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Complete Mass Spectrometry/Mass Spectrometry Data Field Acquisition on the Chromatographic Time Scale by Time-Resolved Ion Momentum Spectrometry with Time-Array Detection

B. A. Eckenrode,[1] J. T. Watson, and C. G. Enke*

Department of Chemistry, Michigan State University, East Lansing, Michigan 48824

J. F. Holland

Department of Biochemistry, Michigan State University, East Lansing, Michigan 48824

Time-resolved ion momentum spectrometry (TRIMS) is combined with time-array detection (TAD) to provide the capability for rapid acquisition of complete mass spectrometry/mass spectrometry (MS/MS) data fields. Observation of all arrival times for all values of the magnetic field strength produces a two-dimensional field from which all MS/MS scans can be obtained. The complete fragmentation map can now be obtained for each component in a mixture, separated by chromatography. Evaluation of the TRIMS-TAD instrument with regard to data quality, integrity, and speed is performed under raw data acquisition and real-time data reduction conditions. A 5-s acquisition of the MS/MS data field for caffeine present in an aspirin tablet demonstrates the gas chromatography (GC)-MS/MS capability of TRIMS-TAD.

INTRODUCTION

Mass spectrometry/mass spectrometry (MS/MS), by its multidimensional nature, has proven to be a key instrumental technique in many analytical problems, especially those involving mixture analysis or structure elucidation (*1-8*). If a mixture of components is present in the source of an MS/MS instrument, the first stage of mass analysis can be used to separate the components by mass and the second stage for the analysis and detection of the selected ion's fragments. Primary mass spectrometric information is thus obtained for each component in the mixture. However, if only a single compound is present in the ion source, the two stages of mass spectrometry can be used to provide a complete fragmentation map for the test compound. If chromatography is used to separate a mixture of components and deliver pure compounds to the source in sequence, the combination of gas chromatography with MS/MS (GC-MS/MS), offers the potential to obtain complete fragmentation information on each component (*9*). The relationships between parent and daughter ions involved in metastable ion decomposition or in collisionally activated dissociation can be established by interpretation of a full MS/MS data field. These relationships are often indicative of a specific ion structure. A goal of this research is to acquire as much fragmentation information as possible about a compound in the brief time it emerges from a chromatographic column. It is important to note that it is not essential to save all of the data in the full field, but it is mandatory that the full field be collected since only time-of-flight gated ions are actually detected. This potential for GC-MS/MS has not heretofore been realized because collecting the full MS/MS data field on the time scale of a packed column chromatographic peak requires an instrument with very rapid collection of mass spectral data and high sensitivity. If packed or wide-bore capillary chromatographic columns are used, only 5-10 s is available for collection of the complete MS/MS data field. Conventional tandem mass filter instruments have not provided the data rates necessary for acquisition of the complete MS/MS data field on the chromatographic time scale.

MS/MS Acquisition with Tandem Mass Filter Instruments. Unfortunately, when tandem mass filter instruments are used, acquisition of the complete MS/MS map (all daughters of all parents) is a relatively lengthy process, at best requiring an exploratory scan to discover potential parent ion masses and then subsequent daughter scans for each potential parent mass found. For state-of-the-art triple-quadrupole instruments, this process can take from 40 to 90 s depending on the mass range, spectral complexity, and ion statistics required. Consequently, much more sample is required for this process than for a single scan in a normal mass spectrometric analysis. A serious disadvantage associated with long acquisition times is the requirement for maintaining a constant sample pressure in the ion source over this interval. This requirement is completely incompatible with sample introduction via a chromatographic inlet.

The multiple scan requirement for full MS/MS data field acquisition is an unavoidable part of the design of tandem MS/MS instruments (*10*). Figure 1 illustrates the strategies for acquisition of a complete MS/MS map on three popular tandem analyzer instruments: a triple-quadrupole mass spectrometer (TQMS), a reverse geometry double-focusing mass spectrometer used as a mass analyzed ion kinetic energy spectrometer (MIKES), and a double-focusing instrument of forward geometry. In TQMS and MIKES instruments, the first mass analyzer transmits a selected parent ion to a collision region, while the second mass analyzer is scanned to detect the daughter ions of that particular parent ion. The process is repeated for the next selected parent ion until the daughter spectra for all the parent ion masses have been collected (*11, 12*). A forward geometry instrument performs collisionally activated dissociation (CAD) in the first field-free region and links a scan of the electric and magnetic sectors in such a way as to allow daughter ion analysis (*13*). Alternatively, with the same geometry, one of the analyzers can be decremented while the other analyzer is scanned through an appropriate mass range (*14*).

