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Nota de aplicación

A Single Injection Screening Method for Tetra-Octa Chlorinated PCDD/FS in Fish and Flyash Matrices Using GC-Triple Quadrupole MS/MS

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Abstract

The aim of this paper is to develop a rapid screening method, allowing the acquisition of all non-toxic and toxic PCDD/Fs in a single injection.

Introduction

Confirmatory dioxin/furan analysis using high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS) is an expensive and time consuming analysis, requiring highly trained instrument operators. The use of a screening method prior to confirmatory analysis can greatly reduce the workload of a HRGC/HRMS laboratory by highlighting samples that are either non-detect, or have concentrations at extremely high levels

that may fall outside the quantifiable range of the HRGC/HRMS method.

Current recommendations are that 10% of screened samples are verified/confirmed by HRGC/HRMS. Some GC-MS/MS methods require multiple injections to acquire toxic and non-toxic PCDD/Fs, or use function switching [1,2,3,4] that ensures that all of the toxic 2,3,7,8-chlorinated PCDD/Fs are detected, but at the expense of some of the nontoxic PCDD/Fs (non-2,3,7,8-chlorinated PCDD/Fs).

The aim of this paper is to develop a rapid screening method, allowing the acquisition of all non-toxic and toxic PCDD/Fs in a single injection. The use of GC Triple Quadrupole MS produces a method that is much easier to utilize when compared with standard HRGC/HRMS methods, with simple instrumental set up reducing the need for such highly trained instrument operators.

Experimental

Methods and Materials

All GC-MS/MS analysis was performed using a Waters Micromass Quattro micro GC Triple Quadrupole mass spectrometer, directly interfaced to an Agilent 6890N GC oven, incorporating a CTC-PAL autosampler. Confirmatory comparison analysis was performed using a Waters Micromass AutoSpec Ultima double-focussing magnetic sector mass spectrometer, directly interfaced to an Agilent 6890N GC oven, incorporating a CTC-PAL autosampler. All data acquisition and processing was performed using MassLynx Software version 4.0, with QuanLynx and TargetLynx Application Managers.

The laboratory acquired two certified reference materials (CRMs), a flyash (Commission of the European Communities, Community Bureau of Reference - BCR, Reference material number 490) and Carp (National Research Council Canada, CARP-2). All standards were purchased from Cambridge Isotope Laboratories and Wellington Laboratories.

The two CRMs were extracted using methods based upon US EPA method 16135 to give a number of extracts in n-nonane. A reduced clean-up method was employed, with the raw extracts being treated with first a multi layer, acid/base activated silica column, followed by partition of the PCDD/Fs using a florisil column. The final extract volume was 20 µL.

A 20 m DB5-ms 0.18 mm ID, 0.18 µm film column was installed in splitless mode. The GC temperature ramp 140 °C for 1.5 min, 18.4 °C/min to 220 °C, 3.7 °C/min to 255 °C, 10.4 °C/min to 310 °C; hold 1 min was used for all injections with a He flow of 0.6 mL/min.

For the GC-MS/MS analysis a five function multiple reaction monitoring (MRM) acquisition system was used to monitor the two most abundant transitions from the molecular ion clusters for native and $^{13}C_{12}$ labelled dioxins and furans, with each function representing a single level of chlorination from tetra through to octa chlorinated. The setting of the time windows for this method would be performed in a similar manner to the HRGC/HRMS analysis, with the exception that the windows for function 1 (TCDD/F's) and function 2 (PeCDD/Fs) were 'overlapped', allowing all congeners to be detected. Figure 1 shows the MRM experiment employed.

