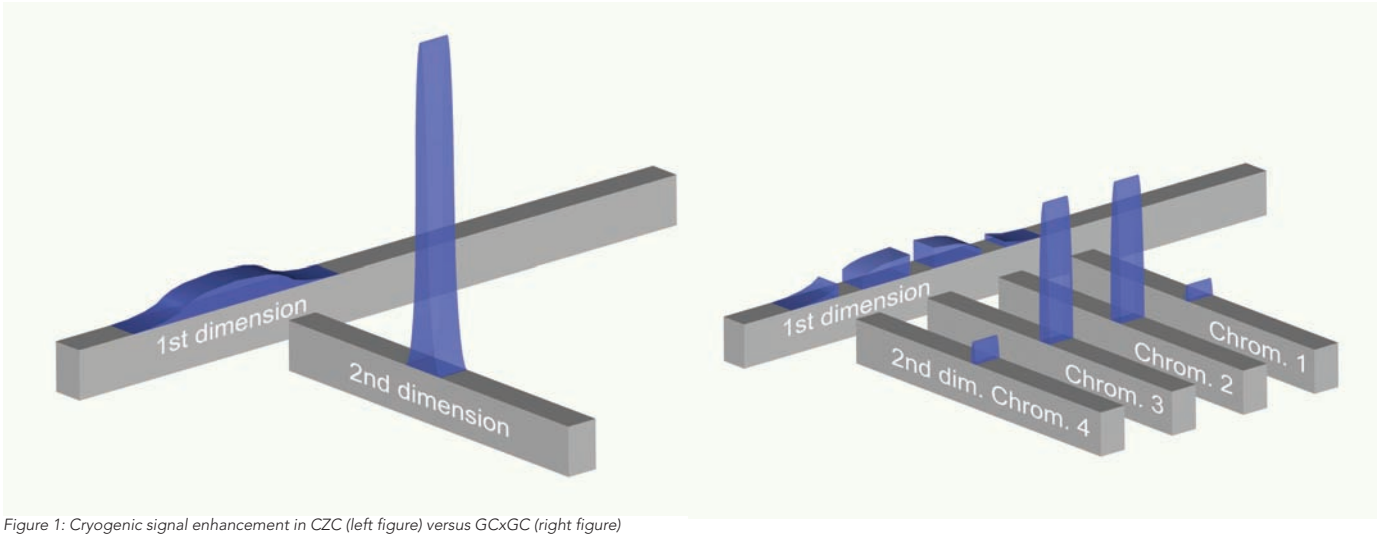


Figure 1: Cryogenic signal enhancement in CZC (left figure) versus GCxGC (right figure)

technique is particularly powerful in the separation of complex mixtures. Marriot et al., demonstrated that combining a cryogenic 'heart-cut' operation by effective timed trapping of particular zones of a primary separation, followed by rapid introduction to a second short column, could lead to improved separation performance and result in significantly increased response sensitivity [5].

Due to the narrow modulated peak widths, comprehensive GCxGC is best used in combination with fast acquisition time of flight (TOF) mass spectrometry [6]; an MS technique that does not have adequate sensitivity and reliability required for trace analysis of compounds such as dioxins and furans [7]. Nevertheless, Focant et al. demonstrated that the modulation process used in GCxGC had an advantage of increased signal intensity for compound such as dioxins. However, because of the inherent limitations of the TOF MS, limits of detection remained insufficient for the application.

The ability to combine the signal intensity increase observed for GCxGC analysis with a more sensitive detection system would present a significant step forward in the pursuit of the low limits of detection required for the analysis of dioxins & furans in DBS samples. Patterson et al., utilised a comprehensive GCxGC modulator on a single, short GC column coupled to a high resolution mass spectrometer (HRMS) – a technique which they termed Cryogenic Zone Compression Gas Chromatography (CZC GC-HRMS) [8,9]. They were able to significantly lower the detection limits for dioxins using this technique.

Cryogenic Zone Compression (CZC) GC operates in a comparable fashion to comprehensive GCxGC. The main difference is that entire 1st dimension peaks are trapped, followed by reinjection of the complete refocused peak onto the 2nd dimension column. Consequently in CZC only one single 2nd dimension chromatogram is produced, whereas in GCxGC each 1st dimension peak is modulated into several 2nd dimension chromatograms. In comparison peak intensities from several 2nd dimension chromatograms from an analogue GCxGC experiment are added up to form one single high peak in a corresponding CZC experiment. This explains why the peak enhancement effect as known from GCxGC is maximised in CZC (Figure 1).

