Fragment Pathway Analysis Using Automated Tandem Mass Spectrometry on an Ion-trap Mass Spectrometer

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A "key-sequence" procedure is presented for the automated tandem mass spectrometric analysis of compounds on a Finnigan ion-trap mass spectrometer. This allows fragmentation pathways of a range of masses or even a complete spectrum to be prepared automatically, obviating the tiresome preparation and optimization of individual scan-editor files. The procedure is limited by the speed of the driving computer; an "IBM-AT", for example, permits more than 10 mass units to be scanned per minute. It is calibrated with perfluorotributylamine and methyl stearate is used to demonstrate its results. The Finnigan ion-trap "programming option" is necessary for implementation of the procedure.

The Finnigan ion-trap mass spectrometer (ITMS) (Finnigan, San Jose, USA) differs from most mass spectrometers in that ions are prepared in batches by gating the electron beam "on" for short periods. The ions are then analysed according to a scan function tailored for a particular analysis. One can either select standard scan conditions, such as for a normal electron ionization spectrum or generate a special function using the scan editor. This latter mode is used for analysis by tandem mass spectrometry (MS/MS) where ions are first retained selectively in the trap, then translationally excited, causing collisions with the helium buffer gas, and are finally ejected from the trap, producing a daughter-ion spectrum.

A typical MS/MS scan-editor profile showing these features is presented in Fig. 1 and the associated scan-editor tables are shown in Table 1. Ignoring settling times, the scan in Fig. 1 may be divided into 4 phases: (a) ionization of the sample with the electron beam gated "on", (b) retention of only the ion of interest, (c) collisional excitation of this ion, causing its fragmentation, (d) sequential ejection of the daughter ions to produce a spectrum.

Under normal electron ionization conditions, the RF potential in step (a) is arbitrarily selected to trap masses above m/z 20 (Table 1, step 3). This mass is selected because it provides the optimum ionization and retention of ions which equate to the normal electron ionization spectrum obtained on a magnetic-sector or quadrupole instrument. The only other variable selected here is the length of time the electron beam is gated "on". A sufficient number of ions must be formed to provide good signals but not so many as to cause space charging. This time is ordinarily chosen either from experience or by use of the automatic gain control (AGC) feature and is inversely related to the amount of compound eluting from the probe, gas inlet or gas chromatographic column. Unfortunately, a fixed ionization time must be used in operations with the scan editor. A suitable estimate can be made by multiplying the AGC time (previously determined) by 5 to 10 since only a single mass is ultimately trapped under MS/MS conditions (the AGC time is based on all ions heavier than a cut-off mass, typically m/z 45).

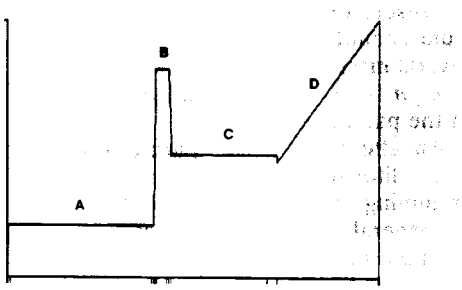

Figure 1. A typical ion-trap mass spectrometer mass-(y-axis)-to-time (x-axis) profile for an MS/MS experiment. Equates to the parameters given in Table 1.

After allowing a short time for the ions to lose excess energy (settling time), the RF trapping potential is abruptly raised to a value causing ejection of all ions lighter than about 85% of the mass of the ion to be retained (Table 1, step 5). At this point a negative (for positive ions) DC potential, arbitrarily about 1.05 times the mass to be retained (instrument dependent), is applied to the ring electrode (Table 1, step 7). This reduces the stability region considerably and therefore, in step (b) of Fig. 1, ions of higher mass and the remaining 15% of masses lower than the parent mass are ejected. After about 1 ms, the DC potential is removed and the RF trapping voltage decreased so as to trap the lowest-mass daughter ion of interest (Table 1, step 9). At this point an AC voltage (tickle) is applied to the end-caps of the cell (Fig. 1, step c) and its frequency is adjusted to match the fundamental secular frequency of oscillation for the ion in the z-direction. If enough voltage is applied, the ion will increase the amplitude of its z-oscillations raising the probability and energy of its collisions with the helium buffer gas, contributing to fragmentation. After a length of time (typically 10–20 ms), the AC voltage is removed and, with the multiplier turned on, the spectrum of the

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Table 1. MSMS4 scan-editor parameters for automated MS/MS analyses[a]