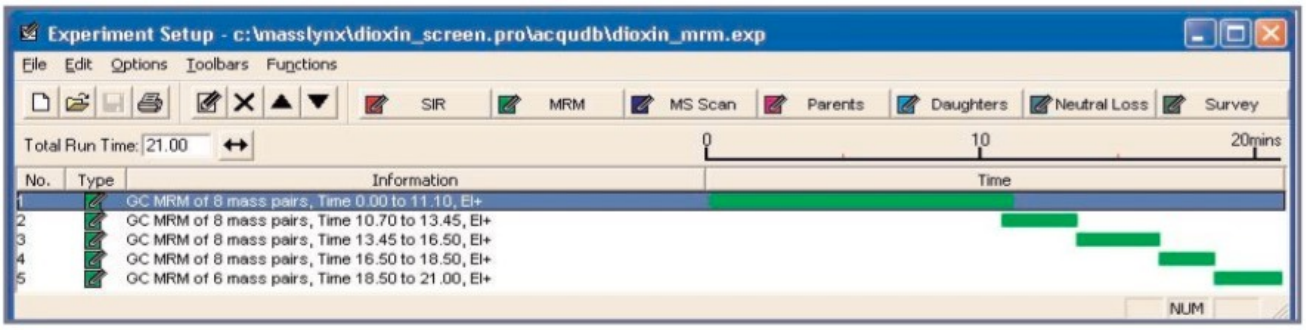

Figure 1. The MRM experiment used for analysis. The green bars depict the time windows employed for analysis. As can be seen, function 1 finishes acquiring at 11.1 minutes, whereas function 2 starts acquiring at 10.7 minutes.

A five point EPA1613 calibration curve, followed by the carp and flyash extracts, and finally EPA1613 calibration standard CS3 was acquired on five separate occasions on the Quattro micro GC, giving a total of 80 injections over 3 days. No GC or instrumental maintenance was performed between batch runs, other than checking of the instrument-tuning parameters.

Results and Discussion

To assess the robustness of the screening method, a number of factors were considered:

1. Stability of response—calculation of %RSD for all rrf's obtained from five replicate calibration curves acquired as described above.
2. Stability of ion ratios—calculation of %RSD for all ion ratios obtained from five replicate calibration curves acquired as described above.
3. Stability of retention times—calculation of %RSD for all retention times obtained from five replicate calibration curves acquired as described above.

Table 1 presents the calculated %RSD values described above.

Congener	RRF %RSD	Ion Ratio %RSD	rt %RSD
2378-TCDF	6.35	5.90	0.14
12378-PeCDF	6.39	3.94	0.16
23478-PeCDF	6.81	4.53	0.13
123478-HxCDF	5.34	4.52	0.14
123678-HxCDF	4.78	4.23	0.15
234678-HxCDF	5.21	3.68	0.14
123789-HxCDF	5.06	3.75	0.13
1234678-HpCDF	4.88	5.27	0.11
1234789-HpCDF	4.07	4.01	0.09
OCDF	3.48	2.96	0.09
2378-TCDD	6.51	4.99	0.13
12378-PeCDD	5.85	3.75	0.14
123478-HxCDD	4.13	4.36	0.14
123678-HxCDD	6.16	3.92	0.14
123789-HxCDD	4.09	3.53	0.14
1234678-HpCDD	3.60	3.46	0.08
OCDD	3.53	2.24	0.09

Table 1. Reproducibility of rrf's, ion ratios and retention times for five repeated calibration curves (total of 25 injections) acquired as part of the sample analysis (total 80 injections including 30 sample injections). The low %RSD values clearly demonstrate the stability and linearity of the method.

A requirement for European Legislation is that the valley between 1,2,3,4,7,8-HxCDF and 1,2,3,6,7,8-HxCDF must not exceed 25%. The chromatographic conditions were optimized such that a rapid run time was obtained (total run time of 21 mins), coupled with suitable separation of these two congeners. The valley obtained between 1,2,3,4,7,8-HxCDF and 1,2,3,6,7,8-HxCDF was 13%. Figure 2 shows the chromatogram for a standard injection taken from the fifth replicate run, for the two congeners.