The sequential nature of the MS/MS analysis imposes a limit on the speed with which the complete MS/MS field can be obtained. In TQMS and MIKES instruments, a complete scan of the second stage of mass analysis is required to yield a single daughter spectrum. For unknown compounds, incrementing along the primary spectrum is followed by scanning of the electric sector or second quadrupole mass filter.

* Person to whom correspondence should be addressed.
[1] Current address: Analytical Chemistry Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831-6365.

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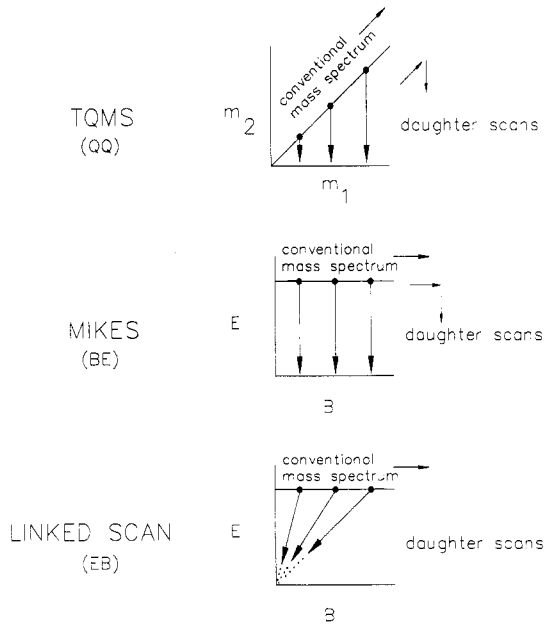

Figure 1. Process of acquiring a complete MS/MS data field with different tandem mass filter instruments. The arrows inside the data boundaries show the incremental, and thus sequential, operation required to achieve a full field acquisition by these techniques. Key: TQMS, triple-quadrupole mass spectrometer; MIKES, mass analyzed ion kinetic energy spectrometer (reverse geometry); EB, forward geometry double-focusing mass spectrometer (B/E linked scan for decrement E and scan B).

Although scanning the second stage requires only 50 to 100 ms, incrementing by 1 dalton over a 500 dalton mass range cannot be accommodated in the time available with chromatographic separation. Linked scanning in a forward geometry instrument can require from 0.25 to 1 s per daughter scan because a magnet is inherently slower to scan and also coordination with the electric sector can be time-consuming. If the whole MS/MS field is to be acquired, the electric sector can be scanned for each value of the magnetic field strength. This process can require several minutes per decade. Clearly, if many different ions could be detected simultaneously, the possibilities for much faster MS/MS data field acquisition could be achieved (*10*).

Frequency and Spatial-Array Detection. MS/MS analysis also can be performed with mass analyzers that are sequential in time rather than space. These, too, have difficulty obtaining the complete MS/MS data field in a time frame acceptable for GC–MS/MS. In a Fourier transform ion cyclotron resonance (FTMS) mass spectrometer, for example, parent ions are isolated in a single analyzer cell from all other ions by broad-band irradiation encompassing all cyclotron frequencies except that of the selected parent ion (*15*, *16*). The isolated ions are then given additional energy (collision energy) for collision with a target gas to produce daughter ions. The target gas must then be pumped out before the daughter spectrum can be obtained. The process is repeated for the next parent ion in the spectrum. In this case, all daughter ions can be measured simultaneously after the selected parent ion has been subjected to collision (*17*, *18*). The entire process (ionization, parent ion selection, collision, and analysis of the resulting daughter ions) for a single parent ion without pumping collision gas in and out can require 100–200 ms/transient. For daughter ion analysis of 10 peaks in the primary spectrum with 25 transients averaged for each daughter spectrum, the total time for a complete MS/MS data field acquisition would be a minimum of approximately 25 s. Nevertheless, two promising FT-MS/MS techniques have been developed recently and soon this analysis time may be reduced (*19*, *20*).

An ion trap can be a very selective and fast scanning technique and could prove promising for high-speed GC–MS/MS. A primary mass spectrum acquisition for a mass range of 30–300 daltons requires approximately 50 ms, and acquisition of an MS/MS spectrum for each parent ion requires approximately 60 ms with the ion trap analyzer. Improvement in the automation of parameter control for the MS/MS scan function could potentially permit acquisition of a complete fragmentation map for 25 to 50 parent ions in less than 5 s. However, this has not yet been demonstrated.