Very low concentration broad conventional GC peaks, that would normally be lost to background noise, can now be detected using CZC. Unfortunately, the approach by Patterson et al [8] – basically using constant modulation with long modulation times to entirely trap peaks - comes with some drawbacks;

- Firstly, the continuous modulation of the cryo-trapping then releasing can 'slice' the elution of a single analyte from the first dimension into several chromatographic peaks in the second dimension. Whereas, in comprehensive GCxGC this is a wanted effect; for CZC this reduces the resulting overall signal intensity, compounds analytical error and complicates data evaluation.
- Secondly, this approach is based on narrow 1st dimension peaks and thus short columns are mandatory. The usage of 30 or 60 m columns - standard in conventional dioxin/furan analysis - cannot or hardly be used.
- Thirdly, the use of constant long modulation times – which corresponds to non- tailored trap timing - can result in unselective trapping of multiple analytes or matrix peaks into the refocused chromatographic band; potentially resulting in loss of information or the focussing of chromatographic noise along with the peak of interest.
- Fourthly, the modulation runs continuously throughout the analytical sequence – resulting in significant consumption of cryogenic gas, adding prohibitive cost and maintenance effort to the analysis.

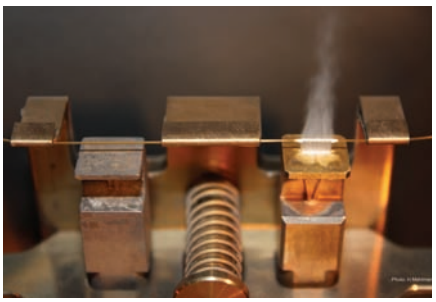


Figure 2: CO_2 Modulator (located inside the GC oven)

To answer these drawbacks and address the challenge of trace analysis from small sample sizes, 'Time Controlled' CZC GC-HRMS was developed and evaluated in this study. Here a novel approach in selective time control of a standard cryogenic GCxGC modulator, rather than constant modulation, to securely capture selected whole analyte peaks was

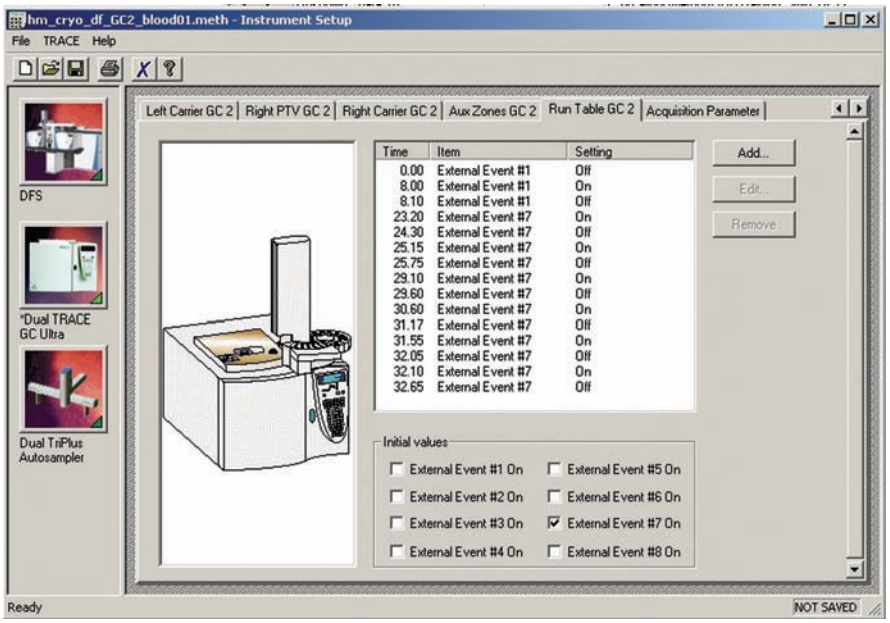
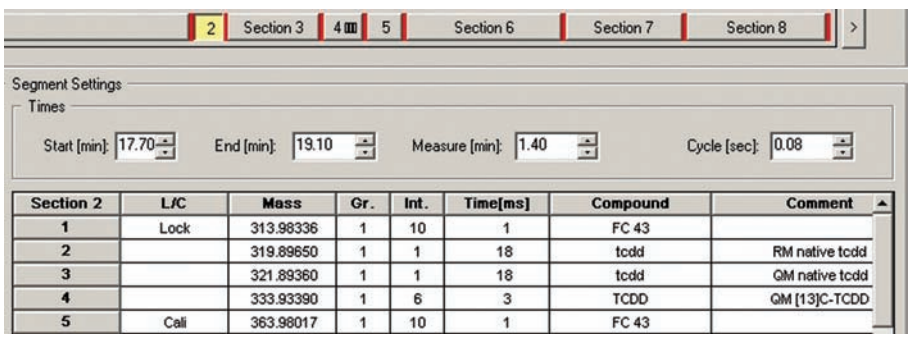