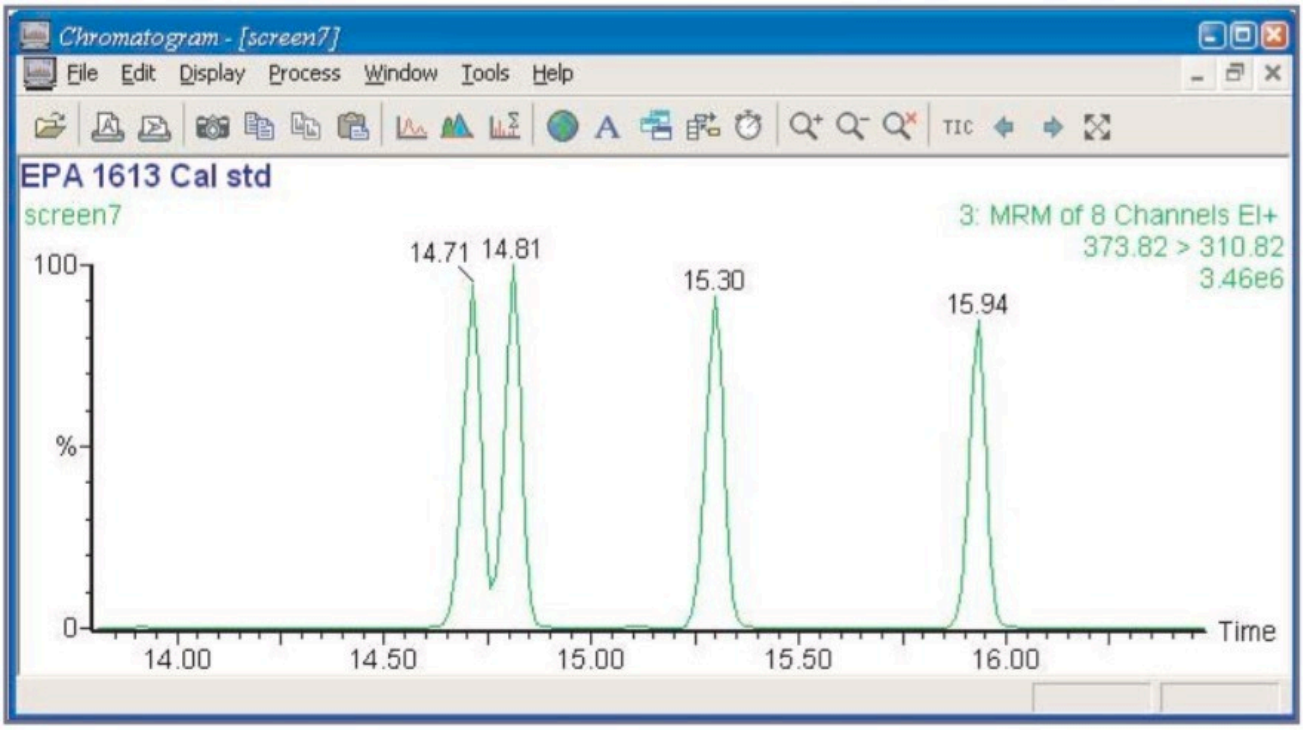

Figure 2. Separation of 1,2,3,4,7,8-HxCDF and 1,2,3,6,7,8-HxCDF, depicted here in the 71st injection of the five replicate batches.

All toxic and non-toxic PCDD/Fs were acquired by 'overlapping' functions 1 (TCDD/Fs) and 2 (PeCDD/Fs). 1,2,8,9-TCDF is the last eluting tetrafuran using a DB5-ms column, and elutes just after 1,3,4,6,8-PeCDF, the first eluting penta-furan. By 'overlapping' functions 1 and 2, both of these congeners could be acquired as part of the totals determination, ensuring that no peaks were missed. Figures 3 and 4 depict the chromatograms, showing the separation and acquisition of these two congeners.

