

A Comparison of Relative Response Factors for Polychlorinated Dibenzo-p-dioxins and Dibenzofurans Using Triple Quadrupole and High Resolution Mass Spectrometry

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Abstract

Stability of the Finnigan MAT TSQ70 triple quadrupole GC/MS/MS instrument is demonstrated for the analysis of polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) using isotope dilution quantitation techniques. Performance was assessed against a VG Autospec high resolution mass spectrometer by statistical evaluations of relative response factors (RRFs) for native and $^{13}\text{C}_{12}$ -labelled analogues. It is shown here that after multiple tunes and thus differing parameter settings between tunes, PCDDs and PCDFs maintain relatively constant RRFs on the TSQ70 indicating good instrument reproducibility and stability. Results concur with the evidence for instrument independent RRFs.

Introduction

The conventional method for PCDD/PCDF analyses in complex matrices such as sediments, effluents and biological tissue is capillary column gas chromatography combined with high resolution mass spectrometry (GC/HRMS). However, the benefits of tandem mass spectrometry for difficult analyses is evident from the enhanced selectivity available¹⁾. In addition, achievable sensitivity by tandem mass spectrometry has been shown to fulfil detection limit requirements set out for GC/HRMS by the EPA for method 1613²⁾. Yost and Enke³⁾ agree that with the triple quadrupole system, the selected ion fragmentation process provides enhanced selectivity and discrimination over normal mass spectrometry without significant loss of sensitivity.

Reiner *et al*⁴⁾ theorized that with specific tuning conditions (eg using 2,3,7,8-substituted congeners) instrument independent RRFs could be obtained. In the present work, we examine the relative response factors (RRFs) obtained on a triple quadrupole mass spectrometer and on a high resolution mass spectrometer of EBE configuration. The suitability of the GC/MS/MS system for the trace analysis of PCDD and PCDF using isotope dilution techniques is discussed.

Experimental

Standard PCDD/PCDF mixtures were prepared from stock solutions obtained from either Cambridge Isotope Laboratories, Inc. or Wellington Laboratories. A 2 μL injection volume

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(versus 1 μ L injections for HRMS) was used for all standards on the GC/MS/MS system in order to obtain comparable sensitivity. Sample sizes were also approximately double those required for GC/HRMS (eg 2L for water, 20g for biota and 10g for sediment samples).

Standards and sample extracts were analyzed by GC/MS/MS (Varian 3400 GC, Finnigan MAT TSQ70 triple quadrupole mass spectrometer, and an ICIS II data system) or GC/HRMS (Hewlett-Packard 5890 Series II GC, VG Autospec at 10,000 resolution, and an OPUS data system). All samples were chromatographed using 60m x 0.25 mm i.d. x 0.25 μ m film thickness J&W DB-5 capillary columns.

The quantitation standard (CS3) was run approximately every 9th injection and verified against the most recent 5-point calibration curve. Quantitation was based on the ratio of the sums of the peak areas for the two ions monitored for each of the native and labelled ions. The ratios for the molecular ion clusters for each congener were used to provide confidence limits during instrument analysis. Achieving these confidence intervals after each tune was a prerequisite for the establishment of acceptable tune conditions.

Confirmation criteria for positive identification of PCDDs and PCDFs via MS/MS included: measurements of relative abundances of the two monitored transition product ions; isotopic ratios \pm 25% of the theoretical values for all monitored ions; and the comparison of analyte GC retention times with those of their ¹³C₁₂-labelled analogues (\pm 2 sec.).

The VG Autospec HRMS instrument was tuned to meet a minimum 10,000 resolution at a 10% valley definition. The tuning procedures and the associated quality assurance / quality control parameters have been described in detailed elsewhere⁵.