Figure 3: GC time events to control cryogenic jet



Section 2	L/C	Mass	Gr.	Int.	Time[ms]	Compound	Comment
1	Lock	313.98336	1	10	1	FC 43	
2		319.89650	1	1	18	tcdd	RM native tcdd
3		321.89360	1	1	18	tcdd	QM native tcdd
4		333.93390	1	6	3	TCDD	QM [13]C-TCDD
5	Cali	363.98017	1	10	1	FC 43	

Figure 4: Multiple ion detection (MID) setup screen. Here showing typical tetrachlorodioxin ions and acquisition times used for CZC

used. Certain peaks were cryogenically focussed to increase signal intensity and directed to a HRMS for ultra sensitive analysis, without the potential risk of peak slicing incumbent with modulation.

Experimental

All experiments were carried out on a Thermo Scientific® DFS™ High Resolution GC-MS coupled to a Thermo Scientific Trace™ GCxGC unit equipped with a Thermo Scientific TriPlus™ auto-sampler. The GCxGC was equipped with a cryogenic GC modulator device using liquid CO_2 as a cryogen (Figure 2). A standard split/splitless injector was employed in splitless mode for all injections.

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

The cryogenic gas operation was timed controlled and fully integrated within the GC method control software, via time event settings (Figure 3). Only one of the two jets of the modulator was used for the experiments (Figure 2). Complete and fully automated timed CZC methods could be created and sequenced using the Xcalibur® data system.

Magnetic sector GC-HRMS is constrained by acquisition rate [10]. In order to achieve the fast sampling frequencies required for this type of analysis, only a reduced number of selected target masses can be used with magnetic sector GC-HRMS detection. Sampling cycle times of 80 – 100 ms were used corresponding to 10 – 12 Hz (Figure 4). Observed typical baseline GC peak widths with CZC activated were ca. 600-700 ms and 6 – 8 MS data points could be sampled over the peak with the settings and columns used.

HRMS settings were equivalent to standard settings typical in routine dioxin analysis: 45 eV, Resolution 10,000 (10 % valley definition), etc.

Certified standards from Wellington Laboratories, Ontario, Canada were employed. The DFS instrument performance specification standard (20 fg/µL 2378-TCDD; 5 pg/µL 2378- ^{13}C TCDD) was diluted down to 2 and 0.5 fg/µL for CZC sensitivity experiments. For multi target CZC experiments a certified 1/10 EPA 1613 CSL standard (Wellington) was employed together with a pooled human serum sample (Figure 6-9).

