COMPARISON OF 2,3,7,8-DIBENZO-P-DIOXIN CHLORINE ISOTOPE RATIO MEASUREMENTS USING ELECTRON MULTIPLIER AND PHOTOMULTIPLIER DETECTORS

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ABSTRACT

We routinely monitor the isotope ratio, m/z 320/322 (M^+/M^{2+}), of 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) as one measure to verify optimum operation of the high resolution mass spectrometry system before and during analysis of trace amounts of 2,3,7,8-TCDD in human serum samples. This is one among several tests that are performed daily to verify operation prior to analyzing unknowns. Among these tests, assuming the instrument has been tuned (1), are a check of sensitivity, chromatography resolution, analyzer resolution, calibration over the selected mass range, and isotope ratio of the m/z 320/322 of the M^+/M^{2+} ion fragment of native 2,3,7,8-TCDD and m/z 332/334 for the C_{13} labeled 2,3,7,8-TCDD internal standard. The attentive mass spectroscopist can readily identify problem areas in need of adjustment before proceeding with the tasks at hand. Although many parameters are considered in establishing the optimum operating profile, we will limit our discussion to the precision and accuracy of measuring the ratio of tetrachlorinated dioxins for simplicity of the model.

KEYWORDS

Electron multiplier, photomultiplier, detectors, chlorine isotope ratio, dioxins, mass spectrometry

INTRODUCTION

The ratio m/z 320/322 of the native (unknown) sample has been estimated to be 0.78 plus or minus 0.1 (2) which has survived several years trial. If this is not the case, often it is assumed that the magnet is not properly positioned or some parameter is improperly adjusted thus causing aberration in mass measurement. But, on the other hand, the mass measurement of ion fragments can be in error even though the instrument controls are apparently properly tuned to yield maximum signal to the detector. This was further compounded in the last year or two, since the photomultiplier was introduced as a detector system on some of the new high resolution mass spectrometers and ambiguous assumptions surfaced indicating that a potential problem had arisen with the

photomultiplier giving somewhat different isotope ratios. We studied this phenomenon while analyzing serum samples using both the electron multiplier and the photomultiplier detectors.

EXPERIMENTAL

The quantitative method of analyzing 2,3,7,8-TCDD in serum on a whole-weight and lipid basis has been previously reported in detail. (3,4,5) Sample extraction and handling has been described in detail. (6) Four magnetic sector high resolution mass spectrometers, ZAB-2F, 70S, 70SE, and the front end of the 70SE-4F, (VG Instruments, Inc., Danvers MA), were employed in this study. All of the mass spectrometry systems had identical VG 11/250J data systems and were integrated into the division data handling network. The instrumental method of analysis has been described in detail (7) along with the quality control and quality assurance program utilized in our laboratory. (6,8).

Mass Spectrometer Detuning Experiments

Detuning the VG 70SE mass spectrometer was studied to determine the effect on sensitivity, isotopic ratios, mass (analyzer) resolution, and the accuracy of reported dioxin concentrations. The detuning was accomplished by altering the voltage on the y focus lens, the ion repeller, the ion energy, or the radial position of the magnet. The mass spectrometer was tuned to obtain optimum sensitivity for the m/z 293 peak of perfluorokerosene (PFK) at a resolution of 10,000. A 75 pg tetra-dioxin standard was injected into the mass spectrometer as a baseline measurement. One parameter of interest was altered to yield a 15-40 % reduction in signal of the m/z 293 PFK peak (observed on the oscilloscope). Then the same standard was analyzed to determine the effects of the altered parameter. This sequence was repeated for each parameter including Y focus, ion energy, repeller voltage, magnet position, Y deflect, and the alpha stop. Each time the parameter previously changed was again optimized prior to offsetting the next choice.

Peak Integration Experiments

Integration parameters (e.g. peak width and threshold in the analyte quantitation file) were varied to determine their effects on the analytical determinations.
The software controlled integration parameters were changed via the methods page and the samples were reprocessed. All instrument analyzer parameters were optimized prior to acquiring data for this part of the experiment. This procedure allowed the assessment of these software parameters without additional injections to avoid instrument variability.

RESULTS AND DISCUSSION:

Detuning the mass spectrometer failed to produce the suspected profound effect on the isotopic ratios. However, among these parameters, the alpha stop was the only adjustment that affected the ratios. On the other hand, detuning varied the calculated concentration of dioxin as much as 20 % in some cases (see Table I). The integration parameters, on the other hand, produced a larger deviation of the isotopic ratios than any of the mass spectrometer analyzer

TABLE I. Effects of Alternated Instrument Parameters

Parameter	% Decrease	Sound/Noise	Ratio 1	Ratio 2	Concentration
Control	0	443	81.9	73.0	75.0
Y-Focus					
Left	35	278	81.9	74.0	77.4
Right	35	384	82.4	72.9	68.3
Ion Energy					
Left	15	350	83.0	70.0	67.2
Right	15	294	82.2	74.6	74.4
Repeller					
Left	15	500	84.3	74.3	83.8
Right	15	278	80.7	72.3	69.0
Magnet					
In	20	100	81.5	71.9	78.4
In	40	100	83.1	75.1	78.6
Out	40	143	82.1	74.8	78.8
Correct.					
In	40	133	85.6	73.9	71.2
Out	40	200	82.6	71.7	70.9
Y-Deflect					
Left	40	80	86.3	75.7	85.0
Right	40	120	85.0	73.9	82.1
Alpha Stop					
Left	40	80	85.3	77.8	88.3
Right	40	70	89.5	71.3	90.0

parameters. The peak integration parameters had a large impact on calculated concentrations shown in **Table II**. The peak width and threshold settings in the analyte file of the quantitative software determine the smoothing applied to the peaks used for quantitation which may explain some of the differences observed.

Table II: Effects of Altered Quantitative Parameters

Peak Width	Threshold	Ratio 1	Ratio 2	Concentration
15	-0.5	80.6	73.9	78.6
21	-0.5	85.6	85.2	96.1
69	-0.5	86.2	86.2	95.9
9	-0.5	87.4	76.6	90.9
15	-0.0	86.7	75.3	87.9
15	-0.2	85.9	74.3	89.2
15	-1.0	86.1	76.4	89.8

The analysis of >3000 serum samples for dioxins in our laboratory using both the electron multipliers and photomultipliers does not support this implication of altered isotope ratios by either detector system. Deviations in isotope ratio measurements arise from low signal input resulting from low analyte concentration, or some interfering compound simultaneously eluting from the chromatographic column.

ACKNOWLEDGEMENTS

We wish to thank Ms. Connie Woodall for her able assistance in preparing graphic illustrations. We acknowledge the suggestions of the VG Engineers and visiting scientists regarding these observations.

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