

GC-APCI-MS/MS Analysis of Polychlorinated Dibenzo-p-dioxins and Furans to Revised US EPA1613 Guidelines

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INTRODUCTION

In 2024, the US EPA approved and promulgated an update to the EPA1613^{1,2,3} guidance on the analysis of polychlorinated dibenzo-p-dioxins and furans (PCDD/Fs) in wastewater, to incorporate Alternative Test Procedures (ATP) making use of tandem quadrupole mass spectrometers (MS/MS), as an alternative to magnetic sector High Resolution Mass Spectrometers (HRMS). One of the incorporated ATPs also made use of an Atmospheric Pressure Chemical Ionization (APCI) source as an alternative to Electron Ionization (EI). This study presents data acquired using these modifications and highlights techniques to adapt the Quality Control (QC) measures of the EPA1613 method to the new alternative system.

There are several QC measures of gas chromatographic separation performance, including retention time stability and chromatographic peak resolution, which are unaffected by the choice of MS/MS instead of HRMS, and these are met by the system used in this study.

However, there are a number of MS performance metrics which require an alternative approach when using the MS/MS platform, specifically:

- Ionization suppression monitoring
- Mass accuracy monitoring
- Mass resolution monitoring
- Ion/isotope ratios accuracy
- Signal to noise evaluation

METHODS

Analysis was performed on a Waters™ Xevo™ TQ-XS mass spectrometer using an Atmospheric Pressure Gas Chromatography (APGC™) source. Samples were introduced using a CTC PAL 3 RSI autosampler, coupled to an Agilent 8890 GC Oven using a Gerstel CIS-4 PTV injector. Chromatographic separations were performed using a Restek Rtx®-Dioxin2 40m x 0.18mm ID x 0.18µm film column. Standards were sourced from Wellington Laboratories, using the EPA1613 CSL to CS5 calibration set, which were diluted tenfold in order to bring them into the working range of the mass spectrometer. The CSL sample was further diluted to a hundredth of its original concentration to assess the limit of detection of the system. All injections were of 4µL volume.

IONIZATION SUPPRESSION MONITORING

A vial of Perfluorotributylamine (PFTBA) was placed in the source enclosure, with a controlled leak to provide a suitable level of a known reference compound in the background spectra throughout the analysis. The MS experiment was modified to include several additional acquisition functions (Figure 1). A Multiple Reaction Monitoring (MRM) function was used to monitor the ionization of PFTBA throughout the separation, to check for suppression events.

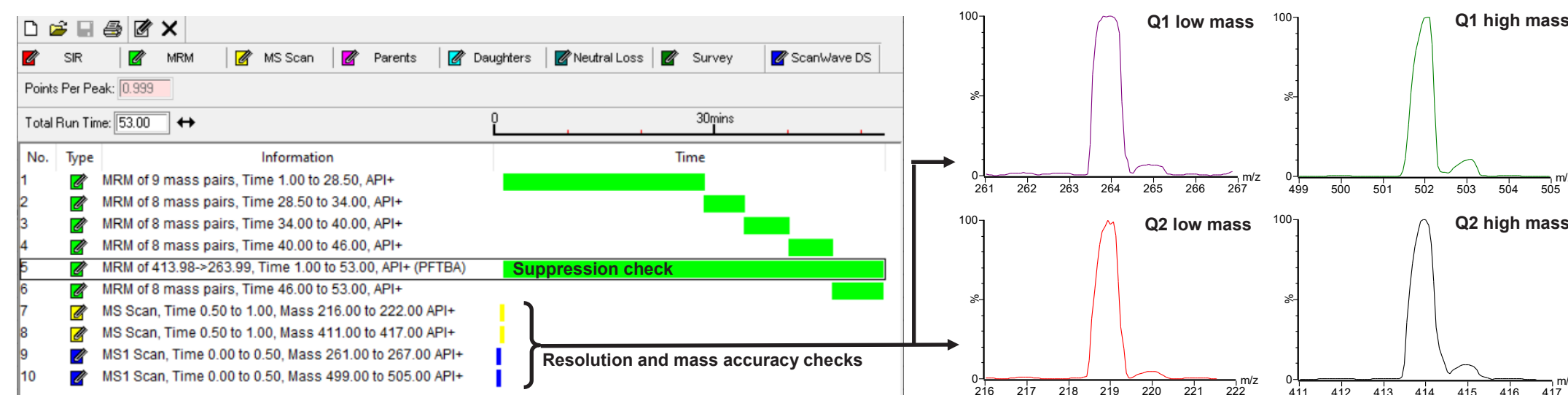


Figure 1. MS/MS experiment details with additional functions

MASS ACCURACY AND RESOLUTION MONITORING

Four additional scanning functions were added to the experiment, all running within the first minute of the separation, before the solvent front elutes. These functions profile the PFTBA masses which bracket the mass ranges used for both quadrupoles. The data gathered are embedded into the acquisition file, and therefore serve as an auditable record of the instrument resolution and mass accuracy. A Python script was created which is able to extract and process these data, and can be run automatically within the MassLynx™ sample list.