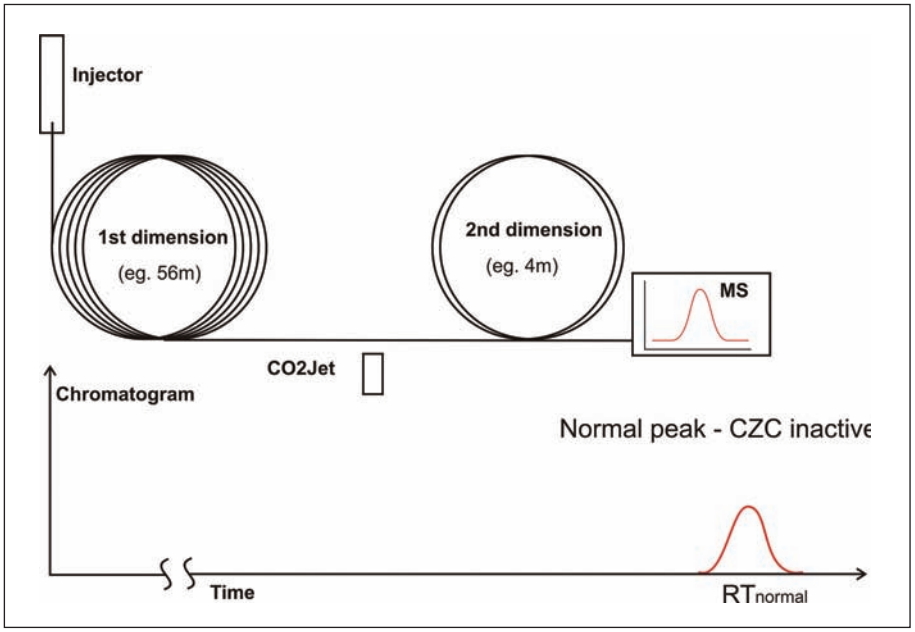


Figure 5a: Cryogenic jet off results in normal peak width for non-target analytes

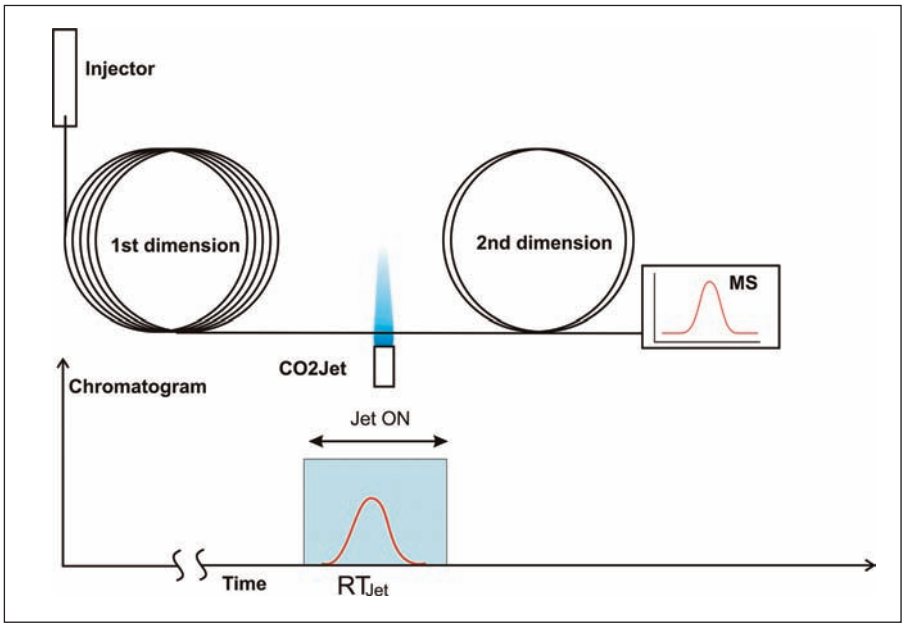


Figure 5b: Cryogenic jet on: step 1 – cryo-traps whole 1st dimension peak of interest

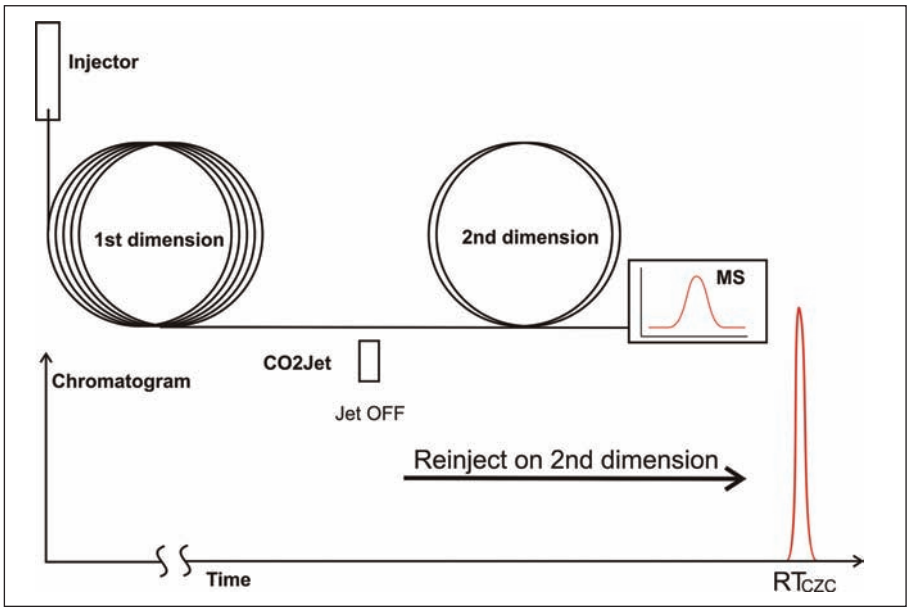


Figure 5c: Cryogenic jet off: step 2 – re-inject peak of interest on 2nd dimension column

