TANDEM MASS SPECTROMETRY SENSITVITY SPECIFICATION FOR 2,3,7,8-TCDD IN A SATURN 2000 QUADRUPOLE ION TRAP

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Introduction

Mass spectrometry techniques are useful in dioxin methods for food only to the extent that they provide a sensitive response to various polychlorodibenzo-p-dioxins/furans (PCDD/Fs) congeners when introduced through high-resolution capillary chromatographic columns. Manufacturers of instruments intended for this use routinely supply a customer with a guarantee that the particular mass spectrometer will respond to a given amount 2,3,7,8-TCDD with a specified signal to noise ratio (SNR). In the case of high-resolution sector mass spectrometers a specific mass resolution is also guaranteed. A typical specification supplied with a Micromass autospec ultima high-resolution mass spectrometer (HRMS) is 100 fg with 100/1 SNR at m/z 321.8937 with a mass resolution of 10,000. A 30 M x 0.25 mm ID is used for the isomer separation while the HRMS monitors a restricted number of ions (4 and a lock mass).

Tandem mass spectrometry techniques have been developed with both high and low resolution mass spectrometers and provide useful data on PCDD/Fs in foods[1-3]. Tandem mass spectrometry instrumental sensitivities have been reported for hybrid high resolution and triple quadrupole techniques[4,5]. Minimum detectable amounts were generally 0.5 to 1 pg. Tandem mass spectrometry in a quadrupole ion trap has been the subject of intensive investigation since 1994[6]. Instrumental minimum detection levels (MDLs) have been reported[6-10] in the 200-250 fg range for TCDD. These MDLs have been demonstrated with fortified and naturally contaminated foods[1,2]. Recently, somewhat lower instrumental MDLs were reported using external ionization mode available with a Thermo/Finnigan GCQ using higher helium pressures. These conditions were tested through analysis of a BCR 607 milk powder[11]. One recent report suggested that the same sensitivity for 2,3,7,8-TCDD would be obtained using MS^3 in a quadrupole ion trap[12].

The SNR for 2,3,7,8-TCDD in a Saturn 2000 was optimized through an examination of 26 parameters. Gas chromatographic and mass acquisition conditions were adjusted to produce the highest SNR possible for a Saturn 2000 instrument using internal ionization and helium as the buffer gas. The goal is to produce a precisely defined benchmark for the Saturn 2000 instrument enabling easy comparisons to other mass spectrometers measuring dioxins and operating under “ideal” conditions.

Methods

A recent model Saturn 2000 ion trap mass spectrometer from Varian Corp. equipped with CI and tandem mass spectrometry options, a CP 3800 gas chromatograph (GC) and a CP 8400 auto-sampler was employed. The Varian CP 3800 GC was equipped with a 1079 multipurpose injector. The injector was operated in the septum programmable on column injection mode and capped with a Merlin Microseal septumless injector cap. The capillary column was a 40 meter 0.18 mm with a 0.18 mm film of DB-5 ms stationary phase. TCDD was injected with the column set at 140 ^{o}C and then GC was ramped at 20^{o}/min. to 260 ^{o}C and then ramped at 1^{o}/min. TCDD standards were purchased from Cambridge isotope as mixtures with other dioxin and furan congeners. All stock and dilute solutions

were in nonane. Stock solutions of 2,3,7,8-TCDD were diluted to provide 200 fg/mL concentrations of 2,3,7,8-TCDD. Precursor ions were stored using two scan functions at m/z 321 and 334. Two mL aliquots (400 fg) were introduced to GC/MS. Adjusting selected parameters from a predetermined set of default starting conditions maximized product ion response resulting from 2,3,7,8-TCDD ions. After optimizing, an average response obtained from 5 repeat injections was used to evaluate MDLs at m/z 257, m/z 259 and m/z 257+259. The average noise was estimated by averaging the peak to peak noise observed 0.5 min. before and after the elution of 2,3,7,8-TCDD.

Table I. Dioxin and PCB adjustable parameters for a Saturn 2000: 27 parameters divided into four parameter groupings: parameter = setting during sensitivity specification (adjustment range).