Louter et al. (*21*) have developed a tandem magnetic sector (BB) instrument in which the second magnet disperses the daughter ions along a multichannel detector for spatial-array detection. Unfortunately, the number of detector channels limits the range of daughter ion masses that can be detected simultaneously at reasonable resolution. Nevertheless, with further developments in detector technology, this type of instrument has good potential for high-speed MS/MS map acquisition.

Time-Resolved Ion Momentum Spectrometry. Time-resolved ion momentum spectrometry (TRIMS) is achieved by determining flight times of ions subsequent to momentum analysis in a magnetic sector mass spectrometer. In a normal magnetic sector instrument, the spectra of stable ions and metastable ions are superimposed. Ions that have undergone collisionally activated dissociation or metastable dissociation in the first field-free region of the single sector mass spectrometer have a longer time-of-flight (lower velocity because they are derived from heavier parents) than that of stable ions of the same momentum. Daughter ions (m_2) will have the same flight time as their parent (m_1) and a lower momentum which causes them to appear at a magnetic field strength corresponding to a lower apparent mass, m^*. The actual mass of the daughter ions can be determined either from the equation $m_2 = (m_1 m^*)^{1/2}$ or from their momentum–velocity coordinates in the TRIMS data field (*22*). Separate observation of stable ions and daughter ions at a particular magnetic field strength is possible by bunching and then time-resolving the ion beam at the exit of a modified LKB-9000 magnetic sector mass spectrometer. Beam deflection is used to create discrete ion packets, and a 1.5-m flight path separates ions according to their velocities. An ion arrival time transient is produced at the detector.

By observation of the ion intensity at all arrival times for all values of the magnetic field strength, a two-dimensional data field is produced (see Figure 2) from which all MS/MS data can be obtained. As the magnetic field is swept in a continuous fashion through the desired mass range, ion flight time transients are collected at many values of the magnetic field strength. If all possible time channels are sampled in every arrival time transient, a complete MS/MS data field acquisition is possible in the time required for a single sweep of the magnetic field strength. For 1000 equal interval samples of the magnetic field strength and a transient repetition rate of 10 kHz, an entire MS/MS map theoretically could be acquired in 100 ms. However, consideration of ion statistics requires the summation of several transients; thus, realistic acquisition times on the order of seconds are required. Subsequent interrogation of the acquired full field will yield all possible parent, daughter, or neutral loss information. For example, a daughter scan can be extracted from this data field by locating all the ions that have the same flight time as the chosen parent ion. This is shown by the vertical line in Figure 2. TRIMS, therefore, can provide the complete MS/MS data field in one sweep of the magnet.

To realize the full potential of TRIMS, a transient recorder capable of collecting all information continuously from each and every deflection pulse (giving rise to a transient) is re-

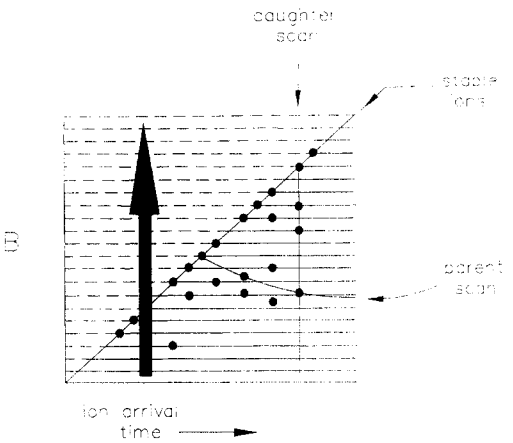

Figure 2. MS/MS data field generated by the TRIMS instrument. The horizontal lines represent ion-arrival-time spectra (scan files) acquired at a particular magnetic field strength (B). The heavy arrow indicates that only one sweep of the magnetic field strength is required to collect the full MS/MS data field and that full arrival time spectra are acquired at successive values of the magnetic field strength. Interrogation of the resulting data field (postprocessing) provides the characteristic MS/MS scans.

quired. Conventional transient recorders are limited either by the maximum repetition rate for signal averaging or by the time required to download the acquired transient before another one can be acquired (*23*). In fact, due to the fundamental limitations in computer bus data transfer rates, the time required to transfer each transient far exceeds the duration of each transient. Only 1–100 transients/s can be obtained presently, or less than one transient out of a thousand in the time-of-flight (TOF) application. To avoid this loss of valuable information, an integrating transient recorder (ITR) was developed that allows the *continuous* acquisition and summation of transient events (*9*, *24*). While incoming transient information is being acquired in one bank of summing registers, a second bank containing previously summed transients (one scan file) is being downloaded to the transfer and processing circuits of the ITR. The banks then reverse their roles and the process continues without interruption. Some of the features of the ITR data system include (a) the capacity to sum 10 to 2000 transients per scan file, resulting in the generation of 1000 to 5 scan files per second, respectively, (b) transient recording at a rate of 200 megasamples/s to provide time resolution of 5 ns, (c) continuous data collection at the maximum rate for at least an hour, and (d) data reduction via parallel processing to mass/intensity pairs in real-time prior to storage (*25*, *26*).