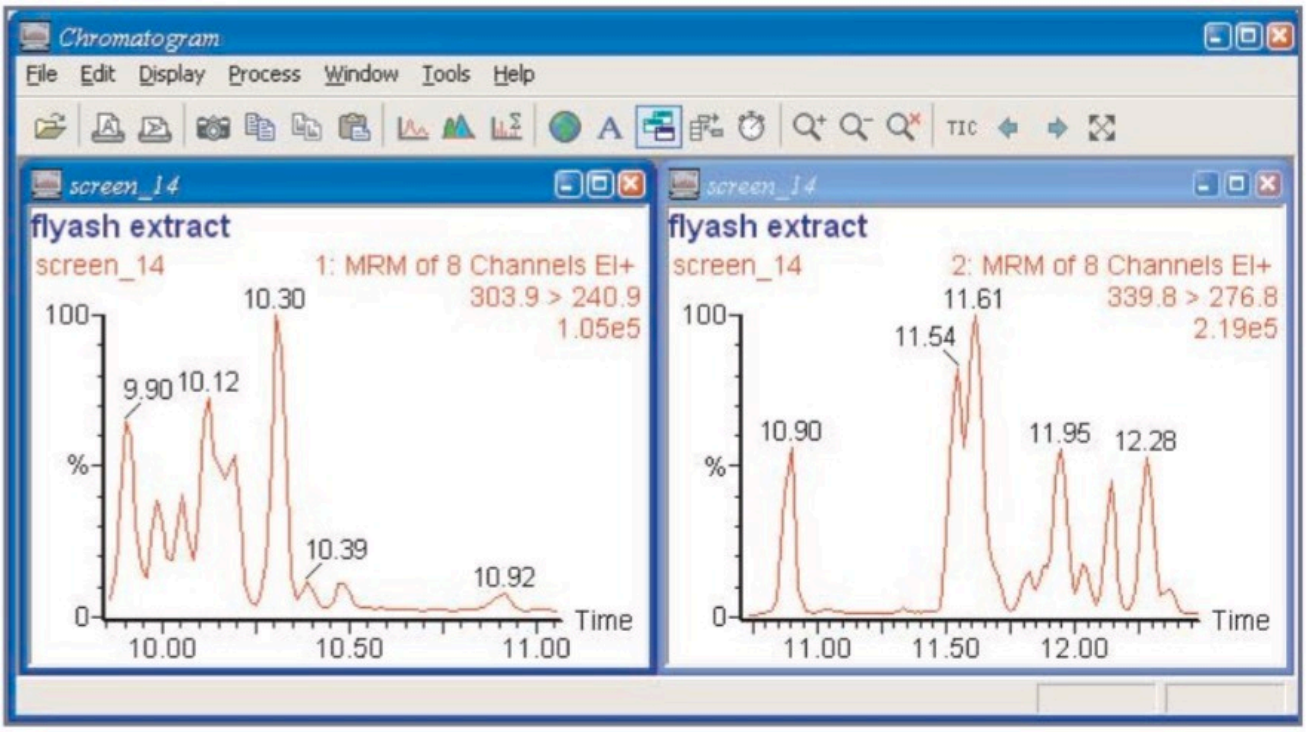

Figure 3. Overlayed chromatograms for TCDF and PeCDF, showing the elution patterns for the non-toxic TCDFs and PeCDFs.