Selectivity using MS/MS was achieved with the use of two stages of quadrupole mass selection, which are separated by a pressurized (rf-only) quadrupole collision cell (q2). In the first quadrupole(q1), ions of the target analyte or parent ions, are mass filtered and subsequently subjected to collisions with Argon gas in the q2 collision chamber producing progeny ions characteristic of the parent ions. The strong focussing nature of the rf-only quadrupole stabilizes the ion after collision and fragmentation⁶. These progeny ions were selected by mass to charge ratio(m/z) in q3 and were detected finally by an electron multiplier. The q1 and q2 quadrupoles were tuned to achieve unit mass resolution over a mass range m/z 60 - 500 amu.

Sensitivity is a direct result of optimal tuning to enhance the signal strength of the progeny ions. A prerequisite to obtaining maximum sensitivity is the use of PCDDs on the solid probe when optimizing instrument parameters. With this technique the instrument is tuned to meet a minimum required detection limit of 5:1 signal:noise, on 1 pg of 2,3,7,8-T4CDD and 10 pg of O8CDD. Tune parameters were optimized with specific attention given to collision gas pressure, collision energy, energy of q3 relative to q2 (collision offset), collision rf potential and electron energy. Optimization of most parameters occurs in an iterative manner since many parameters affect each other.

The daughter ion MS/MS spectrum of a mass selected compound is determined by the internal energy of the parent ion⁷. The RRFs of various isomers are dependant on the crucial parameters which affect the internal energies of the ions, such as collision energy. All analyses were performed at a constant collision pressure as determined at the time of the instrument

tune. Efficient fragmentation was found to occur at collision gas pressures of $2.6 - 3.0 \times 10^{-5}$ mTorr. This pressure was measured with a manifold ion gauge and maintained by the lack of differential pumping in the q2 region.

Once optimization was complete and acceptable sensitivity was obtained, tuning was not generally necessary for approximately 3 to 6 months, depending on the longevity of the source conditions according to sample load and cleanliness. The duration of the tune was extended with the use of exchangeable ion volumes. i.e. used/dirty ion volumes were replaced with clean ion volumes whenever necessary without changing the source conditions and therefore reducing the frequency of tunes.

RESULTS & DISCUSSION

The percent relative standard deviations (%RSD) of the RRFs for the 2,3,7,8-substituted PCDD/PCDF isomers are presented in columns 1 to 5 of Table 1. Columns 1 through 4 tabulate the %RSDs over four consecutive tunes, while column 5 represents the cumulative %RSDs for the duration of 1 year. The calibration of the TSQ70 has produced very consistent RRFs during and between tunes. The stability of the instrument was demonstrated over time, despite having changed many variables (eg replacement of ion volumes or GC columns; venting the instrument on numerous occasions; source removal and cleaning; and removal and cleaning of the quadrupole rods). The RRFs are statistically within acceptance limits as defined by EPA method 1613 for between run comparisons and (%RSD) calculations.

Averages of the PCDD/PCDF toxic isomer RRFs for the two instruments are summarized in columns 6 and 7 of Table 1. We observed excellent agreement for the majority of isomers. The $^{13}\text{C}_{12}$ -2,3,7,8-T4CDD, $^{13}\text{C}_{12}$ -1,2,3,7,8-P5CDD, and $^{13}\text{C}_{12}$ -1,2,3,4,7,8,9-H7CDF RRFs were significantly different. For the native compounds, 1,2,3,4,7,8-H6CDF, 1,2,3,4,6,7,8-H7CDF and O8CDF had the greatest discrepancies.

An analysis of relative response factors for GCMS experiments has been published previously⁸⁾ and this model has also been applied to the isomers of T4CDF⁹⁾. Briefly, the model considers the following five major variables:

- I, the rate of total ion production from each substance within the ion source for a fixed flow rate of sample supplied to the source;
- F, the fraction of total ionization within the source corresponding to the ions at m/z values of interest;
- E, the efficiencies with which the indicated ions are extracted from the source and focussed into the analyzer;
- T, the efficiency with which an ion is transmitted through the analyzer to the detector; and
- G, the current gains of the electron multiplier detector for different ions.

