

## OPTIMIZATION OF THE QUADRUPOLE ION STORAGE MASS SPECTROMETER FOR THE TANDEM MASS SPECTROMETRIC ANALYSIS OF DIOXINS AND FURANS

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**Introduction.** The commercial quadrupole ion trap, introduced in 1983 by Finnigan Corp., has been transformed from a simple gas chromatography detector to a high performance mass spectrometer.<sup>1</sup> Composed of a central electrode having the form of a hyperboloid of one sheet (or ring electrode) located between two hyperboloids of two sheets (or end-cap electrodes), the ion trap is a mass spectrometer whose essential components can be held in one hand. Advances in the application of the ion trap for environmental analysis<sup>2,3</sup> stem, in part, from the operation of the trap in the mass-selective instability mode, in which an rf voltage applied to the ring electrode controls the storage and ejection of ions.<sup>4</sup> Increasing the rf field causes destabilization of the axial motion of ions through holes in the bottom end-cap electrode where they may impinge on an external electron multiplier. The storage and detection of ions is optimized by operating the ion trap with a relatively high background pressure of helium ( $\approx 1$  mTorr) which functions as both a buffer gas and collision gas. As a buffer gas, helium is responsible for the focussing of ions towards the centre of the trap *via* the viscous damping of ion motion through multiple-low energy collisions. As a collision gas, helium is the target species necessary for the collisionally activated dissociation (CAD) of kinetically excited ions. In most GC/ion trap instruments, helium is the GC carrier gas, thus facilitating the introduction of this vital element into the trap.

Conventional analysis of dioxins and furans by EI/MS/MS employs beam-type instruments consisting of two quadrupole rod assemblies for parent ion isolation and daughter ion scanning (Q1 and Q3, respectively) and an rf only octapole collision cell.<sup>5</sup> Such tandem "in-space" mass spectrometers are plagued with a number of drawbacks. Economically, the instruments are expensive to purchase and maintain, they require a large housing facility, and are generally labour intensive with as much as 20% down time. In contrast, the manipulating of ions by changing various instrumental voltages during the course of an experiment (a "scan function") in an ion trap means the analysis occurs sequentially "in time" rather than space. The result is a benchtop GC/MS/MS system that can represent a 60% cost savings alternative to beam-type instruments for MS/MS.

In addition to economic benefits, two other advantages pertaining to the MS/MS mode of operation exist for ion traps: (1) as all stages of analysis are performed in the same analyzer, losses associated with transporting ions from one analyzer to the next are negated, leading to greater MS/MS efficiency (and, therefore, sensitivity) in the ion trap. In fact, MS/MS efficiencies approaching 100% are achievable with ion traps,<sup>1</sup> whereas in beam instruments it is uncommon to have efficiencies greater than 10%.<sup>6</sup> (2) the number of parameters for controlling the dissociation pathway in an ion trap is greater than for a beam-instrument. In the latter, one controls the acceleration voltage (and thus the translational kinetic energy, TKE, of an ion) and the collision cell pressure. In an ion trap, one must consider  $q_z$  (and thus the trapping potential, *vide infra*), the irradiating frequency, the duration of irradiation, and the pressure of helium. With these

parameters, a high degree of reaction channel specificity can be achieved in ion trap MS/MS.

In a continuing research program concerning the applications and limitations of the GC/quadrupole ion trap mass spectrometer, we have focussed on the development of a GC/EI/MS/MS protocol in which a waveform generator and prototype software may be used to advantage for the ultra-trace detection of dioxins and furans. Previously, one of the software limitations of non-research grade ion traps for analytical applications was the inability to quantitate using internal standards which chromatographically co-elute with their native analytes.<sup>2</sup> Further, the analysis by MS/MS of a number of analytes which differ in parent ion mass was impossible in a single chromatographic run, since only one scan function could be applied per acquisition. These two impediments to quantitation have recently been alleviated; a small number of commercially-available GC/ion trap instruments can now be applied to dioxin and furan analysis using isotopic dilution techniques.

In order to harness the specificity conferred by MS/MS analysis in an ion trap, a four-step process is required involving ionization, parent ion isolation, CAD, and mass analysis. In the current investigation, the ionization time is regulated by an automatic gain control (AGC) algorithm which moderates the population of ions stored in the trap. Thus ionization time varies inversely as a function of concentration. Next, parent ion isolation is achieved by the application of a number of notched multi-frequency waveforms. The process of resonant ejection of undesired matrix ions increases the sensitivity of the trap and mass-resolution.<sup>2,3</sup> Parent ion isolation is followed by CAD, one of the most important capabilities of the ion trap. As this investigation is concerned largely with the CAD episode, and as an understanding of CAD is central to an appreciation of the MS/MS process in the ion trap, a brief introduction to CAD theory is presented.