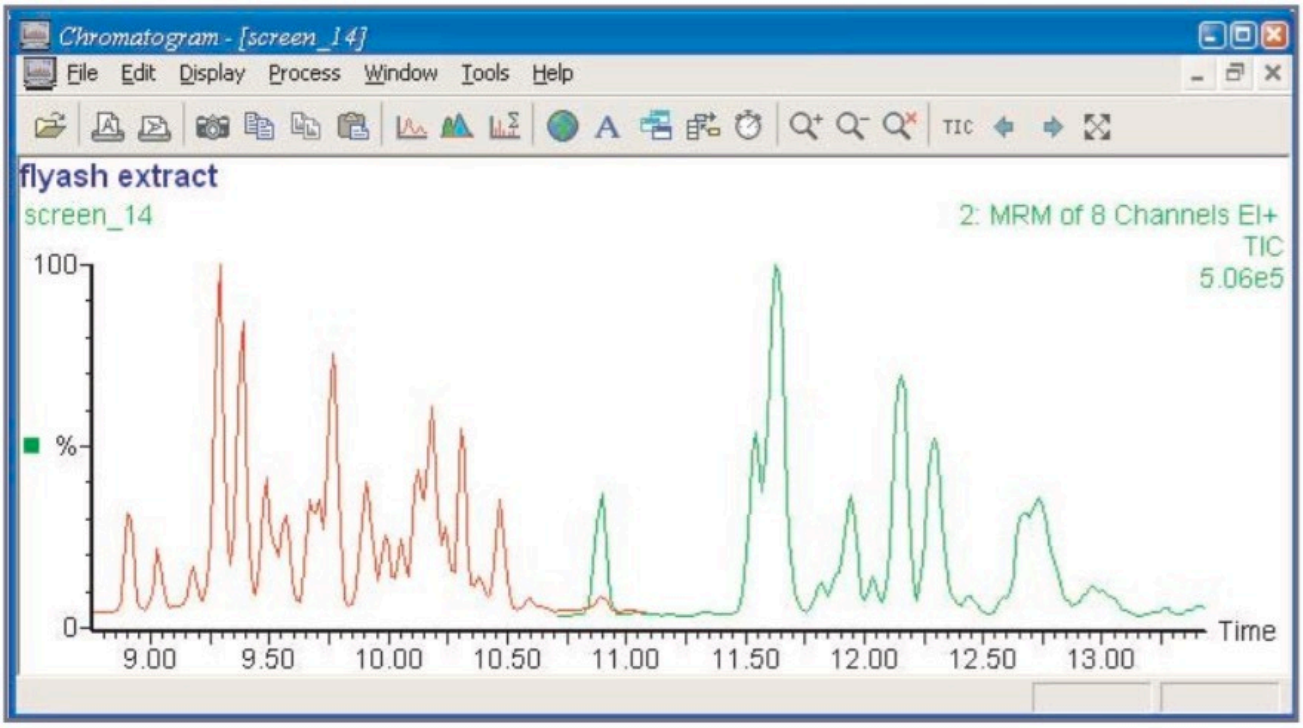

Figure 4. Expansion of the retention time range showing clearly the acquisition of 1,2,8,9-TCDF in function 1, after the acquisition of 1,3,4,6,8- PeCDF in function 2.

For processing of the daily acquisitions, the calibration curve was generated, with a CS3 injection after the samples being processed against the calibration curve to generate a % response deviation. The deviations of the calculated responses for all congeners are below 8%, demonstrating excellent stability of the entire system during a batch run. Table 2 presents the calibration curve results for a typical EPA1613 five point curve, and the CS 3% response deviation for the CS3 standard injected after the sample extracts had acquired. Figure 5 presents the 2,3,7,8-TCDD calibration curve taken from the above five point curve.

Native Congener	RRF Mean	RRF %RSD	CS3 % deviation	LOD (ng /kg) WHO-TEQ
2378 -TCDF	0.91	5.66	-0.1	0.008250
12378 - PeCDF	0.93	1.93	-3.1	0.005500
23478 - PeCDF	0.97	2.49	-3.6	0.053000
123478 - HxCDF	1.13	2.57	0.2	0.009900
123678 - HxCDF	1.10	3.09	-3.5	0.010100
234678 - HxCDF	1.10	4.72	-1.9	0.009700
123789 - HxCDF	1.10	2.26	-2.4	0.010200
1234678 - HpCDF	1.30	3.11	-0.8	0.000806
1234789 - HpCDF	1.23	2.53	-0.6	0.000860
OCDF	1.64	3.26	7.4	0.000005
2378 -TCDD	0.81	2.17	-0.9	0.107000
12378 - PeCDD	1.00	3.13	-1.1	0.137000
123478 - HxCDD	1.02	5.60	-1.3	0.013800
123678 - HxCDD	1.01	5.04	0.1	0.013700
123789 - HxCDD	0.98	5.30	-1.4	0.014300
1234678 - HpCDD	1.10	4.84	-1.8	0.001480
OCDD	1.01	3.91	5	0.000014
			Total WHO -TEQ(ng/kg)	0.40

Table 2. Typical five point calibration curve statistics. The CS3 % deviation column refers to the comparison of a CS3 injection, after the samples had acquired, against the calibration curve. The calculated LODs are based upon a 10 g Carp extract.