A. Ion Formation:
1) Ionization time = 65,000 msec (10-65,000)
2) Emission current = 100 mamps (10-100)
3) AGC target = 5000 (10-65,000)
4) AGC prescan type = parent (product or parent)
5) AGC prescan time = 1500 msec (10-2,000)
6) Ionization LMCO = 48 m/z (35-160)
7) Ejection amplitude = 20 V (0-60 V)
8) Helium bath gas pressure (ion guage readings from 20 torr to 100 torr)
9) Trap temperature = 220ºC (80-250)

B. Ion isolation:
1) Isolation time 5 ms (1-10)
2) Broadband amplitude 30 V (0-60)
3) Mass window = 3 m/z (1-12)
4) High edge offset = 2 (1-20)
5) Low edge offset = 4 (1-20)

C. Collision induced dissociation:
1) CID LMCO equivalent to a q = 0.4 (35-300 m/z)
2) Excitation voltage = 1.7 volts (1-60 V)
3) Excitation time = 10 (1-1000)
4) Modulation range = 0 DACs (1-12)
5) Modulation rate = 3000 msec/step (DAC step) (29-5600)
6) Number of frequencies (waveforms) = 5 (1-121)
7) Frequency offset = 0 (0-1000 Hz)

D) Mass scanning:
1) Chlorine isotopic peak selection = M+ and M+2 for TCDD, M+ and M+2 for $^{13}C_{12}$-TCDD
2) Analytical scan rate = 0.27 sec (0.27 –5.00)
3) Number of scan functions/segment = 2; one for native and one for the $^{13}C_{12}$-TCDD (1-10)
4) Electron multiplier offset = +300 volts (0-300)
5) Product ion scan range = 25 m/z (0-650)
6) Background mass = 150 m/z (35-300)
7) Single or double MS/MS scan mode = MS^2 (MS^2 or MS^3)

Results and Discussion

Tandem MS experiments in the quadrupole ion trap proceed sequentially through four sections each done with differing RF potentials and ramps. They are ion formation, ion isolation, collision-induced

dissociation (CID with helium) and mass scanning. Several parameters can be adjusted in each section. The final settings used in these experiments are given in Table 1. Ten parameters were critical for high TCDD sensitivity in the Saturn 2000 optimized for the detection of m/z 257 and m/z 259. They are precursor ion selection, mass window, high/low edge offset, ionization time, multiplier offset, filament emission current, excitation amplitude, CID RF amplitude, helium pressure and CID bandwidth. Optimal CID RF amplitudes resulting in a trapping parameter (q_z) between 0.3-0.5 have been reported previously for TCDD[8]. Trap temperature was set initially at 220 °C, however no increase in response was observed at 200 °C as opposed to 220 °C, a temperature that more effectively reduces tailing of higher chlorinated congeners[9]. Reducing the multiplier offset from 300 V to 150 V or reducing the maximum ion time under automatic gain control from 65 ms to 35 ms produced similar two-fold reductions of the SNRs for TCDD ions. An emission current of 50 mamps instead of 100 had a lesser effect on SNR. Therefore, emission current values of at least 50 mamps could be used to extend filament lifetimes as has been purposed[8,9] with only minimal loss in SNR.