When a potential is applied to the ring electrode of the ion trap, and the end-caps are earthed, the mathematics of ion trajectory stability obey the Mathieu second-order differential equation.<sup>7</sup> The stable solutions to the Mathieu equation map an ion's trajectory in  $a_z$ - $q_z$  space, which are defined as  $q_z = 8eV/m(r_0^2 + 2z_0^2)\Omega^2$  and  $a_z = 16eU/m(r_0^2 + 2z_0^2)\Omega^2$ ;  $U$  and  $V$  represent the amplitudes of the dc and rf potentials applied to the ring electrode, respectively,  $r_0$  is the radius of the ring electrode (10.00 mm),  $z_0$  is half the separation of the end-cap electrodes (7.83 mm),  $\Omega$  is the angular frequency of the RF drive potential (1.05 MHz),  $e$  is the electronic charge and  $m$  is the mass of the ion. For an ion to be trapped, its trajectory must be stable. A stable trajectory resembles a Lissajous figure which is defined by the radial and axial frequencies of ion motion. The fundamental axial secular frequency of stable ion trajectories is given by  $\omega_{z,0} = \beta_z \Omega/2$ , where  $\beta_z$  is a function of the stability parameters  $a_z$  and  $q_z$ . When the frequency of a waveform (applied across the end-cap electrodes) matches  $\omega_{z,0}$ , the ion gains TKE in the axial direction. The TKE of the excited ion is converted to internal energy *via* multiple low-energy collisions with helium. Simulation studies indicate that the mean ion kinetic energy may be taken as  $\approx 5eV$ .<sup>7</sup> In the centre of mass system this corresponds to, on average, 0.05 eV that may be transferred as the result of a single collision with helium. As a general rule of thumb, ions in the trap suffer about 20,000 collisions per second.<sup>7</sup> CAD episodes lasting 20-30 ms are thus sufficient to access a range of reaction channels of differing activation energy barriers. Of course, the amplitude of the irradiating waveform has a profound effect on the collision energy, as it affects the rate of increase in the amplitude of the ion's trajectory.

We have examined the factors necessary for the optimization of an ion trap for dioxin and furan analysis by GC/EI/MS/MS. The major fragmentation channel observed for these compounds when analyzed by MS/MS with beam-instruments is the loss of COCl.<sup>5</sup> Three modes of resonant excitation have been examined on the ion trap in an attempt to optimize this major dissociative channel; these modes include single-frequency irradiation (SFI), secular-frequency modulation (SFM), and multi-frequency irradiation (MFI). The last two techniques obviate the laborious procedure required for empirically matching an applied single-frequency waveform to the fundamental secular frequency of the ion; further, secular frequency shifts due to space charge effects<sup>7</sup> are less problematic.

## Experimental.

(a) **Instrumental.** All ion trap experiments were performed on a Varian Saturn 4D GC/MS equipped with

a Varian waveform generator, autosampler (model no. 8200), and septum programmable injector with a high-performance insert. Saturn version 5.0 software was used for data acquisitions while prototype software ("Toolkit") was required for the application of multiple scan functions in a given chromatographic run. The ion trap was operated in mode II' (i.e. with the end-cap electrodes grounded) with dipolar resonance ejection at a fixed frequency of 485 kHz to give mass-selective ejection at  $q_z \approx 0.89$ . The trap manifold temperature was held constant at 260°C with helium buffer gas present at  $\approx 1$  mTorr. The sensitivity of the ion trap was optimal with a filament emission current of 100  $\mu$ A and an electron multiplier voltage of 1800 V (the latter tuned to give a  $10^5$  gain). The tunable target ion current for the AGC algorithm was 65,000, thus ensuring the longest possible ionization time for the detection of low concentrations of analyte. Mass calibration (i.e. dacs/amu) was performed using perfluoro-tributylamine (PFTBA). Automated frequency calibrations were derived using  $m/z$  69 and 131 from PFTBA. The latter procedure requires some explanation. The proper calculation of  $\omega_{z,0}$  requires the determination of a "trap factor". This factor is a trap calibration factor that may vary slightly from instrument to instrument or as a function of time. The factor is calculated automatically using a calibration ion (from PFTBA), the theoretical frequency of an applied waveform required to excite that ion, and the empirically determined rf storage dac value required to bring the applied waveform into resonance with the ion.