TRIMS with Time-Array Detection. Connecting the output of the TRIMS instrument to the ITR permits high-speed GC–MS/MS analysis. The instrumental limit for the acquisition of data on the time axis is 1000 TOF scan files per second. If each TOF scan file acquisition is to be correlated with a point on the magnetic field axis scan of the magnet, the magnetic field scan rate will determine the resolution of scans along the magnetic field axis. For example, if one wishes to scan the magnetic field (B) from mass 200 to 300 in 1 s, up to 1000 complete TOF scan files can be recorded over this mass range. If this resolution is greater than needed, the magnetic field scan rate could be increased to give multiple MS/MS spectra per second, or the number of TOF transients summed per TOF scan file could be increased to improve the signal-to-noise ratio (S/N).

The trade-offs for TRIMS-TAD in resolution, detectability, and acquisition time can be related with the following equations:

$$(\text{transients/scan file})/(\text{transients/s}) = \text{s/scan file} \quad (1)$$

$$\text{mass range/mass resolution } (\leq 1) = \text{TOF scan files/sweep} \quad (2)$$

$$\text{TOF scan files/sweep} \times \text{s/scan file} = \text{s/sweep} \quad (3)$$

A scan file is defined as the summation of a given number of transients, and a sweep represents a single magnetic field transit over the desired mass range. Equation 1 is related to the S/N at each momentum setting. Equation 2 is related to the desired mass resolution (adjacent masses are separated in both velocity and momentum dimensions) and is dependent on the nature of the sample and the objectives of the analysis. Equation 3 represents the time for a complete MS/MS map acquisition with TRIMS-TAD and is determined by the temporal characteristics of sample introduction. For example, if one desires to obtain a complete data field over the mass range 50–500 from a sample emerging from a GC column during an interval of 10 s, the following logic would be applied. The exponential scanning mode for the magnetic field strength would be used at a rate of 10 s/decade. This mode will produce approximately identical mass spectral peak characteristics throughout the mass range, presenting an equitable proportion of time for data collection for the ion current of each mass in the spectrum. The mass spectral peaks from this instrument at these settings are approximately 10 ms wide at base line. Utilizing 5 ms to generate each scan file would require the acquisition of 2000 TOF scan files. Because the magnet is scanned continuously and the ITR integrates all of the ion current striking the multiplier during each summation period, reasonably accurate peak height intensities are obtained at this resolution, and fragmentation patterns adequate for structural analysis are created. The summation of 50 transients provides a theoretical S/N improvement of greater than 2 over that with only 10 summed transients. In addition, the integrating feature of the ITR provides the capacity for measurement of peak intensities at the low end of the mass range where the mass spectral peaks are typically somewhat narrower in time.

The potential for acquiring the entire MS/MS data field from compounds as they elute from capillary columns could be realized on an instrument other than the LKB-9000 employed in this research. With improved ion sources and, thus, improved signal levels, the time for an analysis could be reduced. Also, temporal changes in analyte concentration in the ion source can be monitored more accurately and efficiently with improved instrumentation.

EXPERIMENTAL SECTION

Experiments were performed on an LKB-9000 single-focusing magnetic sector mass spectrometer modified for postsector beam deflection (*27*). A beam deflection assembly mounted after the exit slit of the mass spectrometer produces packets of ions. The assembly is built such that ion packets can be produced at a variable rate (typically 10 kHz) and with a variable time width (dependent on the slew rate). A pulse from the ITR control circuitry is used as the deflection trigger for time synchronization of the ion beam. A block diagram of the TRIMS-TAD configuration is shown in Figure 3. An optically isolated 16-bit digital-to-analog converter (DAC) or an optically isolated 12-bit analog-to-digital converter (ADC) provides control/sensing for the magnet and an interface to a status-in, command-out circuit board allows incrementing/sensing (by DAC/ADC) of the magnetic field strength after each scan file has been prepared. The output current of a channel electron multiplier array (CEMA) is amplified by a model 342 wideband noninverting dc-coupled current amplifier (Analog Modules, Inc., Longwood, FL) in tandem with an inverting CLC 220 dc amplifier (Comlinear Corp., Loveland, CO). The second amplifier serves to provide the ITR with the proper signal polarity and also increases the gain. The signal is terminated with 50 Ω at the ADC input of the ITR.