Table Number	1	2	3	4	5	6	7	8	9	10	11	12	13
Title:	Start	Pre-ionize	Ionize	Settle	Ramp to DC	Settle	DC	Settle	Ramp to tickle	Tickle	Settle Mult/on	Acquire daughter	Clean. . . End
Acquire data												X	
Multiplier "ON"											X	X	
Ionize			X										
Trigger	X												
RF ramp					X				X			X	
Tickle										X[b]		X	
DC							X						
Start Mass	0	20	20	20	20	85[c]	85[c]	85[c]	85[c]	50	50	50	
End Mass	0	20	20	20	85[c]	85[c]	85[c]	85[c]	50	50	50	106[c]	0
Time (μsec)	50	200	15000[d]	200	200	500	1000	200	300	10000[b]	1000	0[e]	100
Tickle Mass										100[f]			
Frequency delta									0				
Tickle (mV)										400			
DC (Volts)							−105[c]						

[a] The tables are shown for an MS/MS spectrum of m/z 100. The programming option modifies the values noted.
[b] On the "tickle" step, the time should be zero and the tickle "off" for DC tuning as in the program under Table 2.
[c] These values are modified by the MS/MS program option every 1.0 mass unit (5 scans under MS/MS).
[d] The filament time must be determined for a sample as described in the text.
[e] This value is left at zero and the time is calculated by the computer based on a scan speed of approximately 5555 masses/s.
[f] This value is modified by the MS/MS program option every 0.2 mass unit.
[g] An "X" in the parameters from "Acquire Data" to "DC" indicates the function is on. Otherwise it is off.

fragment ions is obtained by increasing the RF ring voltage steadily (Fig. 1, step d). During this period, enhanced resolution of the fragment-ion spectra is achieved through the use of a small auxiliary RF oscillation also applied to the end caps[1] (Table 1, step 12). The theory of the ion trap and the above processes has been reviewed fully in a recent publication.[2]

When a scan-editor file is developed for a particular fragmentation, conditions must first be optimized to trap efficiently only the ion of interest. This ion is then selectively "tickled" without being ejected in the process. However, mass values entered in the scan-editor (start mass, end mass, tickle mass) only cause the application to the trap of frequencies calculated from first principles and, consequently, the actual mass values they produce are unlikely to be exactly correct. If a compound can be bled into the machine constantly, the scan-editor file can be optimized while observing the screen and adjusting the parameters. Even though this approach works well, the process must be repeated for every ion of interest and progress can be slow. An automated method is described below that can scan an area or even the whole mass spectrum, correctly assigning masses to daughter ions produced from each ion in the original spectrum.

EXPERIMENTAL

A Finnigan ion-trap mass spectrometer (ITMS) equipped with a DC ring-electrode-voltage attachment and a conversion-dynode detector was used to obtain all spectra. Samples could be desorbed from the solids probe with a cooled source (to cause very slow evaporation) or, as in one example below, through a standard reference gas inlet or gas probe. Data were acquired to, and processed on, an IBM AT (tm) computer under standard ITMS software with enhancement by the Finnigan Programming Option.

RESULTS AND DISCUSSION

A typical standard ion-trap MS/MS mass-to-time profile is shown in Fig. 1 and the associated scan-editor tables are shown in Table 1. As discussed above, the only masses in calibration are those actually derived during the scan process. In order to optimize the MS/MS analysis of a particular ion, it is first necessary to adjust the DC to trap selectively a relatively large number of ions of that one species. In practice, it is preferable to sacrifice selectivity in order to maximize trapping by retaining a small range of masses (±1–2 mass numbers). Use of a low tickle-voltage, carefully stepped across the region, will ensure selective fragmentation of the major ion species in the trap. Two parameters are entered in the scan-editor to determine this optimum trapping condition. From experience on our instrument, they appear to be related in magnitude. The first is the mass (RF voltage) at which the ion is trapped (which should be 85% of the mass to be analysed) and the second is the amplitude of the applied DC voltage once the ion has stabilized at that mass. For our instrument, from manual trapping experiments on a range of masses of ions, the latter optimizes at a number arbitrarily −1.05 times the mass. To ensure observation of all fragmentation, the spectrum should be scanned from a nominal mass value several mass units lower than the lowest mass expected (e.g., m/z 50) to a value ca 6 mass units above the mass of the parent ion.

