Multiplexed Rectilinear Ion Trap Mass Spectrometer for High-Throughput Analysis

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A multichannel mass spectrometer based on the rectilinear ion trap (RIT) analyzer was designed and constructed for simultaneous high-throughput analysis of multiple samples. The instrument features four parallel ion source/mass analyzer/detector channels assembled in a single vacuum chamber and operated using a common set of control electronics, including a single rf amplifier and transformer coil. This multiplexed RIT mass spectrometer employs an array of four millimeter-sized ion traps (x_o = 5.0 mm and y_o = 4.0 mm, where x_o and y_o are the half-distances in the x and y dimensions, respectively). Mass spectra are acquired from four different samples simultaneously. The available mass/charge range is m/z 15−510 with excellent linearity of the mass calibration (R^2 = 0.999 999). The peak width is less than 0.3 mass/charge units at m/z 146, corresponding to a resolution of ~500. Simultaneous MS/MS of ions due to four compounds (3-fluoroanisole, 4-fluoroanisole, 2-fluorobenzyl alcohol, 2,6-dimethylcyclohexanone) with the same nominal molecular radical cation but distinctive fragmentation patterns was demonstrated. Isolation and fragmentation efficiencies were ~25 and ~75%, respectively, measured in the typical case of the molecular radical cation of acetophenone. Preacquisition differential data were obtained by real-time subtraction of the ion signals from two channels of the multiplexed mass spectrometer. The differential experiment presented offers proof of principle of comparative mass spectra in high-throughput screening applications while reducing data storage requirements.

High-throughput mass spectrometry is potentially valuable in a number of scientific fields, including drug discovery,[1] metabolomics,[2,3] hazardous chemical detection,[4] and process monitoring.[5] This realization has resulted in the design, construction, and evaluation of several fully multiplexed mass spectrometers.[6−9] For the purposes of this discussion, the term multiplexed applies to instruments in which multiple samples are mass analyzed simultaneously, typically by using a mass spectrometer with equal numbers of sources, analyzers, and detectors, contained within a single vacuum system and operated using a common set of electronics. In this way, parallel analysis using the same method is achieved efficiently.

Fully multiplexed mass spectrometers reported to date have utilized either ion trap[6−8] or time-of-flight[9] mass analyzers. Arrays of cylindrical ion trap (CIT) mass analyzers have been successfully coupled to arrays of electrospray ionization (ESI) sources[6] as well as electron ionization/chemical ionization (EI/CI) sources[7] in two separate instrumental platforms. These fully multiplexed instruments were used to acquire mass spectra from multiple samples in parallel using multiple identical source/analyzer/detector channels. Also, a multichannel experiment was reported using an array of miniature CITs (r_o = 2.5 mm) in which a single sample was analyzed simultaneously by EI and methane CI in adjacent channels.[8] The experiment served as proof of principle that this type of multichannel instrumentation is capable of executing unique, parallel analyses on a single sample, yielding complementary data sets and offering a greater degree of confidence in detection. In a related experiment, data were recently published from a multichannel matrix-assisted laser desorption/ionization-time-of-flight mass spectrometer.[9] A single laser beam was split in order to simultaneously ionize two spots, each a mixture of the same bioagent simulants but exposed to different sample preparation reagents.

The trapping capacity of the CIT is reduced with any reduction in the size of the mass analyzer.[10−12] To overcome this limitation, miniature CITs have been arrayed to distribute the ion population among the ion traps, increasing the effective trapping capacity of the mass analyzer and regaining lost sensitivity.[12,13] These miniature arrays have the advantage of the lower rf voltage requirements inherent to miniaturized ion traps[13−18] and linear quadrupoles.[19−21] An alternative to the CIT, the linear ion trap

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(LIT) has a much greater ion trapping capacity and can be used instead. In fact, the LIT has been shown to be a superior mass analyzer in terms of trapping efficiency of externally generated ions, trapping capacity, and detection efficiency in comparison to similarly sized Paul-type ion traps, including cylindrical ion traps.[22,23] Utilization of the linear ion trap, both as a stand-alone mass analyzer and as a component of hybrid instruments, was recently reviewed.[24] A simplified version of the LIT, the rectilinear ion trap (RIT),[25] shows the expected improved performance over the CIT with regard to each of these figures of merit.[26]

To move multiplexed mass spectrometry beyond proof-of-principle experiments toward more routine high-throughput sample analysis, design considerations for robustness and quality of analytical performance are paramount. One of the factors that requires attention to achieve high performance in multiplexed mass spectrometers is cross-talk. Neutral cross-talk between adjacent channels of the EI/CI miniature CIT array mass spectrometer was the most severe problem encountered with previous instruments.[7,8]

This paper reports the construction and characterization of a multichannel mass spectrometer based on an array of rectilinear ion traps for high-throughput mass analysis. The instrument consists of four parallel source/analyzer/detector channels, each housed in one quadrant of a specialized vacuum manifold, designed to minimize neutral cross-talk. The vacuum manifold is divided with a welded stainless steel cross into four quadrants, entirely isolated from one another except near the flange to which the sole turbomolecular pump is connected. A single set of modified commercial electronics is used to control the simultaneous operation of all channels. Though the RITs are nominally identical, precision-machined devices, they will necessarily differ slightly in terms of higher-order field content due to mechanical dissimilarities. The multichannel experiments reported here include the simultaneous, high-throughput mass analysis of four unique analytes as well as tandem mass spectrometry (MS/MS) experiments performed in parallel on four different samples. The ion signals from two parallel channels in the multichannel instrument were subtracted in real time, prior to acquisition or normalization, in a new differential experiment. The multiplexed RIT mass spectrometer was characterized in terms of its mass/charge range, mass resolution, cross-talk, and isolation and fragmentation efficiencies in MS^n experiments. A detailed description of its design and assembly is given.