Results & Discussion

Already during first experiments in late 2010 it became soon clear that time controlling the activity of a single modulator jet rather than using constant modulation seemed to be the safer, simpler and far more flexible approach for selective peak signal enhancement. Following this was further developed into the final concept as described below.

Figures 5a - 5c illustrate the basic concept of time controlled CZC. If the CO_2 modulator jet is not in use throughout an analysis run a normal chromatogram will be obtained (Figure 5a).

For all CZC target analytes – analytes for which signal enhancement is required – the CO_2 modulator jet must be activated shortly before the peak reaches the position of the jet (Figure 5b). Then the jet is turned off and the cryo-focussed chromatographic band is re-injected onto the second dimension column (Figure 5c). The 2nd dimension chromatography is fast, due to the short length of the 2nd dimension column and high oven temperature. This results in very narrow GC peaks.

Due to the simplicity and flexibility of this approach the combination of CZC and standard GC analysis is easily achievable even within one analytical run (Figure 6).

Column dimensions and GC parameters define the 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.

Jet activation times are determined from preceding experiments where standard analyte retention times and 2nd dimension column retention time need to be determined.

Figures 7 – 9 compare relative signal enhancement between a standard analysis experiment and a time controlled CZC experiment. The standard chromatogram is shown on the left and compared to the CZC chromatogram on the right. Mass traces shown in all chromatograms from upper to lower trace are: ratio / qualifier isotope mass for native (^{12}C) 2378-TCDD; quantification mass for native 2378-TCDD and internal standard quantification mass trace ^{13}C-TCDD respectively (chromatogram label AA

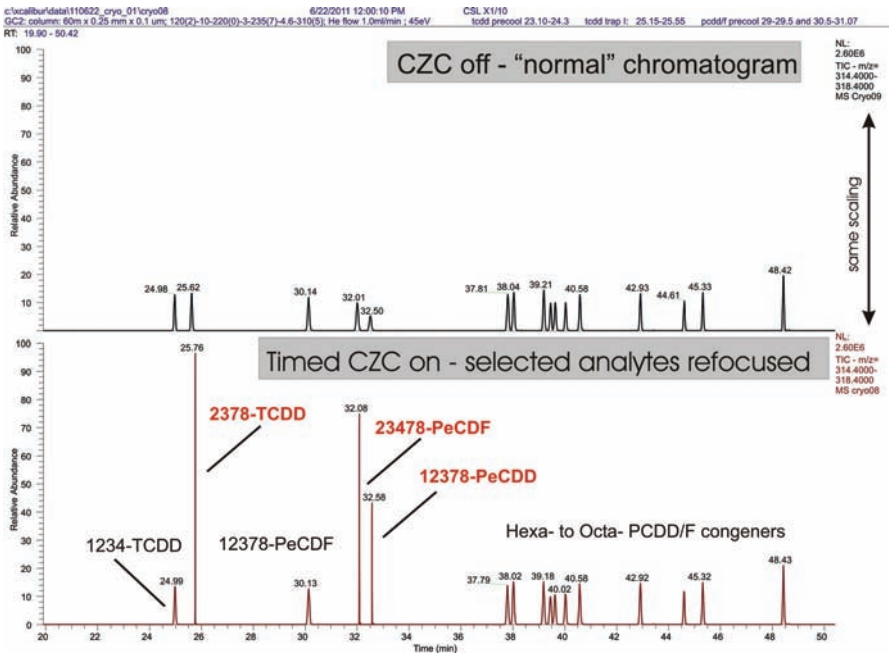


Figure 6: upper mass trace - standard dioxin/furan chromatogram; lower mass trace: CZC chromatogram with 3 CZC cryo-focussed target analytes; same scaling for both traces

= peak area) . This setup corresponds to the isotope dilution technique typically used in dioxin/furan analysis. Based on experimental data retention time (RT) stability seems comparable between CZC and standard GC analysis. RT differences as shown here, e.g. between Figures 7 and 8, are due to different parameter settings used during method optimisation.