The interface between the Hall probe and the ITR was constructed to remove the step-function inherent with DAC control

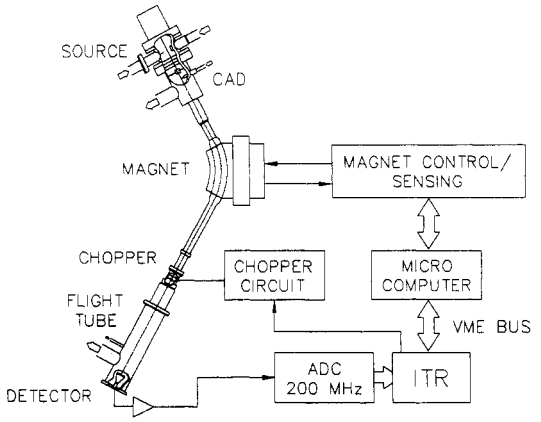

Figure 3. A schematic of the TRIMS–TAD instrument. The TAD data system is comprised of an integrating transient recorder (ITR) and control hardware.

of the magnet. This allows the magnet to be scanned in its conventional (analog) manner. Transient summation occurs on a much shorter time scale than the change in magnetic field strength over a range of one mass unit and, therefore, a factor of approximately 8 in speed is gained relative to DAC operation.

A 200 megasample/s ADC (LeCroy Research Corp., Spring Valley, NY) digitizes the transient data followed by custom sum and store circuitry, which contains two banks of memory and adders. This circuitry sums the ion current at each arrival time following each deflection of the ion beam for a preset number of digitized transients. While one bank of memory collects and sums successive transients, the other bank outputs the previous scan file to the reduction hardware. As the ITR collects the ion current in each transient with 5-ns resolution over an 80-μs range, all the spectral information from each pulse is recorded.

The number of transients to be summed, scan files (magnet increments) to be collected, and mass range per transient (related to the flight time for the ions of interest) are predetermined and input into a parameter file for the ITR. A VME 68000-based (Motorola, Inc.) control system communicates with the ITR through a common bus. Raw data are stored on a 300-Mbyte (Priam, Inc.) hard disk and postprocessing is performed in software. Data reduction to triplets (Hall voltage, flight time, intensity) can also be performed in real-time by parallel processing with three VME133A 20-MHz (Motorola, Inc.) microprocessors on a common bus.

The ion source pressure for steady-state samples leaked into the instrument was typically 1.2×10^{-6} Torr measured by a Penning gauge directly below the source housing and the source temperature was 230 °C. The trap current was 60 μA and the accelerating voltage was 3500 V. The front plate of the CEMA detector was typically maintained at a voltage of −2.65 kV.

n-Decane and 1-decanol were obtained from Chem Service (West Chester, PA) and not further purified. These compounds were introduced via a heated gas inlet. A 1-g sample of Excedrin (Bristol-Myers) was dissolved in 50 mL of methanol and the resulting supernatant was chromatographed at a column temperature of 150 °C. The column used was a methyl silicone wide-bore capillary column (5 m × 0.53 mm × 2.65 mm). The capillary column was fitted to the LKB-9000 with a conversion kit purchased from Supelco, Inc. (Bellefonte, PA).

A program was written that plots the intensity range as a function of scan number (related to the magnetic field strength). The resulting plots, which resemble total ion current plots, were used to determine where in the large data field significant fragmentation reactions might be found. Figure 4 in the next section is an example of such a plot.

Graphical Display Algorithm. Peak-finding is done on the VME system and subsequently the data are downloaded to an IBM-AT for further processing. A three-dimensional (3-D) plotting algorithm facilitates display of the MS/MS data. Software was written to present the data in a graphical form with perspective viewing capability. The multidimensionality of the data require its presentation on a two-dimensional x,y plane with intensity projecting into the z direction (*28*).