Gas chromatographic conditions necessary for the optimal separation of all dioxins and furans have been described elsewhere.<sup>2,8</sup> For the current study, a 30 m x 0.25 mm i.d. x 25  $\mu$ m DB-5 column was utilized in order to expedite MS/MS optimization. The transfer line temperature between the gas chromatograph and ion trap was 260°C.

**(b) Parent Ion Isolation.** A template scan function (Figure 1) was used for ionization, parent ion isolation, CAD, and daughter ion mass scanning. 70 eV ionization was performed at an rf storage voltage of 1000  $V_{o-p}$  (low-mass cut-off of 160 amu). Parent ions ( $M^+$ ) from both native and  $^{13}C_{12}$ -labelled dioxins and furans were partially isolated during ionization using two multi-frequency waveforms (WF 1 A and B), each 30  $V_{p-p}$ . These waveforms contain frequency components which correspond to the secular frequencies of a range of ions which bracket  $M^+$ . Those ions whose secular frequencies are not included in the waveforms are stored. All other ions formed during the period of ionization are ejected axially. The parent mass isolation was fine tuned by increasing the amplitude of the rf voltage applied to the ring electrode so as to eject axially ions  $< M^+$  at  $\beta_z \approx 1.0$  ( $q_z \approx 0.89$ ). A diminution of the parent ion  $q_z$  value followed by the application of a broadband multi-frequency waveform (WF 2, 5 ms, 20  $V_{p-p}$ ) results in the ejection of any remaining ions of mass  $> M^+$ .

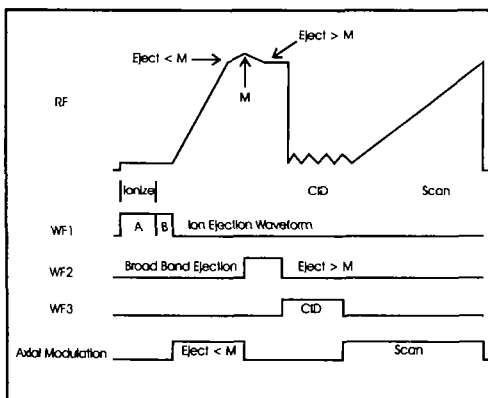


Figure 1: Template Scan Function for Ionization, Parent Ion Isolation and Dissociation and Daughter Ion Mass Scanning

### (c) Resonant Excitation Studies

**(i) Single-frequency Irradiation.** Parent ions were irradiated for 30 ms at  $q_z \approx 0.4$  with a single-frequency sine waveform applied across the end-cap electrodes in dipolar fashion. In order to match the applied waveform frequency with  $\omega_{z,0}$ , it was necessary to offset the applied frequency from its calibration based value by as much as +400 Hz.

**(ii) Secular-Frequency Modulation.** SFM was implemented by varying the amplitude of the rf voltage applied to the ring electrode. As the rf voltage changes,  $\omega_{z,0}$  changes. The axial translational energy of the parent ion increases when  $\omega_{z,0}$  matches an applied single-frequency waveform. Thus SFM effectively moves

an ion in and out of resonance. To optimize the conversion efficiency associated with the loss of COCl, SFM experiments were carried out under two sets of conditions: (1) the parent ion was modulated over a range of 6  $V_{0,p}$  with an ion dwell time of 5 ms/ $V_{0,p}$ , giving 5 CAD segments lasting 150 ms, and (2) the parent ion was modulated over 4  $V_{0,p}$  with an ion dwell time of 5 ms/ $V_{0,p}$  for a total of 5 CAD segments lasting 100 ms. In both experiments, a sine waveform was applied at  $\omega_{z,0}$  for an ion at  $q_z \approx 0.4$ , around which the ions were modulated.

(iii) **Multi-frequency Irradiation.** MFI involves the construction of a waveform consisting of frequency components, the number of which are specified by the bandwidth (BW). Conversion efficiencies were examined as a function of waveform BW and amplitude. Both 1 kHz (3 frequency components) and 2 kHz (5 frequency components) BW waveforms, centred at  $\omega_{z,0}$  for an ion at  $q_z \approx 0.4$ , were applied in separate experiments for a period of 30 ms.