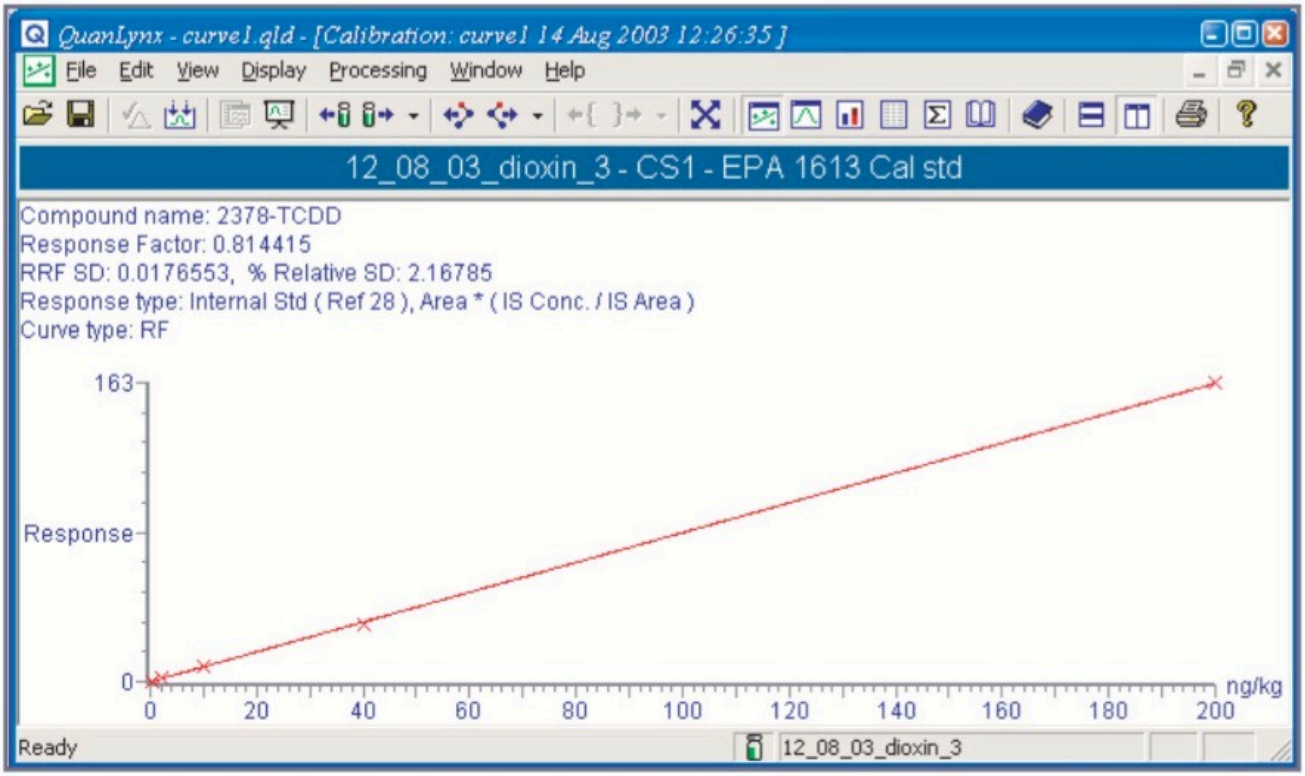

Figure 5. Example calibration curve for 2,3,7,8-TCDD.

The calculated concentrations for 2,3,7,8-TCDF in the carp and flyash samples were compared with both the high resolution confirmatory runs, and the certified concentrations (ng/kg). The carp has a certified concentration of 18.2 ng/kg, and the flyash has a certified concentration of 900 ng/kg. Confirmatory analysis on an AutoSpec Ultima NT verified these concentrations in the extracts processed. Table 3 presents the results obtained from the screening method for this target congener from each of the five batch analyses. Figures 6 and 7 present the chromatograms for 2,3,7,8-TCDF in flyash (Figure 6) and carp (Figure 7).

Run No	TCDF conc. (Carp) ng/kg	δ ng/kg
1	17.1	-1.1
2	17.5	-0.7
3	18.2	0
4	17.4	-0.8
5	18.2	0
%rsd	2.81	
Run No	TCDF conc. (ash) ng/kg	δ ng/kg
1	914	14
2	858	-42
3	876	-24
4	958	58
5	954	54
%rsd	4.93	

Table 3. Calculated concentrations for 2,3,7,8-TCDF in the carp and flyash extracts, for a single injection of each matrix in five repeat analyses. The deviation from the certified value, and the %RSD of the calculated concentrations are also displayed.