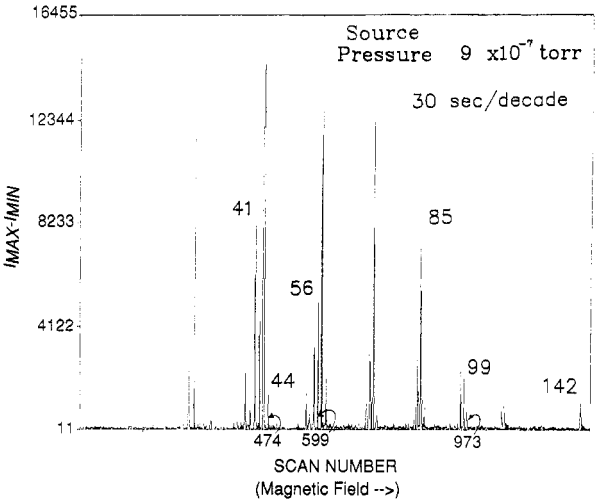

Figure 4. Composite plot of the intensity range as a function of the magnetic field strength. At each value of the magnetic field strength the difference between the maximum and the minimum intensity in that scan file is plotted. (Because the most intense peak in most scan files is that due to a stable ion, this analogue spectrum ($I_{MAX} - I_{MIN}$ vs scan number) has the appearance of a conventional histogram spectrum.) This type of plot is convenient for examining the full MS/MS data field.

The MS/MS data field represented in commercial software packages could accommodate only a data grid measuring 100 × 100. Even with such a limited grid size, several hours were required to perform the necessary calculations. The graphics software developed in this laboratory can accept data grids typically as large as 1000 × 1000 with display generation execution time on the order of seconds (*29*). The software permits multiple variables governing tick mark frequency, tick mark length, axis label sizes and placement, grid line frequency, global scaling, and data thresholding. The software will accept user supplied vectors in an ASCII file in the x,y,z data space. This allows generation of isomass lines or other user-defined annotations.

RESULTS AND DISCUSSION

Evaluation of the TRIMS–TAD instrument was performed in two stages. Confirmation of MS/MS data quality was assessed with *n*-decane; data integrity and speed were assessed with 1-decanol. Both analyses were performed without real-time peak finding. (The recording of intensity for every mass value is inherently slower, due to data transfer rate limitations, than recording only the mass/intensity pairs for peaks.) The large number of metastable ions reported for *n*-decane (*30*) made it a good candidate for a test compound. The *n*-decane sample was introduced into the mass spectrometer at a constant rate via heated inlet. Under these conditions the TRIMS–TAD instrument was evaluated for daughter ion detection, mass range, and signal-to-background capabilities.

The complete MS/MS data field was collected for *n*-decane; 1280 scan files were acquired at regular intervals along the magnetic field axis for a mass range of 15–150 daltons (with the magnet under DAC control). The ITR summed 200 transients at each magnetic field strength to provide a good signal-to-background (S/B) ratio for each scan file. The complete MS/MS field was acquired in 30 s. Figure 4 is a plot of the maximum minus the minimum intensity versus scan file number.

Scan files acquired at three different magnetic field strength values are shown in Figure 5. Scan files 599 (Figure 5a) and 973 (Figure 5b) show only one peak each at m/z 56 and 100, respectively, representing stable ions. Scan file 474 (Figure 5c) shows two peaks; the first corresponds to a stable ion of mass 44, the second (at a longer arrival time) corresponds to the metastable decomposition of m/z 113 to m/z 71 at an apparent mass of approximately 44.

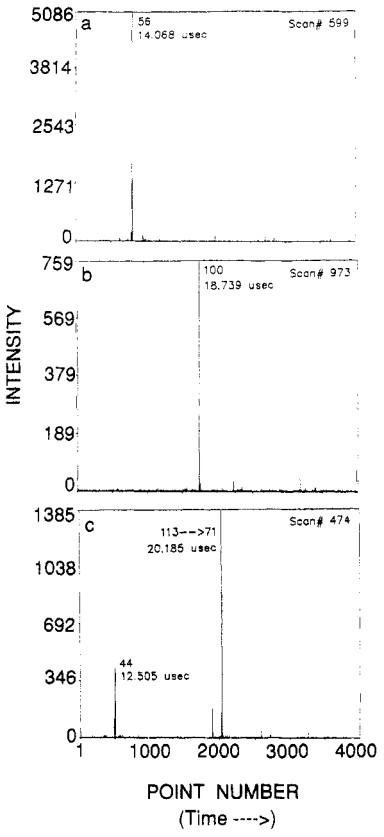

Figure 5. Three scan files selected from the composite *n*-decane data represented in Figure 4. Scan file 599 shows a peak at *m/z* 56 for a stable ion. Scan file 973 illustrates the signal-to-background for ion current of reasonably low intensity at *m/z* 100. Scan file 474 shows a peak at *m/z* 44 for a stable ion, as well as a peak at nearly the same apparent mass value which corresponds to the metastable reaction 113 → 71.