Variables E, T, and G may be assumed to be completely dominated by purely physical effects dependent on m/z only, such as ion optics and mass discrimination. It is known that parent ion transmission is poorer in quadrupole systems. However since quadrupoles are not dependant upon ion velocity for the mass filtering action, the kinetic energy release does not degrade the daughter ion resolution as it does in HRMS instruments with the BE geometry¹⁰⁾

TABLE 1 - Percent Relative Standard Deviations of Continuing Calibrations Between Tunes for GC/MS/MS and Average RRFs for GC/MS/MS vs. GC/HRMS

	%RSDs for MS/MS Continuing Calibrations Between Tunes					Average RRF's (Consecutive CS3s)	
	05/93 to 06/93 (n=19)	06/93 to 11/93 (n=51)	11/93 to 03/94 (n=44)	03/94 to present (n=30)	05/93 to present ^a (n=144)	MS/MS (n=35)	HRMS (n=35)
	¹³ C ₁₂ -2,3,7,8-T4CDD	2.8	2.9	6.4	4.0	6.7	1.38
¹³ C ₁₂ -1,2,3,7,8-P5CDD	5.8	8.5	12	10	12	1.21	0.75
¹³ C ₁₂ -1,2,3,4,7,8-H6CDD	3.7	4.5	4.5	5.5	4.9	0.89	0.89
¹³ C ₁₂ -1,2,3,6,7,8-H6CDD	3.5	4.1	5.4	5.0	4.9	1.03	1.03
¹³ C ₁₂ -1,2,3,7,8,9-H6CDD	1	1	1	1	1	1.00	1.00
¹³ C ₁₂ -1,2,3,4,6,7,8-H7CDD	23	11	8.8	5.8	13	0.82	0.81
¹³ C ₁₂ -1,2,3,4,6,7,8,9-O8CDD	20	22	16	13	20	0.58	0.48
¹³ C ₁₂ -2,3,7,8-T4CDF	17	6.7	19	5.4	18	1.52	1.63
¹³ C ₁₂ -1,2,3,7,8-P5CDF	15	9.1	22	9.1	21	1.26	1.21
¹³ C ₁₂ -2,3,4,7,8-P5CDF	15	10	22	11	22	1.36	1.30
¹³ C ₁₂ -1,2,3,4,7,8-H6CDF	11	7.2	14	5.7	16	0.96	1.16
¹³ C ₁₂ -1,2,3,6,7,8-H6CDF	18	6.8	14	8.5	18	0.99	1.23
¹³ C ₁₂ -2,3,4,6,7,8-H6CDF	12	5.3	14	5.1	15	0.95	1.16
¹³ C ₁₂ -1,2,3,7,8,9-H6CDF	18	6.6	12	8.5	15	0.85	1.19
¹³ C ₁₂ -1,2,3,4,6,7,8-H7CDF	28	12	13	5.5	19	0.80	0.98
¹³ C ₁₂ -1,2,3,4,7,8,9-H7CDF	36	15	15	8.5	20	0.76	1.35
2,3,7,8-Substituted Isomers							
2,3,7,8-T4CDD	6.7	8.2	11	3.6	9.6	1.08	0.99
1,2,3,7,8-P5CDD	4.4	4.7	8.8	2.9	8.0	1.01	1.03
1,2,3,4,7,8-H6CDD	4.8	11	7.4	5.0	8.6	1.02	1.02
1,2,3,6,7,8-H6CDD	6.1	6.8	14	4.1	10	0.98	0.95
1,2,3,7,8,9-H6CDD	4.1	7.9	7.3	4.2	7.4	0.95	0.93
1,2,3,4,6,7,8-H7CDD	7.1	5.0	9.2	2.6	7.6	0.99	0.96
1,2,3,4,6,7,8,9-O8CDD	1.7	4.5	11	3.1	8.0	1.32	1.31
2,3,7,8-T4CDF	4.0	6.6	8.4	3.8	8.5	1.06	0.97
1,2,3,7,8-P5CDF	3.4	8.2	9.3	4.9	9.7	1.09	1.05
2,3,4,7,8-P5CDF	4.0	5.1	11.6	2.8	9.4	1.06	1.03
1,2,3,4,7,8-H6CDF	5.1	6.2	8.5	3.2	7.7	1.04	1.37
1,2,3,6,7,8-H6CDF	5.9	13	7.9	4.6	11	1.11	1.26
2,3,4,6,7,8-H6CDF	7.5	7.2	7.9	4.1	8.7	1.05	0.97
1,2,3,7,8,9-H6CDF	6.7	5.7	7.3	4.2	7.7	1.10	1.19
1,2,3,4,7,8,9-H7CDF	6.1	4.4	9.2	2.3	7.2	1.09	1.42
1,2,3,4,7,8,9-H7CDF	5.9	6.1	9.0	2.3	7.6	1.05	1.35
1,2,3,4,6,7,8,9-O8CDF	7.6	5.5	11	4.5	12	1.22	1.56