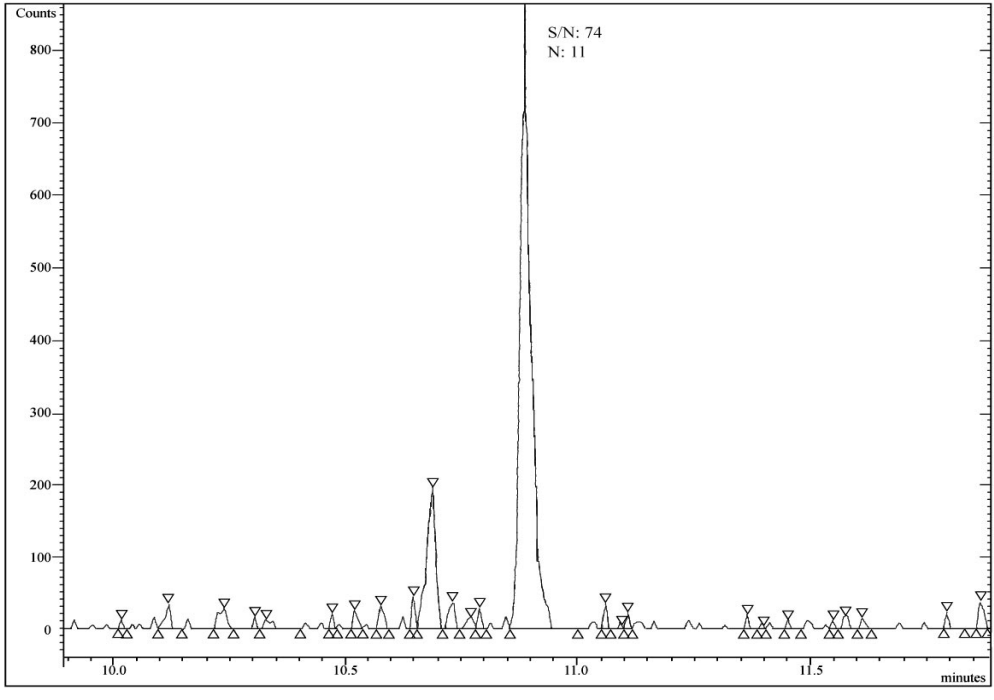

Figure 1. An example of the summed product ion chromatograms for m/z 257 $(M\text{-}COCl)^+$ and m/z 259 $(M+2\text{-}COCl)^+$ 2,3,7,8-TCDD; 400 fg with a SNR = 61.

Scan rate and GC temperature ramping work inversely. Fast GC ramps produced narrower peaks as expected that required faster scanning to obtain sufficient data points to prevent distortion of the ion chromatographic peak shape. Micro-scan rates less than 200 ms are not obtainable using the Saturn 2000 regardless of the maximum ionization time setting, CID excitation times or mass scan range. A "micro-scan" rate below 270 ms was not possible under conditions used (Table 1) even with short CID

excitation times (<10 ms). Computer hardware and software limitations are partly responsible for the slower scan rates. An older Saturn 4D is able to scan at a 100 ms rate per micro-scan (1 data point) while using the maximum AGC ionization time for the older software (25 ms), a short excitation time (10 ms) and a small scan range (e.g. m/z 250-290). This commercially available Saturn 4D is unable to scan at a 50 ms micro-scan rate (20 micro-scans/sec) under any conditions of mass scan range, ionization time or CID duration, as was previously reported[13]. Mass window and high/low edge offsets were adjusted to store M^+ and $(M+2)^+$. There are a few combinations of precursor ion selection, mass window (1-3 m/z) and high/low edge offset that will effectively store the targeted molecule chlorine isotopic ions for TCDD (m/z 320 and 322) for subsequent CID. Application of between 1 and 9 waveforms with sufficient amplitude will completely fragment both stored targeted molecular ions (M^+ and $(M+2)^+$ with the majority of the ion current appearing at m/z 257 and 259. RF modulation was not adjusted at any waveform number, but left at zero.

The average SNR for 400 fg of TCDD at m/z 257+259 was 49, corresponding to an instrumental MDL of approximately 25 fg at 3/1 SNR. An example of a summed product ion chromatogram for m/z 257+259 is presented in Figure 1. The SNR for m/z 257 and m/z 259 were lower at 29/1 and 19/1, respectively. The results generally agree with Eppe[11] using an external ionization system found on a Thermo/Finnigan GCQ, with a somewhat lower MDL. The MDL measured with Saturn 2000 is approximately 7-10 times higher than the specification sold currently with a Micromass Autospec Ultima high-resolution sector instrument. An MDL for either instrument will be somewhat higher under actually analytical conditions with sample matrix effects, slower temperature programming (both) and a longer column (Micromass instrument).

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