The CZC signal enhancement effect can be seen unambiguously in all examples. Peak height increases inversely in function of the peak width; e.g. 2378-TCDD standard analysis 9 – 10 sec baseline peak width versus 600-700 ms in CZC. The observed CZC signal intensity increase was almost the same for solvent standard analysis and matrix sample analysis. This is due to the high selectivity of HRMS. Less selective detectors would be more likely to have compromised response in matrix. Interestingly, observed peak areas were often not completely equivalent between normal GC-HRMS analysis and CZC GC-HRMS. CZC typically showed a ca. 20 - 30 % increase in peak area over normal GC-HRMS, with the effect more pronounced for low concentration peaks. A possible hypothesis for this is as follows:

A chromatographic peak can also be seen as a concentration profile with signal intensities being lower towards each of the flanks of the peak. If now the overall compound concentration is decreased successively it can be expected that the detectability of the peak flanks will be affected first resulting in underestimation of peak area. CZC remedies this effect to some extent.

Time controlled CZC requires complete separation of congeners and isomers in the 1st dimension for refocusing of a single target analyte. So for the field of dioxin analysis CZC will be applicable to types of samples which only contain the toxic 2,3,7,8 chlorinated congeners. This includes samples of biological origin where increased chromatographic separation power to separate non toxic congeners from toxic 2,3,7,8 chlorinated congeners - as often found in environmental samples for example - is not mandatory.

Future experiments will include further CZC method optimisation in order to evaluate minimum instrument detection limits (iLODs) for different target analytes and investigate quality parameters like analytical precision at the very low femtogram or even attogram range. This is of special interest for analysis of selected indicator persistent organic pollutants (POPs) in very low volume human blood/serum samples – including dried blood spot analysis with sample volumes as low as 100 µL.

Conclusions

'Time controlled' CZC GC-HRMS has been demonstrated for the first time. This technique achieves the signal intensity improvements of CZC as described by Patterson et al. [8], without the drawbacks resulting from inadvertent 'peak slicing', matrix focussing and high cryogenic gas consumption. Individual components can be selected, based on retention time; trapped, focussed and released into the second

dimension of the column without affecting the elution characteristics of neighbouring peaks. Early indications are that this technique can yield at least a factor of tenfold increase in signal intensity. Another major advantage of the time controlled CZC approach is that due to its simplicity and flexibility standard analysis and CZC experiments can be combined within one analysis sequence and even within the same measurement.

Although further work in optimisation of this technique is required; combining time controlled CZC with the sensitive and selective detection of high resolution mass spectrometry could push absolute instrumental detection limits from low femtogram down into the attogram range for specific POPs including 2378-TCDD.

Such detection limits are congruous with those required for detection of trace persistent organic pollutants (POPs), such as dioxins, in dried blood spots. Further investigations are planned to investigate the feasibility of this technique for this application.