ION / ISOTOPE RATIO ACCURACY

In traditional HRMS analysis of dioxins and furans, the isotopic ratios of these polychlorinated compounds are well known and are a crucial quality control check. A ratio error tolerance of ±15% is applied to all peak detections, typically based on the two most abundant isotope of each species. In the MS/MS analysis there is an added consideration when forming expectations for isotopic ratios; the probability of the loss of the chlorine isotope selected as the neutral fragment of the MRM. The most abundant loss observed for PCDD/Fs is the loss of COCl. As the most intense parent ions contain a greater abundance of ³⁵Cl atoms, the targeted loss is more specifically the loss of ^{CO³⁵Cl}. To calculate the theoretical isotopic ratios, the relative proportion of ³⁵Cl and ³⁷Cl atoms in

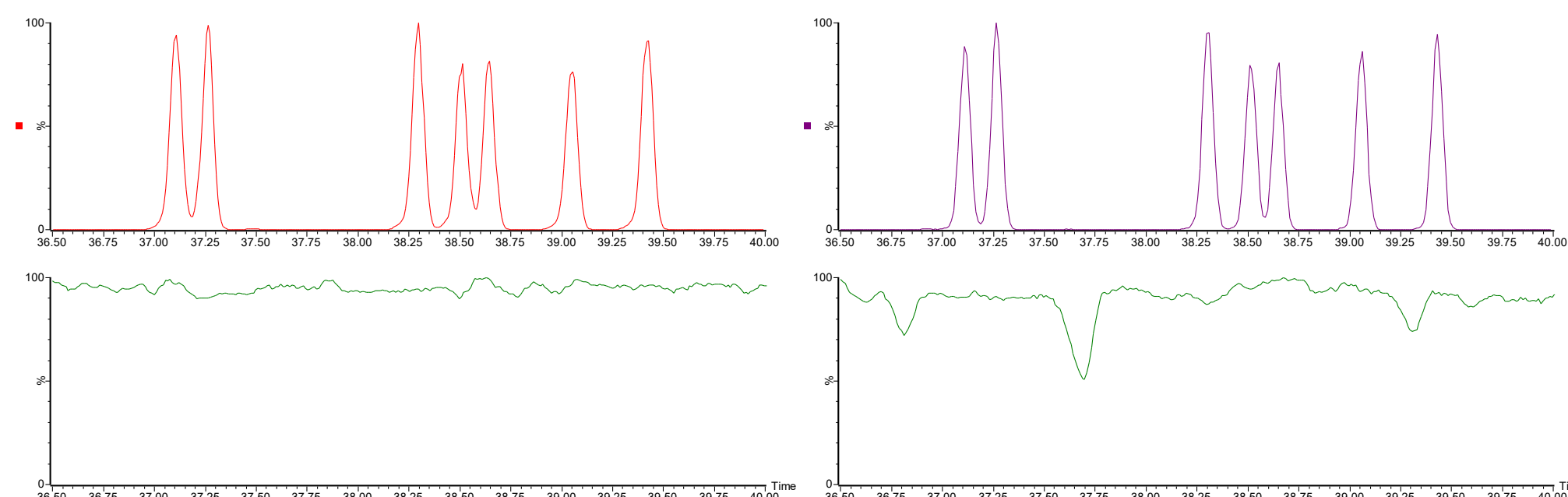


Figure 3. Monitoring for ionization suppressing events during the acquisition. Traces on the left are the Hexa-dioxin and Furans peaks (top) and the suppression check (lower) for a solvent standard. Traces on the right are for a sample with a heavy matrix load, showing ionization suppression events due to the elution of high concentration matrix components

the parent ion needs to be factored in. The revised ratio expectation are shown in Table 1, along with the upper and lower values which represent a ±15% error.