An alternative to preparing a series of individual scan-editor files, involves the use of the Finnigan programming option (using FORTH computer language). A general scan function can be loaded (in the example below, this is called "MSMS4") and adjustments made (a) to the RF and DC voltages used to trap a given mass, (b) to the "tickle" mass, and (c) to the upper mass of the scanned spectrum. Table 2 shows such a program for scanning the spectrum of perfluorotributylamine and optimizing the ion-trapping conditions (note: the "tickle" must be "off" in Table 1, step

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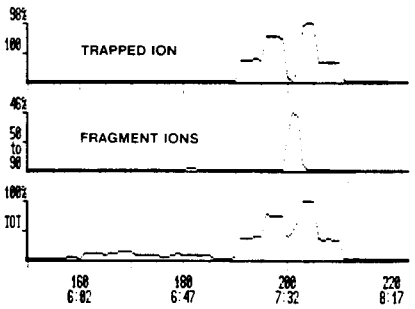

Figure 2. Trapping and MS/MS fragmentation of mass 100 under automated "key sequence" program analysis. Upper:trapping of mass 100 and optimum tickle around scan 200; middle: the formation of fragment ions in the range *m/z* 50 to *m/z* 90; and lower: the total ions trapped. All three ion intensities are normalized and plotted against scan number.

10).

The first 9 lines of the Forth program instruct the instrument to acquire data to a file called "REF" from a scan function called "MSMS4", after which the instrument is turned on and allowed to stabilize for 10 s. At this point a loop is set up to step from mass 60 to mass 520, scanning a spectrum at each step (one mass unit). Remembering that many parameters used in the programming option are loaded at ten times their value into the scan-editor tables (Table 2), the mass of the ion to be trapped is multiplied by 8.5 ("17*2/") (rounded up with the "4+") and the double precision value loaded into the relevant start and end mass values of steps 5 to 9 as required. The end mass of step 12 is then set to 6 mass units ("60+") above that of the trapped ion and the DC voltage set to −1.05 times the mass ("−21*2/"). A single scan is then taken and the process repeated until a mass of 520 has been trapped. At the end of this loop, the instrument is turned off and the valve for the calibration compound closed. It must be stressed again that the masses entered above have had no calibration table adjustment and that only during the spectrum scan (Table 1, step 12) will the detected fragment ions be adjusted to correct mass (calibrated).

This program allows the DC trapping voltage to be optimized (by varying the DC-voltage to trapped-mass ratio) until a minimum range of masses is trapped with little loss of ion intensity.

When producing an MS/MS spectrum on the ITMS, the best results are obtained when a low level of supplementary AC ("tickle (mv)") is applied to the end caps and when the RF of the "tickle mass" is centered on the mass to be fragmented within 0.4 mass numbers of the correct value. To best achieve this, the "tickle mass" should be adjusted in steps of 0.2 mass numbers and a scan taken at each step. It is not necessary to adjust the DC trapping conditions this precisely, because a small range of masses is permitted to remain in the cell (as discussed above). A program to perform this operation is shown in Table 3.

The "tickle mass" is stepped 0.2 mass numbers ("2+") for every mass, and separate spectra are obtained. After each mass unit has been "tickled", the trapping mass is stepped and the process begun again, resulting in a mass unit step for every 5 scans. This would work perfectly if the RF calculation for the tickle mass and trapping mass were calibrated (no use of underlined code in Table 3). This is not possible on our instrument and so a linear adjustment has been added to the function to overcome this problem (including underlined section, Table 3). To establish this "calibration", the underlined section can first be deleted and a full-mass MS/MS scan performed. An examination of the tickle position for varying trapped masses allows the correction to be calculated. The optimum tickle position is when the center of the trapped mass coincides to where the most fragment ions are detected. For instance, as shown in Fig. 2, the ion *m/z* 100 is trapped and scanned from scan number 190 to 210 (lower axis: total ions; upper axis: *m/z* 100) while the fragment ions (middle axis: combined intensity of ions of mass 50 to 90) are detected between scan numbers 200 and 203. The optimum tickle mass for *m/z* 100 is therefore approximately at scan number 200, forty mass units (5 scans per mass unit) above the start at *m/z* 60 (i.e., mass 100). However, mass 264 is best "tickled" at scan number 1036, some 16 scans higher

