

An Evaluation of MS/MS for Quantitative Analysis of Polychlorinated Dibenzo-*p*-dioxins and Dibenzofurans.

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Introduction. The value of mass spectrometry/mass spectrometry (MS/MS) to enhance selectivity in the analysis of environmental samples has previously been demonstrated, most notably for the analysis of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDDs and PCDFs). The use of MS/MS as a routine tool has been slow to develop, however, due to the cost of the instrumentation and the view that quantitative data cannot be obtained by using MS/MS¹. This study was undertaken to address the latter concern. Our approach was to develop and validate a method for the analysis of PCDDs and PCDFs by investigating the linearity and reproducibility of the response of the analyte to the internal standard. In addition, we compared the results of quantitative analyses of environmental samples by using high-resolution mass spectrometry and mass spectrometry/mass spectrometry. These data were then used to suggest criteria (including acceptable isotope ratio limits, variation in relative response factors) for the identification and quantitation of PCDDs and PCDFs.

Experimental. Mass spectrometry/mass spectrometry and high-resolution mass spectrometry (HRMS) experiments were performed on a VG70SEQ (EBqQ) hybrid mass spectrometer. For HRMS, the instrument was operated in the selected ion recording mode with resolving power of 10,000 (10% valley definition). A high sensitivity electron ionization source as supplied by the manufacturer was used throughout the study (source temperature of 275 C, electron energy of 34 eV, and filament emission current of 1 mA). For MS/MS, the instrument was operated in the selected reaction monitoring mode at a resolution of 500. A collision energy of 20 eV and an argon pressure of 1.8×10^{-2} mbar (true pressure) were used because previous experiments demonstrated that these conditions were optimum for the production of (M-COCl)⁺ product ions². Parent ions of native and isotopically labelled PCDDs and PCDFs were transmitted into the collision region from MS1. Selected reaction monitoring of the (M-COCl)⁺ daughter ions formed during the collision induced dissociations were completed in MS2. The quadrupole mass spectrometer was set at unit mass resolution. In both HRMS and MS/MS, PCDD

and PCDF ions were monitored in five windows. Standard solutions of native and isotopically labelled polychlorinated dibenzo-*p*-dioxins and dibenzofurans were prepared by making dilutions of standard materials (2,3,7,8-TCDD, $^{13}\text{C}_{12}$ -2,3,7,8-TCDD, 2,3,7,8-TCDF, $^{13}\text{C}_{12}$ -2,3,7,8-TCDF, 1,2,3,7,8-PeCDD, $^{13}\text{C}_{12}$ -1,2,3,7,8-PeCDD, 1,2,3,7,8-PeCDF, 2,3,4,7,8-PeCDF, 1,2,3,4,7,8-HxCDD, 1,2,3,6,7,8-HxCDD, $^{13}\text{C}_{12}$ -1,2,3,6,7,8-HxCDD, 1,2,3,7,8,9-HxCDD, 1,2,3,4,7,8-HxCDF, 1,2,3,6,7,8-HxCDF, 1,2,3,7,8,9-HxCDF, 2,3,4,6,7,8-HxCDF, 1,2,3,4,6,7,8-HpCDF, $^{13}\text{C}_{12}$ -1,2,3,4,6,7,8-HpCDF, 1,2,3,4,7,8,9-HpCDF, OCDD, $^{13}\text{C}_{12}$ -OCDD and OCDF) obtained from Cambridge Isotopes Laboratory and Northrop Environmental Services. The final concentrations of these solutions contained 2.5, 10, 50, 200 and 1000 pg/uL of the native PCDDs and PCDFs and 100 pg/uL of the isotopically labelled materials (except $^{13}\text{C}_{12}$ -OCDD which was at a concentration of 200 pg/uL). Environmental samples were obtained and spiked with labeled standards. The samples were then Soxhlet extracted in toluene for 16 hours and solvent exchanged to hexane. The PCDDs and PCDFs were isolated by using column cleanup procedures based on the use of silica gel, alumina and AX-21/Celite 545 carbon columns.

Five calibration curves were constructed by measuring the response of the analyte to the internal standard. These analyses were completed over a period of 8, 24 and 92 hours and 3 months after the analysis of the standards used to construct the first calibration curve. The stability of the instrument to perform quantitative measurements and the linearity of the response of the analyte to the internal standard was determined by calculating the mean, standard and percent relative standard deviation. No re-tuning or calibration of the instrument was performed from the time of the first analyses and those analyses performed 92 hours later.

Results and Discussion. The data were analyzed by two ways. First the per cent relative standard deviation (%RSD) among the relative response factors and the correlation coefficient (r^2) from linear regression analysis of the data were obtained to determine the linearity of the response of the analyte to the internal standard for each day. Secondly, the overall %RSD and r^2 values among the calibration curves were calculated to determine the reproducibility of the response of the analyte to the internal standard. The average %RSD among the relative response factors (RRFs) was less than 15% and r^2 values of 0.90 or higher for the calibration curves constructed from data obtained in the first three curves. The results obtained from the analyses performed after the instrument was allowed to remain idle for 60 hours indicate that instrument conditions had changed as a %RSD for the RRFs of the OCDD and OCDF congeners increased from 9% to 41%. Regression analyses of the collective data (response factors for all 5 curves) yielded r^2 values from 0.80 to 0.98 for all of the 17 congeners and the mean %RSD for the relative response factors was 27%. This value decreased to 19% and the r^2 value range increased to 0.86 to 0.98 after excluding the data obtained after 92 hours, thereby again demonstrating instrument instability after this period. The measured isotope ratio of

the two ions that were monitored for each analyte (native and labeled) were also compared with the theoretical values. The mean of the percent difference between the measured and theoretical values ranged from 5% to 27%. The highest mean percent difference was observed for OCDF. These results compare favorably with accepted isotope ratio variability limits for HRMS (± 15 %RSD).

The results of analyses of a Hudson River sediment extract by HRMS and MS/MS demonstrate that both methods yield similar results when interferences are not present. For two Hudson river sample extracts, we obtained a total mean PeCDF concentration by using HRMS of 1.8 ng/g (dry weight). Analysis of the same extracts by MS/MS yielded an mean concentration of 1.9 ng/g (dry weight). The most interesting result was that MS/MS allowed quantitation of the 1,2,3,7,8 PeCDD isomer whereas this isomer could not be identified or quantified by using HRMS. In this case, we obtained a mean concentration of 8.6 ug/kg (dry weight) for the 1,2,3,7,8-PeCDD isomer and a total mean PeCDD concentration of 77ug/kg (dry weight) by using MS/MS. The total mean PeCDD concentration by using HRMS was 18ug/pg (dry weight). A value for the 1,2,3,7,8 PeCDD isomer could not be obtained from the HRMS data.

Summary and Conclusions. We have established that MS/MS can yield linear and reproducible response factors of the analyte to the internal standard for standard solutions containing PCDDs and PCDFs over a 24 hour period. These results are different from those of other researchers who reported a concentration dependence of the analyte to the internal standard¹. We have also established that when instrument instability occurs, it is easily regained by re-tuning the instrument. From these data, we suggest that when performing MS/MS analyses, the operator re-tune the instrument every 24 hours and that a %RSD of $\pm 20\%$ between the relative response factors and a 20% difference between measured and theoretical isotope ratios be accepted.

References Cited

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