^a represents cumulative average for first four columns.

The remaining variables, I and F, are concerned solely with the processes occurring within the ion source. These factors might be expected to show significant variations when comparing an HRMS ion source operating at 8 kV to a quadrupole source operating at a potential in the region of 10 V. Indeed, discrepancies in ion abundances in magnetic sector versus quadrupole instruments are well known for reference calibration compounds^{11,12}. It has also been shown that quadrupole spectrometers may be operated so as to produce fragmentation patterns similar to those observed on other types of instruments¹³.

Given the complexity of the processes which are involved, the sets of RRFs on the two instrument shows a surprisingly high level of correlation. With regards to the quantitation of real samples on the two instruments, the single most important issue is whether or not instrumental differences in RRF values have any bearing on analytical results. The importance of accuracy is obvious for the detection and subsequent quantitation of PCDD/PCDFs when one takes into consideration the health concerns and legal ramifications associated with the presence of these contaminants in our environment. We regularly inject sample extracts on both instruments and have found the analytical data to exhibit excellent agreement when using isotope dilution techniques. We have also evaluated the performance of the two instruments during round robin performance evaluation tests¹⁴.

In general, quadrupole detection limits are often dependent on the level of interferences rather than on instrumental limitations. The constant bleed of perfluorokerosene through the septum inlet of the HRMS is not required with the GC/MS/MS system, thereby eliminating any noise problems that may result from this constant flux of reference compound. Likewise the GC/MS/MS ion source is exposed to a considerably less amount of "contamination" effects and thus remains clean for longer periods of time.

The HRMS instrument requires daily tuning, or at the very least verification of resolution and transmission. The GC/MS/MS tuning procedure requires 2-3 days of a qualified operators time for optimum results and tuning parameters may remain valid for 3 - 6 months. The manufacturer is presently working on an autotune procedure that may reduce the tuning time to less than a day. The quadrupole instrument also shows more stability with respect to ambient conditions as compared to the GC/HRMS. The heated manifold on the triple quadrupole MS helps to provide a more controlled system which is less susceptible to ambient conditions. When similar GC conditions are used, the difference in analysis time between GC/HRMS and GC/MS/MS is nominal. The TSQ utilizes double the volume of standard solutions with 2 μ L injections. However, old standard aliquots which are unusable quantitatively can be used for tuning purposes. Most importantly, interferences seen on HRMS are not seen in multiple reaction monitoring techniques (with the exception of diphenyl ethers).

Conclusions

This work demonstrates the capability of tandem mass spectrometry in the performance of accurate quantitative trace analysis of PCDDs and PCDFs using isotope dilution techniques. The overall performance of the MS/MS system depends on many instrumental parameters. With proper attention given to the optimization of these parameters, control monitoring and essential maintenance, instrument independent RRFs can be obtained which are very reproducible. The GC/MS/MS system has the ability to achieve the required detection limits of EPA method 1613 with potentially less interferences as compared to HRMS methods.

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