SIGNAL TO NOISE EVALUATION

The calculation of detection limits for the analysis of PCDD/Fs, as laid out in the EPA1613 regulations, are performed through assessment of the signal to noise (S/N) of analyte peak detections for the lowest concentration calibration standard. During MS/MS analysis, a frequent issue with applying this technique is the absence or extremely low levels of background noise in the MRM traces, leading to unrealistically low Limits of Quantification (LoQ). EU legislation⁴ mitigates this effect by setting the LoQ as being equal to the lowest concentration standard that conforms to the other QCs metrics. This can be problematic and can result in the over-reporting of toxicity for the sample, when compared to HRMS.

The LoQ of MS/MS systems are commonly specified by establishing the Instrument Detection Limit (IDL), an evaluation of the statistical reproducibility of a low concentration calibration sample.

The system used in this study was adjusted to reduce the degree of detector thresholding applied, to provide a

Acquired Name: 250415 PCDDF 08
Acquired: 15-Apr-2025 : 21:18:04
Processed: 15-Apr-2025 : 22:20:41
Function 9: MS AI+
Target Mass 263.99
Centroid Found Mass: 263.92
Mass Error Da: -0.07
PASS: Threshold, Da (+/-): 0.15
Width at 50% = 0.66, PASS: Threshold = 0.75
Width at 10% = 0.84, PASS: Threshold = 0.99

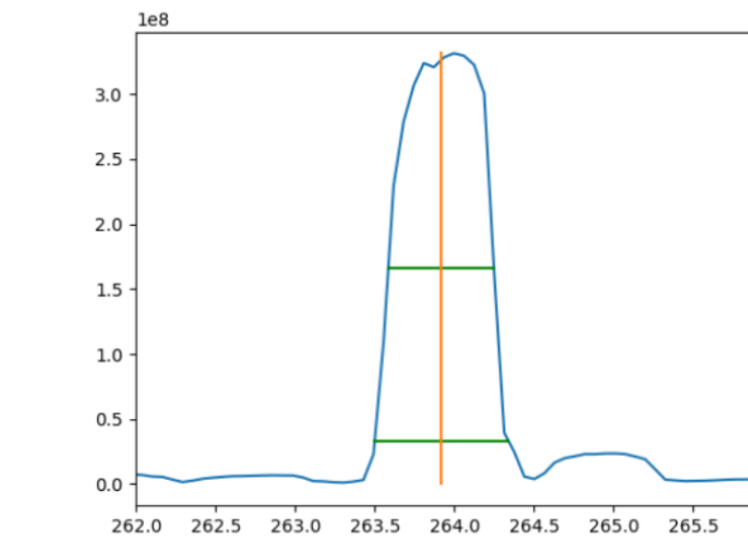


Figure 2. Example of a Python script printout from the automated resolution and mass accuracy checking process

persistent baseline of noise, akin to that observed in traditional HRMS analysis. 10 injections of a hundredfold diluted CSL standard were made, and a comparison of the detection limit was made using both the IDL and the S/N based approach.

RESULTS

A triplicate injection of CSL to CS5 standards yielded a standard deviation in the Relative Response Factors (RRFs) of 1.61%, from a total of 594 RRF calculations in the sample set. The highest single deviation was +8.8%, comfortably within the ±20% limit permitted by the EPA regulations. Data for the individual congeners analysed are shown in Table 2.

Chlorine Number	Quan & Qual	Ratio (qual/ quan)	Lower Limit (-15%)	Upper Limit (+15%)
4	M & (M+2)	0.96	0.82	1.10
5	(M+2) & M	0.78	0.66	0.90
6	(M+2) & (M+4)	0.64	0.54	0.74
7	(M+2) & (M+4)	0.80	0.68	0.92
8	(M+2) & (M+4)	0.96	0.82	1.10

Table 1: Most abundant MRM transitions, their theoretical ratios and tolerances

The average magnitude of the isotope error for all 612 peaks analysed (included Internal and Recovery Standards) in this study was ±1.16%. The total averaged error for the same detections was 0.02%, demonstrating no systemic bias in the mass spectrometer and verifying that the theoretical ratios given in Table 1 are correct. All measurements were comfortably within the ±15% limit permitted by the regulation.

The automated measurement of the mass accuracy and quadrupole resolutions by the custom Python program produced individual pdf report files for each data file acquired, an example of which is shown in Figure 2. These files are labelled with either "pass" or "fail" to draw the operator's attention to any problems encountered. For the duration of the study, all data files passed the criteria specified.