Each of the scan files shown provides information on the performance of the TRIMS–TAD instrument. The stable ions of mass 56 and 100 are found in a conventional mass spectrum of *n*-decane to be approximately 40% and 1% of the base peak, respectively. The same relative intensities are observed with the TRIMS–TAD instrument. In addition, the background is expected to be low due to the MS/MS measurement process and consequently the signal-to-background ratio (S/B) is very good. The stable and daughter ions represented in Figure 5c indicate the time resolution capability of the ITR. A 20-μs scan file (with data acquisition beginning at 10 μs) is illustrated, with 5-ns resolution. With an accelerating voltage of 3500 V, and a flight length of 1.5 m, this time interval corresponds to a mass range of approximately 27–266 daltons. Only a fraction of the time channels was used in these scan files; the complete mass range extends beyond 1000 daltons. These data also indicate, from the high S/B, that less summing was necessary (eqs 1–3) and, thus, the total MS/MS map acquisition time could have been reduced by approximately a factor of 3. For this steady-state sample, the acquisition time was not a concern and the 30-s acquisition time for this MS/MS map was quite sufficient for the initial assessment of TRIMS–TAD.

Data integrity and speed of the complete MS/MS data field acquisition were studied with the unimolecular decompositions of 1-decanol. Farncombe and co-workers had successfully mapped the fragmentation reactions of this compound by employing a forward geometry double-focusing mass spectrometer (*14*); these data provided a good basis of comparison.

The results for the MS/MS map acquisition for 1-decanol under steady-state conditions are shown in Figure 6. The large data field presented was drawn by using the graphical

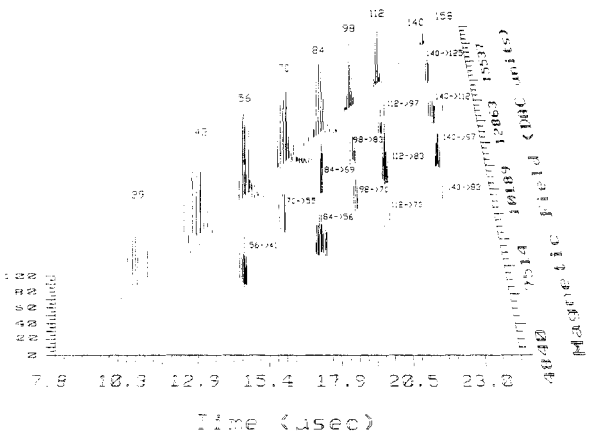

Figure 6. Complete unimolecular fragmentation field observed for 1-decanol. The peak intensities for metastable ions have been multiplied by 5.

algorithm discussed in the Experimental Section. This is a tilted view with perspective of the full field with the masses and observed reactions labeled. The mass range is slightly greater than 1 decade (15–168 daltons); the magnetic field strength was controlled by the DAC. The acquisition time was 43 s as compared to the several minutes by Farncombe and co-workers. Under raw data acquisition conditions (no real-time peak finding) this experiment produced 12.1 Mybtes of data, forcing the hard disk to operate at a sustained data rate of 2.26 Mbits/s.

Most of the metastable reactions observed in the literature for 1-decanol were observed during this 43-s experimental acquisition period with TRIMS–TAD. The relative intensities of these data agree very well (the errors are estimated to be within a few percent) with those determined by Farncombe et al. One important difference observed is that for the TRIMS–TAD instrument to detect low mass decomposition reaction products, the magnetic field strength start value would have to be lowered because their apparent masses would be lower. Because of this, a few 1-decanol fragmentation reactions were not observed by TRIMS–TAD in this experiment. This was found to be the shortest acquisition period possible for 1-decanol (under DAC control, raw data acquisition and processing, and with the LKB-9000) at which the less abundant metastable ions could still be observed. Newer instruments with improved sensitivity would allow increased acquisition speeds for comparable data integrity. A factor of 4 or 5 is estimated as a practical degree of improvement in acquisition speed.