Table 2. Key sequence program to selectively trap and scan at each mass of a spectrum (used in conjunction with sequence-editor file "MSMS4", see Table 1)

```
R EXP ⟨CR⟩
ACQUIRE-TO: REF
LOAD-SCAN-FUNCTION: MSMS4
ACQUIRE-MODE-ON
MULTIPLIER-ON
FILAMENT-ON
RF-ON
CAL-GAS-ON
10  SEC-WAIT
    [[520 60]] ⟨DO⟩ ⟨I⟩ [[DUP 17*2/4+S>D
5   SET-TABLE  DDUP  SET-END-MASS
6   SET-TABLE  DDUP  SET-START-MASS   DDUPSET-END-MASS
7   SET-TABLE  DDUP  SET-START-MASS   DDUPSET-END-MASS
8   SET-TABLE  DDUP  SET-START-MASS   DDUPSET-END-MASS
9   SET-TABLE        SET-START-MASS
12  SET-TABLE  DUP   10*60+S>D        SET-END-MASS
7   SET-TABLE        −21*2/S>D        SET-DC-VOLTAGE 1 SCAN-ACQUIRE
    ]]⟨LOOP⟩[[
    MULTIPLIER-OFF FILAMENT-OFF CAL-GAS-OFF RF-OFF]]⟨ESC⟩ ⟨'R⟩
```

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Table 3. Key sequence program to selectively MS/MS and scan a complete spectrum (used in conjunction with sequence-editor file "MSMS4")

```
    R EXP ⟨CR⟩
    ACQUIRE-TO: REF
    LOAD-SCAN-FUNCTION: MSMS4
    ACQUIRE-MODE-ON
    MULTIPLIER-ON
    FILAMENT-ON
    RF-ON
    CAL-GAS-ON
10  SEC-WAIT
    [[520 60]] ⟨DO⟩ ⟨I⟩ [[DUP 17*2/4 + S > D
5   SET-TABLE  DDUP  SET-END-MASS
6   SET-TABLE  DDUP  SET-START-MASS  DDUPSET-END-MASS
7   SET-TABLE  DDUP  SET-START-MASS  DDUPSET-END-MASS
8   SET-TABLE  DDUP  SET-START-MASS  DDUPSET-END-MASS
9   SET-TABLE        SET-START-MASS
12  SET-TABLE  DUP   10* 60 + S > D        SET-END-MASS
7   SET-TABLE  DUP   −21*2/S > D           SET-DC-VOLTAGE
10  SET-TABLE        10* DUP 1000 − 50/ +
                     DUP        S > D SET-TICKLE-MASS 1 SCAN-ACQUIRE
                     DUP  2 +   S > D SET-TICKLE-MASS 1 SCAN-ACQUIRE
                     DUP  4 +   S > D SET-TICKLE-MASS 1 SCAN-ACQUIRE
                     DUP  6 +   S > D SET-TICKLE-MASS 1 SCAN-ACQUIRE
                          10 +  S > D SET-TICKLE MASS 1 SCAN-ACQUIRE
    ]]⟨LOOP⟩[[
    MULTIPLIER-OFF FILAMENT-OFF CAL-GAS-OFF RF-OFF]] ⟨ESC⟩ ⟨ˆR⟩
```

than expected (1020 = (264–60)*5). The above correction (underlined in Table 3), therefore subtracts the 100 mass units from the actual, divides the difference by 50 and adds this to the tickle mass. This is a correction of 2 mass units for every 100 mass units and brings the tickle mass in phase with the DC trapped mass. This type of correction will vary with the instrument and source assembly. However, once determined for a particular instrument, it appears to remain correct for all samples run subsequently until source changes or disassembly are necessary. An alternative to this full spectrum "calibration" can be obtained from individual MS/MS analysis of two relevant ions (in a calibration compound) using manual adjustment of separate scan-editor functions. Once this "key-sequence" has been so calibrated, a complete MS/MS spectrum can be obtained on any compound with no operator intervention after initiation. The "chromatogram" for an automated MS/MS analysis of perfluorotributylamine is shown in Fig. 3. This equates to the mass spectrum with loss of resolution coming from the range of masses trapped in each MS/MS step. Within any "chromatogram" peak, a spectrum of the trapped ion is obtained and when the "tickle" frequency coincides with the resonance of the desired ion, the daughter-ion spectrum is observed. The "key-sequence" MS/MS spectrum of the parent ion of methyl stearate is shown in Fig. 4 with the "tickle" (a) off the peak, (b) at the edge of the peak (small amount of fragmentation) and (c) centered on the mass 298 ion (full daughter-ion spectrum). The fragmentation pathway determined from this procedure is shown in Fig. 5. The source of the most intense electron-ionization ion at m/z 74 was not observed. While this is known to come from the parent ion in electron ionization (EI), its non-appearance under MS/MS conditions is common and a

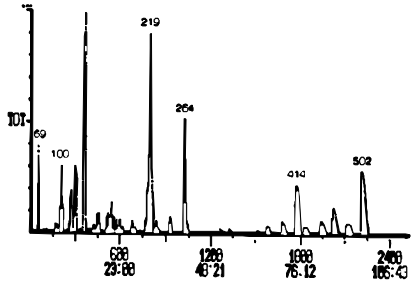

Figure 3. "Chromatogram" from automated MS/MS analysis of perfluorotributylamine. Each peak represents trapping and MS/MS analysis of a particular m/z value (some shown).