(iv) **Internal Standard Calibration.** Ion preparation methods (IPMs) were constructed using "toolkit" for each of the 10 congener groups examined ( $T_4$ CDD/F-OCDD/F). Each IPM contained 4 scan functions which were responsible for native and  $^{13}C_{12}$ -labelled parent ion isolation and dissociation using SFI. Only four ions from each congener group were examined: the native and internal standard from both dioxin and furan. The most intense ion from each isotopic cluster was selected as the parent ion. All calibration standards (CS series<sup>9</sup>) contained positional isomers from each congener group that have a 2,3,7,8-chlorinated substitution pattern. Concentrations of dioxin and furan analytes ranged from 1 pg- 4,000 pg, depending on the congener group.<sup>9</sup> All chromatographic profiles were acquired using a scan time of 0.5 s. The combination of scan time and scan function duration specifies the number of microscans per scan, where a microscan is defined as one application of the entire scan function. In order to maintain the accuracy of each chromatographic data point, at least 4 microscans/scan were required.

**Results and Discussion.** Parent ion isolation efficiencies were on the order of 90%. Thus 500 fg of the isolated parent ion of 2,3,7,8- $T_4$ CDD/F could be observed routinely with S/N ratios > 5:1.

Overall, each resonant mode of excitation accessed, to varying degrees, the energy channel corresponding to the loss of COCl. Tables 1 and 2 summarize the distribution of daughter ion current for both the dioxins and furans obtained under optimal SFI conditions. Waveform amplitudes required for optimal conversion efficiency (CE) were higher for the furans compared to the dioxins, suggesting that a larger activation energy barrier exists for the loss of COCl in the former species (CE is defined as the sum of the daughter ion current divided by the abundance of the parent ion prior to dissociation). Moreover, higher activation energy channels corresponding to the loss of COCl<sub>2</sub> and COCl<sub>3</sub> were observed for the furans; this contrasts with the high energy loss of 2(COCl) in the dioxins. The abundance of furan daughter ions formed through higher activation energy processes was greater than that for dioxin. One noteworthy difference between the fragmentation channels of dioxin and furan concerns the lowest energy channel, the loss of Cl. As much as 17% of the dioxin parent ion current (6% in furan) is conserved in the daughter ion resulting from loss of Cl. Nonetheless, CE's for the loss of COCl are comparable between dioxin and furan congeners. Surprisingly, the amplitudes for optimal CE amongst the dioxin congeners remain essentially constant, and yet in the centre-of-mass frame, the energy available for internal excitation should decrease with increasing mass. The expected relationship between mass and decreasing available energy is exemplified for the furan congeners, where increasing amplitudes are required for optimal CE as parent mass increases. All the dissociation channels observed in the ion trap confirm those reported in earlier TSQ studies.<sup>3</sup>

Optimization of SFM required an examination of the the range of modulation and ion dwell time per rf voltage step. For all dioxin and furan congeners, an ion dwell time of 5 ms was required for optimal CE, regardless of the modulation range. Furthermore, 5 CAD scan segments were required for the complete dissociation of isolated parent ions. This latter condition translates into CAD episodes lasting 100 ms for a 4  $V_{0,p}$  modulation range, and 150 ms for a 6  $V_{0,p}$  modulation.

The distribution of daughter ion current in SFM closely paralleled that of SFI when the optimal modulation range was used, but larger waveform amplitudes were required for furan dissociation

compared to dioxin. In contrast to SFI, slightly larger amplitudes were required for COCl loss using SFM, since the irradiating process was not continuous;  $\omega_{z,0}$  constantly changes. This can be rationalized as follows: When the frequency components of the ion are off-resonance with the applied waveform, collisional de-excitation can occur for internally excited ions which would otherwise undergo dissociation. Of course there will be instances when frequency components other than  $\omega_{z,0}$  will be on-resonance. Since the amplitude of the applied waveform influences the collision energy by affecting the rate of increase in the amplitude of an ions trajectory, excitation at a frequency other than  $\omega_{z,0}$  requires a larger waveform amplitude to achieve the same rate of increase in the amplitude of the trajectory. The optimal modulation range required to effect the loss of COCl by SFM was not predictable. A  $\pm 1V_{0,p}$  rf change corresponds to a  $\pm 200$  Hz shift in  $\omega_{z,0}$  at  $q_z \approx 0.4$ . Since empirically determined values of  $\omega_{z,0}$  were never more than +400 Hz from the waveform calculator value, a  $6V_{0,p}$  modulation range is extreme, though required in

TABLE 1: Dioxin Daughter Ion Current Distribution Obtained Under Optimal SFI Conditions.

CONGENER	% [M-COCl]	% [M-Cl]	% [M-2COCl]	Waveform ( $V_{p-p}$ )
T <sub>1</sub> CDD	72	16	4	0.6
P <sub>3</sub> CDD	80	17	7	0.6
H <sub>6</sub> CDD	80	16	6	0.6
H <sub>7</sub> CDD	74	12	6	0.7
O <sub>8</sub> CDD	65	10	2	0.6

many instances for optimal CE. Clearly, a complex relationship exists between energy deposition and the ratio of time that an ion spends on- and off- resonance, and is the subject of further investigation.