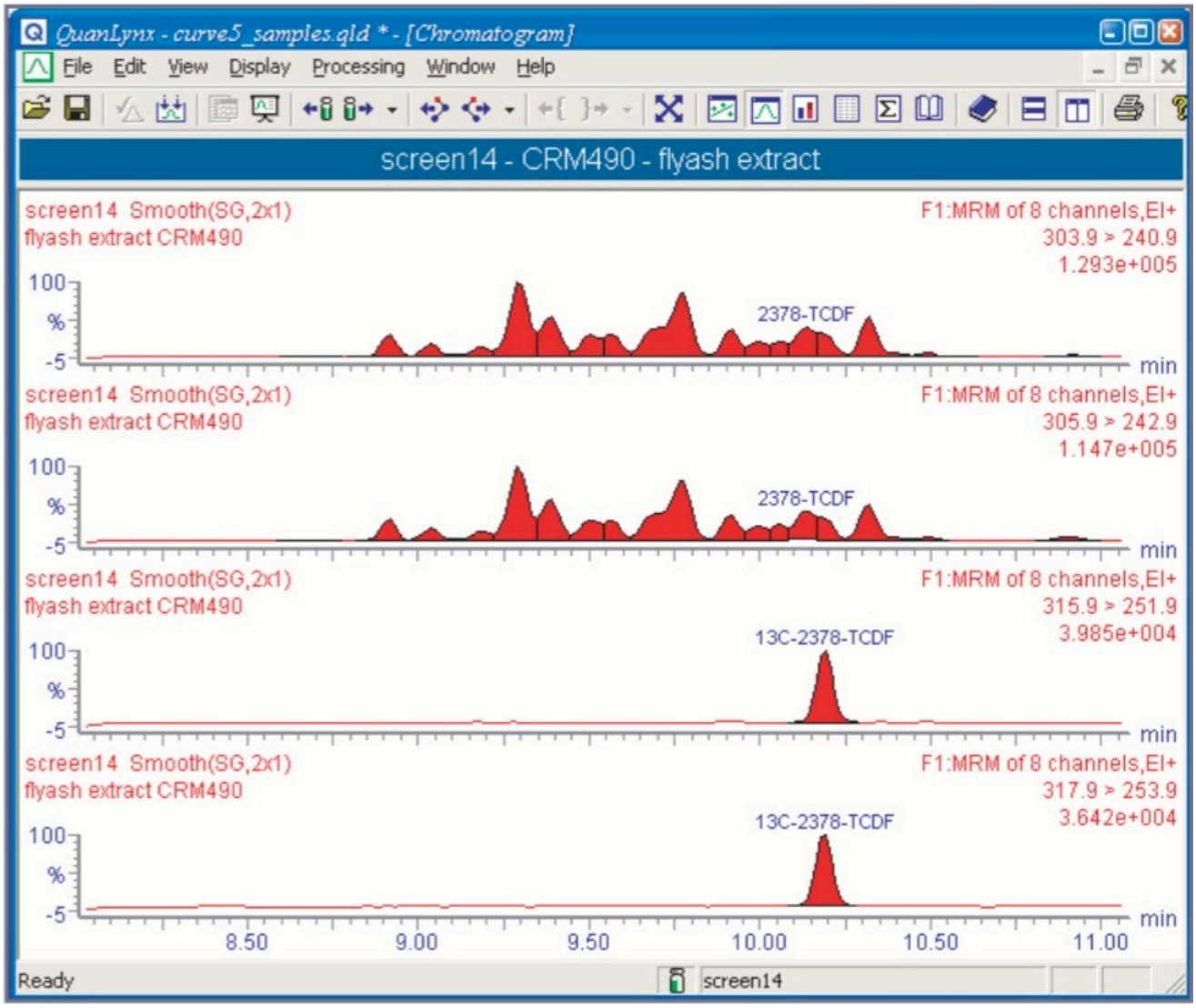

Figure 6. 2,3,7,8-TCDF chromatogram, flyash extract. 1,2,8,9-TCDF is visible to the high retention time portion of the chromatogram.