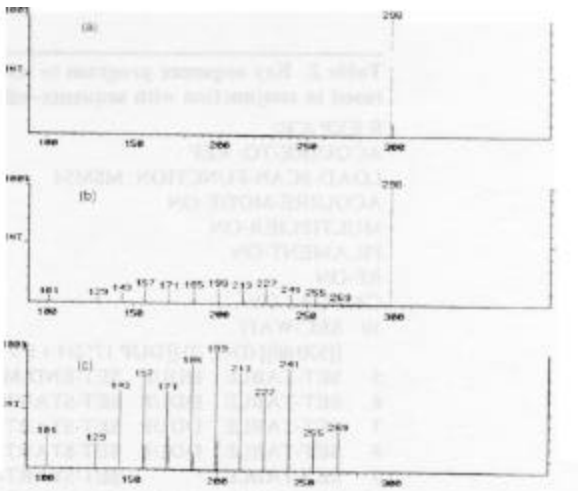

Figure 4. Examination of the automated MS/MS analysis of m/z 298 (M^+) of methyl stearate showing the result (a) with the tickle missing the peak (no tickle on m/z 298, top axis), (b) at the edge of the peak (inefficient tickle of parent ion, middle axis) and (c) exactly on top of the peak (full daughter-ion spectrum, lower axis).

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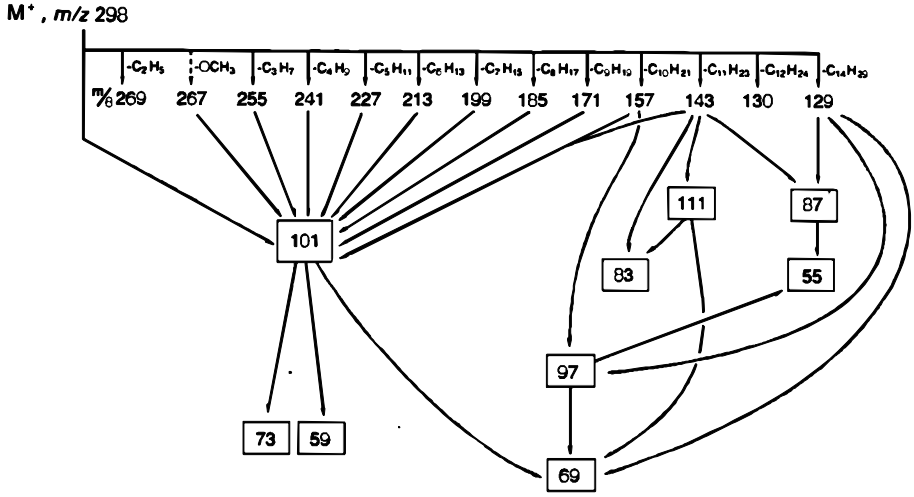

Figure 5. Fragmentation pathway determined for methyl stearate using the automated MS/MS procedure on the ion trap mass spectrometer.

separate publication is in preparation concerning this phenomenon.

Conclusion

By using a "key-sequence" under the programming option of the Finnigan ion-trap software, an automated MS/MS analysis of all the daughter ions from a region of an electron ionization spectrum is possible, with no operator intervention once the spectrum is started. With a scan speed of about 10 mass numbers per minute, this method compares favorably with an MS/MS about 4 peaks per hour obtained by using the manual separate scan function technique and occupying full operator time. The described procedure has been applied to a number of compounds, including a range of steroids and compounds related to artemesinin (results to be reported elsewhere). A complete MS/MS spectrum on ions spread over 200 mass units has been obtained on as little as 2 micrograms of material. However, as the scan speed on our computer was 10 masses per minute (50 scans per minute), it can only be applied to compounds where the sample can be desorbed slowly from a probe or admitted via a gas inlet (on a fast '386 computer, almost twice the rate has been obtained). In practice, we have found it easiest to adjust the source temperature to bleed the compound very slowly into the ionizer. Use of a relatively long ionization time provides the intensity required to produce good daughter-ion spectra with little interference from intermolecular reactions. The technique could easily be extended for use under chemical ionization (CI) conditions by using a suitable CI-MS/MS sequence-editor table and necessary adjustment of the table change numbers in the "key sequence" program. It would be difficult to apply the program described to the MS/MS of a gas chromatographic peak unless the mass-scan range was very small and the program modified to run in a cyclic fashion.

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