The following experimental observations characterize the MFI of dioxins and furans: (a) higher activation energy channels are more highly populated, regardless of the waveform BW; (b) in contrast to SFI and SFM, larger amplitudes are required for optimal CE since each frequency component in the waveform receives only a portion of the specified voltage; (c) larger amplitudes are required to effect COCl loss in the furans; and (d) CE's for COCl loss are on average 5-15% less than observed with optimized SFI and SFM. Although the frequency pattern used to generate the waveform is designed to distribute the excitation power evenly over the bandwidth, the true power spectrum may contain an irregular power distribution that can lead to ion losses and reduced CE. Due to such irregular power distributions, waveform amplitudes which were expected to effect dissociation often failed to do so. Thus the relationship between BW, empirically determined  $\omega_{z,0}$ , and MS/MS efficiency was often blurred.

Having optimized the three modes of resonant excitation, a judicious decision was made as to which technique was most applicable for the analysis of dioxins and furans. The application of SFM was unlikely since the CAD episode was too long (100-150 ms) and thus lowered the number of scan functions which could be applied across a GC-peak, as well as the number of microscans/scan. Parenthetically, a minimum of 4 microscans/scan were required to maintain the accuracy of each chromatographic data point. MFI satisfies the need for short CAD episodes (30 ms), however given the erratic waveform behaviour at certain amplitudes, further characterization of this resonant technique is required before it can be applied to dioxin and furan analysis. Therefore, SFI was chosen as the resonant mode of excitation since (a) CAD episodes were short, (b) CE's were high for the loss of COCl, and (c) the MS/MS process was reliable.

Preliminary results for the quantitation of dioxins and furans using toolkit software and optimal SFI conditions were impressive. The daughter ion extracted chromatograms for 1 pg of native 2,3,7,8-

**TABLE 2: Furan Daughter Ion Current Distribution Obtained Under Optimal SFI Conditions.**

CONGENER	% [M-COCl]	% [M-Cl]	% [M-COCl <sub>2</sub> ]	% [M-COCl <sub>3</sub> ]	Waveform (V <sub>p-p</sub> )
T <sub>4</sub> CDF	89	6	6	4	0.6
P <sub>3</sub> CDF	81	2	13	16	0.7
H <sub>6</sub> CDF	79	5	6	4	0.7
H <sub>7</sub> CDF	78	3	2	1	0.9
O <sub>4</sub> CDF	56	1	0	0	1.0

tetrachlorodibenzofuran and 1 pg of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, with their respective co-eluting <sup>13</sup>C<sub>12</sub>-internal standards exhibited an acceptable appearance, even though 4 scan functions were applied across each GC-peak (at a scan time of 0.5 s). S/N ratios of 15:1 for 1 pg of native analyte on the ion trap compared favourably with those observed on a TSQ 70 for the same calibration standard. Plots of response vs. concentration were linear for many of the congener groups. For example, 2,3,4,7,8-pentachlorofuran analyzed with the ion trap by MS/MS over a concentration range of 2.5 pg-2,000 pg had a correlation co-efficient of 0.99973 with a 17% RSD; analysis with the TSQ 70 also furnished a linear response, with a correlation co-efficient of 0.99975 and an RSD of 11%. Mean relative response factors were close to 1 over the calibration range examined with both instruments.

**Conclusions.** A preliminary yet extensive investigation has been carried out concerning the ability of the ion trap to function acceptably as a tandem mass spectrometer for the complex analysis of dioxins and furans. The sensitivity of the ion trap parallels that of beam-instruments for 1 pg of native analyte, thus illustrating the high MS/MS efficiency of the ion trap. With prototype software, we have illustrated the capability of constructing calibration plots using co-eluting labelled internal standards on an ion trap. Since response is linear over the range of concentrations inspected, space charge effects are not detrimental to resonant excitation by SFI. As this investigation is preliminary, further studies required for a complete method include: (1) increasing the number of daughter ions monitored to include the loss of COCl from both [M]<sup>+</sup> and [M+2]<sup>+</sup> parent ions from each dioxin and furan species; (2) the determination of the stability of irradiation frequencies with respect to trap calibration and sample load, and (3) testing the ability of the ion trap to produce accurate quantitative results in the presence of interfering matrix ions from a variety of sample types.

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