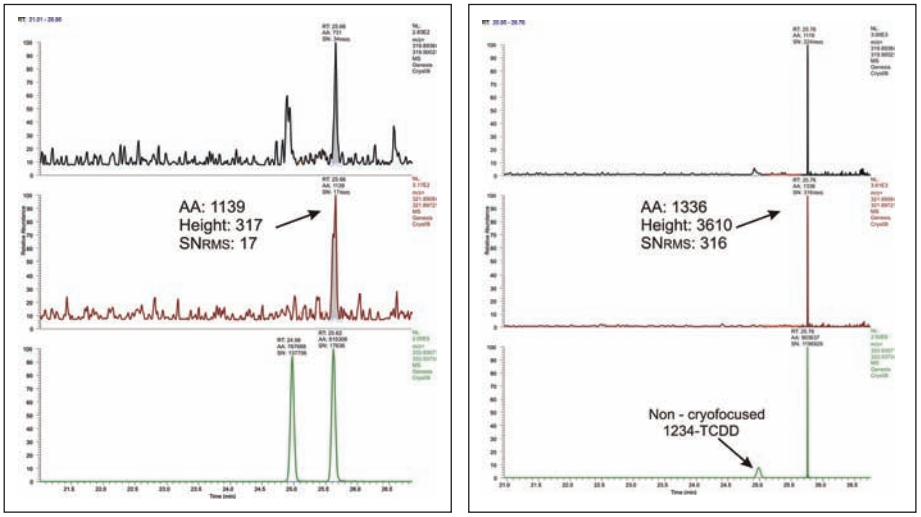


Figure 7: sensitivity increase with CZC in 1/10 EPA 1613 CSL standard (10 fg/ µL 2378-TCDD); left - standard chromatogram TCDD (mass trace 1 and 2: ratio /qualifier and quantification m/z for native (^{12}C) TCDD; trace 3: quantification m/z ^{13}C TCDD); right – chromatogram with cryo-focussed 2378-TCDD peaks, note: 1234-TCDD is not focussed.

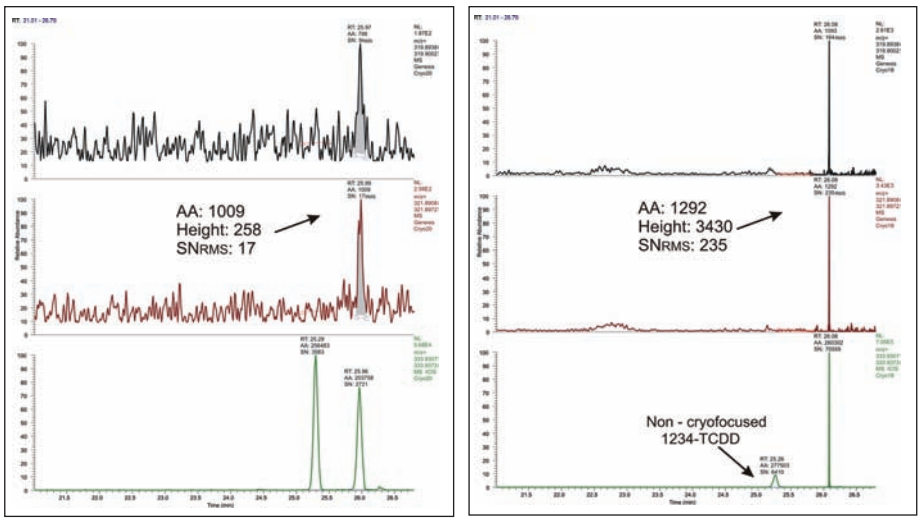


Figure 8: sensitivity increase for 2378-TCDD with CZC in low level pooled blood sample (ca. 10 fg/µL native TCDD); left - standard analysis; right - CZC experiment; all mass traces as explained in Figure 7

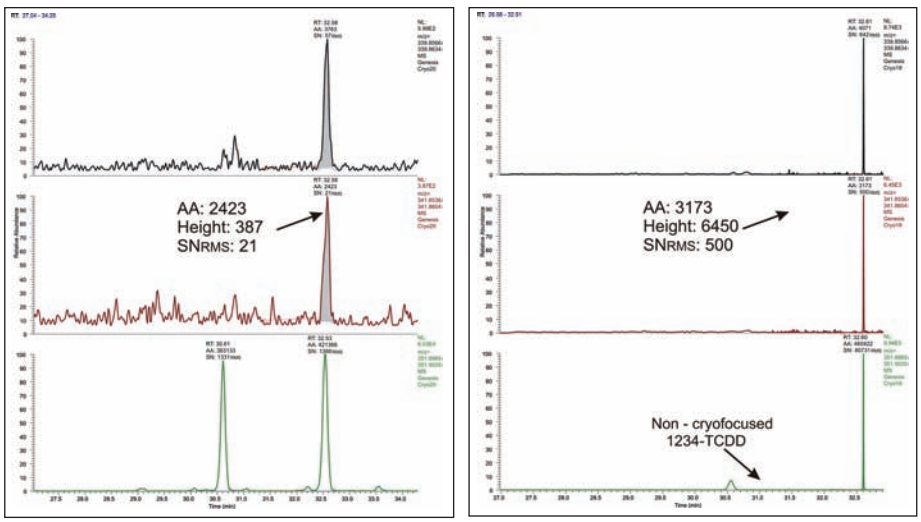


Figure 9: sensitivity increase for native PCDF with CZC in low level pooled blood sample ca. 30 fg PCDF;); left - standard analysis; right - CZC experiment; mass traces analogue to Figure 7, only for PCDF

Acknowledgements

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