From these experiments it was apparent that the write-speed of the hard disk for data storage is an additional time factor to consider with TRIMS–TAD when collecting data in the raw data acquisition mode. After each scan file is downloaded to the storage circuitry of the ITR data system, a signal is generated to increment the magnetic field strength. It was found that the total acquisition time can vary by as much as 2 s, depending on the distance the disk head has to move and the amount of data being downloaded. A loss of data can result if the hard disk is required to sustain a data rate much greater than 4 or 5 Mbits/s. Peak finding with time centroiding and integration in real-time via parallel processing improves data throughout and eliminates disk write speed as a contributing factor to the MS/MS acquisition time. Parallel processing is required for high-speed GC–MS/MS analyses.

GC–MS/MS. The quantity of sample injected into the GC dramatically affected the amount of time available for MS/MS data acquisition. Sample sizes of less than 1 μg caused sample pressure changes (represented by GC peaks) in the source that were too rapid for a full MS/MS map to be acquired. The

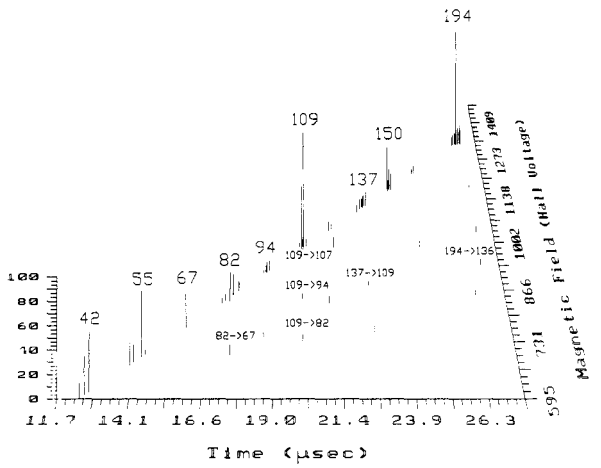

Figure 7. A complete unimolecular fragmentation field for caffeine collected with TRIMS–TAD subsequent to chromatographic separation from other aspirin table components. This is a 5-s acquisition with the summation of 100 ion flight time transients for each of 500 scan files (incremented along the magnetic field strength axis). Some representative metastable reactions have been labeled.

actual sample amount required by the TRIMS–TAD instrument for a discernible MS/MS map for caffeine, after separation from the solvent and other components of the aspirin tablet by the GC, was 4.4 μg. A tilted view of the complete MS/MS data field for this compound is shown in Figure 7. The mass range is 1 decade, and 18.4 μs of TOF information (3689 data points over a mass range from m/z 20 to 200) was acquired for each of 500 scan files. The total time for acquisition of this MS/MS data field (metastable decompositions) was 5 s in the real-time peak finding data acquisition mode with a mass resolution of 500 along both the magnetic field and time axes. The ITR was synchronized with the sensing of the magnetic field strength instead of controlling the magnet with the DAC. The stable ions and some of the observed fragmentation reactions have been labeled. The minimum sample requirement of 4.4 μg represents at least 2 orders of magnitude lower sample than that reported in the literature for the same type of analysis. More typical are the milligram quantities reported by Farncombe and co-workers (*31*, *32*).

The precision of the intensities in the data field of Figure 7 is approximately 10%. This result is estimated from comparisons of the fragmentation maps from several different GC experiments. The data acquisition start point was manually determined during the caffeine elution profile and was subject to error. Automation of the MS/MS data acquisition start time for each chromatographic peak would most likely improve the reproducibility. Nevertheless, these experiments indicate the present capability for the analysis of single components in mixtures with the TRIMS–TAD approach providing complete MS/MS data acquisition on the time scale of packed column chromatography. With a more automated data system, the complete MS/MS data field could be acquired for any component of a complex mixture in a single GC run.

CONCLUSION

The rapid acquisition of an MS/MS data matrix opens new opportunities for the full use of MS/MS. The ability to collect MS/MS spectra without interference from other components in an impure sample will facilitate the construction of MS/MS data bases (high energy) (*33*). These data bases would be in the usual format: complete daughter spectrum for each m/z value having significant intensity in the normal spectrum. The conversion of the intensity vs B and t data to daughter spectra is readily accomplished. Other applications of MS/MS not utilizing on-line chromatographic separation, such as structure elucidation and studies of fragmentation pathways, could be carried out significantly more rapidly and consequently with much less sample. As liquid or supercritical fluid chromatographic–mass spectrometric interfaces become common, it will be possible to map constituents generated from biochemical digests. The data rate advantage of TRIMS–TAD will improve accuracy in the structural differentiation of compounds as well as aid in mixture analysis and, thus, increase the analytical utility of GC–MS/MS.

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