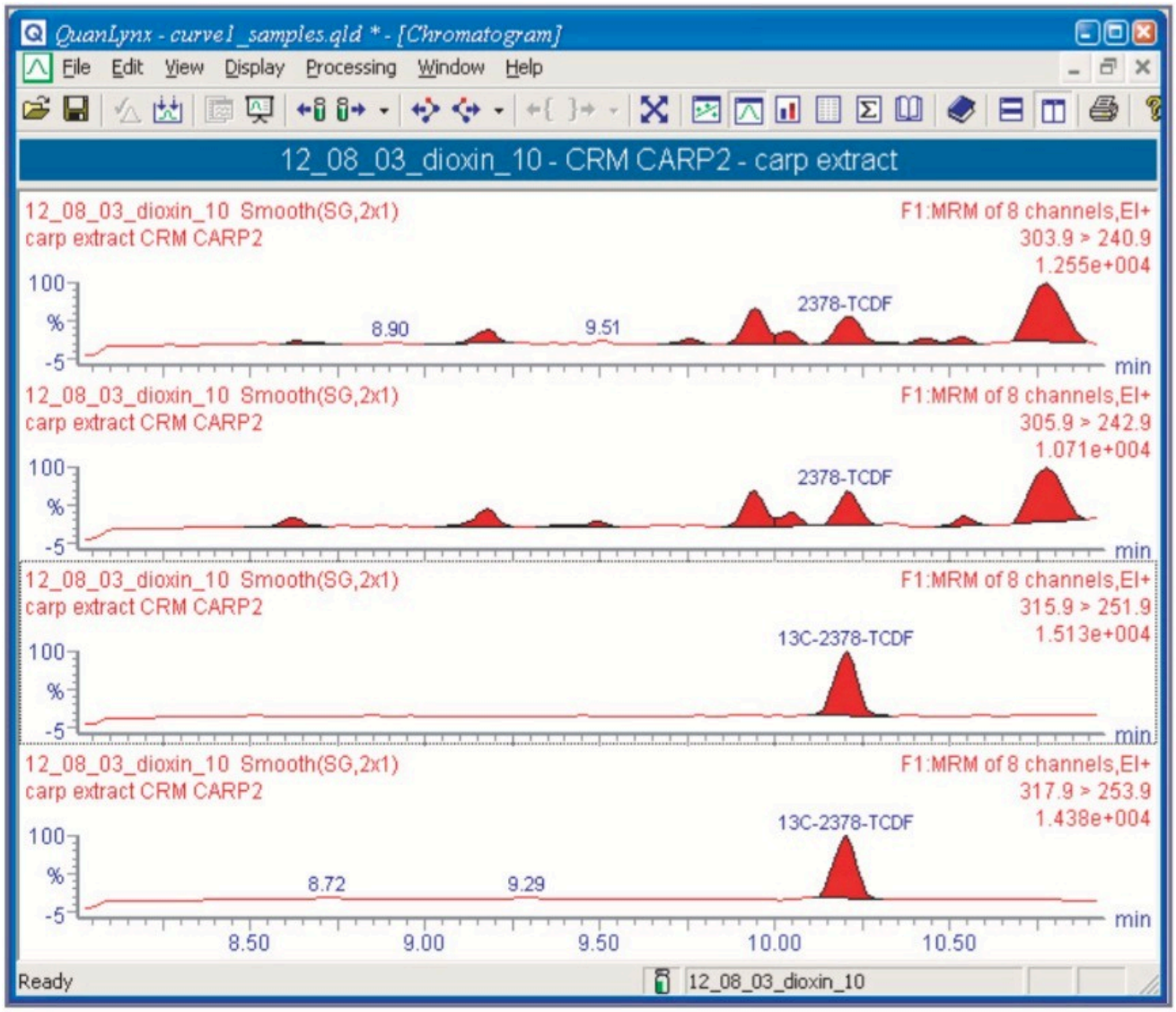

Figure 7. 2,3,7,8-TCDF chromatogram, carp extract.

Conclusion

GC Triple Quadrupole Mass Spectrometry offers a relatively cheap and rapid screening method for dioxins and furans. The Stability of response and ion ratios, imperative for certainty of results confirms that the method is

robust and reliable, and can offer the analyst a useful tool in the laboratory. The limit of detection offers the ability to determine relatively low concentrations of the target analytes in difficult matrices; coupled with a total run time of 21 min, giving the analyst a greater degree of confidence in the results obtained with greatly reduced analysis time.

References

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