An example of the source ionization suppression checking function is shown in Figure 3. Here a solvent standard is compared with an extracted sample with known heavy matrix load. The negative deflections of the signal coincide with the elution of large matrix components, and mirror the behaviour that was noted when this sample was run on a high resolution magnetic sector MS instrument. This diagnostic check can be used to highlight sample issues and prompt remedial actions (such as further clean up), as required by the regulations.

A comparison of the two approaches for the measurement of signal to noise is given in Table 2. The two techniques gave similar results on a per congener basis, demonstrating that with a suitable adjustment of the MS detector parameters, the traditional HRMS technique for evaluation of the Limit of Detection can be used in MS/MS analysis.

DISCUSSION

This study shows that it is possible to migrate the well-established quality control and instrument performance checks from the EPA1613 regulations from a high resolution magnetic sector mass spectrometer to an APCI source tandem quadrupole system.

Isotope ratio expectations require adaption to account for the neutral losses in MS/MS analysis but are as precise and as accurate as for HRMS.

The APCI source can provide stable ionization, resulting in reproducible RRFs for all analytes across a broad concentration range. The stability also permits monitoring for ionization suppression during the analysis, highlighting problematic samples.

The modifications to the detector set-up, to provide a persistent positive level of noise throughout the analysis, allows traditional S/N assessment techniques to be used. This prevents the need to run multiple injections of a low level standard in order to calculate the IDL using the statistical reproducibility method, which can be time consuming but remains the only approach in the absence of noise.

CONCLUSION

- The workflows of the APCI-MS/MS system were successfully adapted to incorporate the quality control measures for HRMS systems that are required for the analysis of PCDD/Fs following the EPA 1613 regulations
- The system demonstrated precise and accurate Relative Response Factors and Isotopic Ratios, adhering to the regulatory limits
- Mass accuracy and resolution were automatically measured and recorded
- Ionization-suppressing chromatographic matrix peaks can be detected
- S/N measurements allow Limits of Detection to be determined using traditional HRMS methods

	RRF RSD (%)	Average Isotope Ratio Error (%)	Instrument Detection Limit (fg on-column)	LoD from Signal:Noise Measurement (fg on-column)
2,3,7,8 TCDF	1.1	1.9	1.1	1.4
1,2,3,7,8 PeCDF	1.7	0.9	2.8	1.6
2,3,4,7,8 PeCDF	1.2	1.0	2.4	1.5
1,2,3,4,7,8 HxCDF	1.2	1.4	3.0	2.4
1,2,3,6,7,8 HxCDF	1.2	1.2	3.4	2.3
2,3,4,6,7,8 HxCDF	1.5	1.6	3.4	2.3
1,2,3,7,8,9 HxCDF	3.2	1.1	3.4	2.4
1,2,3,4,6,7,8 HpCDF	0.9	1.1	4.6	2.7
1,2,3,4,7,8,9 HpCDF	1.0	1.3	2.4	3.3
OCDF	3.1	1.4	4.4	6.1
2,3,7,8 TCDD	2.5	2.4	1.6	1.8
1,2,3,7,8 PeCDD	1.2	1.0	3.6	2.0
1,2,3,4,7,8 HxCDD	1.2	1.5	4.0	3.8
1,2,3,6,7,8 HxCDD	2.7	1.4	2.0	3.4
1,2,3,7,8,9 HxCDD	2.0	1.3	2.7	3.7
1,2,3,4,6,7,8 HpCDD	2.6	1.1	4.9	4.4
OCDD	0.9	0.7	6.2	6.7
Average :	1.7	1.3	3.3	3.1

Table 2. Individual congener performance measures for the triplicate CSL to CS5 calibration line, along with limits of detection assessment from the 100 x diluted CSL standard

References

1. EPA Method 1613 (Revision B), U.S. Environmental Protection Agency (USEPA), Washington, DC, Sept. 1994.
2. Methods for Measurement of 2,3,7,8-substituted tetra through octa-chlorinated dibenzo-p-dioxins and dibenzofurans in Wastewater | US EPA (<https://www.epa.gov/owow/methods/methods-measurement-2378-substituted-tetra-through-octa-chlorinated-dibenzo-p-dioxins>)
3. SGS AXYS Method 16130 Rev 1.0 (<https://www.sgsaxys.com/wp-content/uploads/2022/09/SGS-AXYS-Method-16130-Rev-1.0.pdf>)
4. Commission Regulation (EU) 589/2014, laying down methods of sampling and analysis for the control of levels of dioxins, dioxin-like PCBs and non-dioxin-like PCBs in certain foodstuffs.