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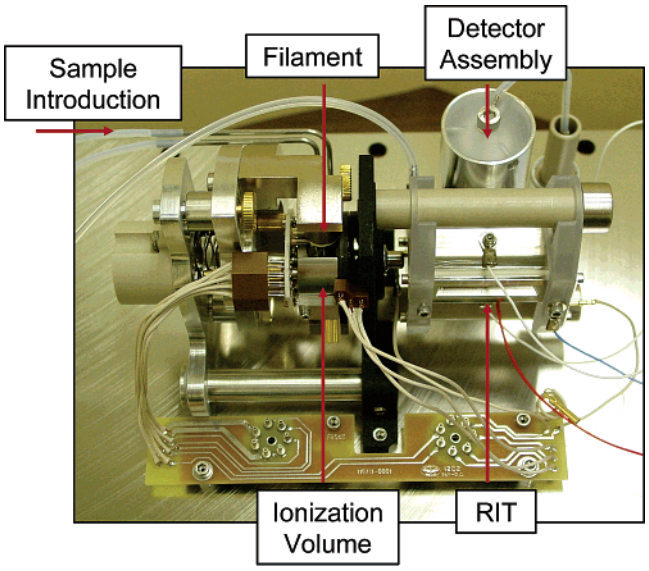

Figure 1. Source/analyzer/detector assembly of one channel of the multiplexed rectilinear ion trap mass spectrometer.

EXPERIMENTAL SECTION

The instrument consisted of multiplexed arrays of identical sample inlets, EI sources, ion-transfer optics, RIT mass analyzers, and channeltron electron multiplier detectors. The source/analyzer/detector assembly of one channel is pictured in Figure 1. The samples were introduced as vapors or gases into the ionization sources. Electron beams were produced from rhenium filaments orientated orthogonally to the ion optical paths of the analyzers. Magnets were positioned on either side of the ion sources to induce rotational motion of the electron beam and increase the ionization efficiency. Ions were extracted from the ion sources and transferred to the RIT mass analyzers through Einzel lenses. The RIT mass analyzers were each composed of three pairs (x, y, z) of rectangular electrodes; the pair of x electrodes had slits for radial ion ejection. The trapping rf voltage was applied to the four x,y electrodes, with the phase applied to the y pair 180° out of phase with that applied to the x pair. The small end cap (z) electrodes were positioned, one at each end of the RIT. Direct current voltages were applied to the end cap electrodes to create an axial potential well to trap ions. Ions enter the RITs axially through a hole in one of the end cap z electrodes, and as the rf amplitude is increased linearly, they are ejected mass-selectively and radially through the slits in the x electrodes and then allowed to impinge upon dynode/multiplier assemblies (one per channel of analysis) for detection. Simultaneous acquisition of data from each of the four channels utilizes a PCI-based data acquisition (DAQ) card (NI PCI-6071E, National Instruments Corp., Austin, TX) and a custom program written with LabVIEW software.[7] The instrument was designed for flexibility and not optimized for minimum footprint; future versions could be engineered to be much smaller with the same performance. A photograph of the multiplexed RIT mass spectrometer is shown in Figure S-1 (Supporting Information). Please note that the Supporting Information provides experimental details and figures not included herein for the sake of concision.

Vacuum System. The stainless steel vacuum manifold (custom-made, Nor-Cal Products, Inc., Yreka, CA), illustrated in Figure 2, was designed to minimize cross-talk between the four parallel ion

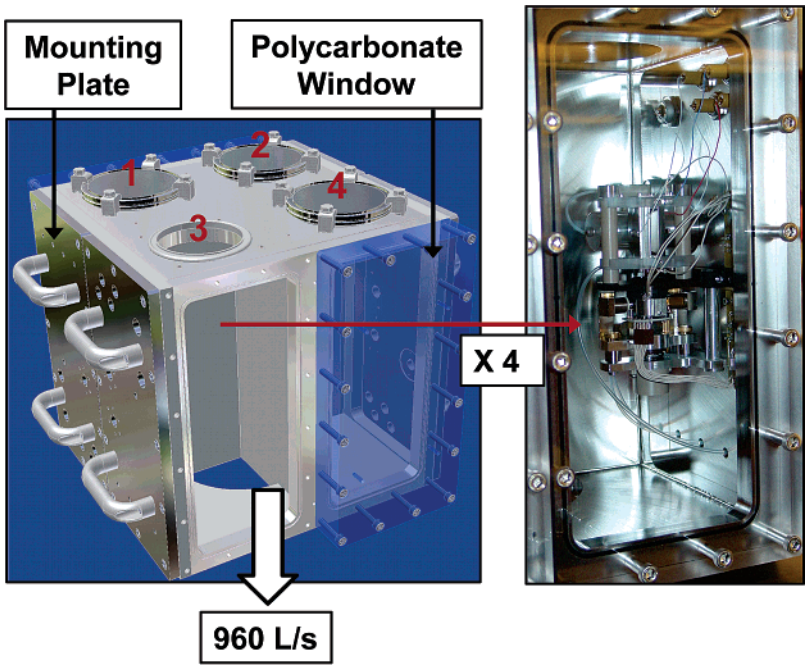

Figure 2. Mechanical drawing of stainless steel vacuum manifold designed to minimize cross-talk between the four parallel channels of analysis. An elongated cross was welded in place to divide the chamber into four quadrants while still permitting a single turbomolecular drag pump to be used. One source/analyzer/detector assembly is pictured as viewed through the polycarbonate window of one channel.

source/mass analyzer/detector channels. The overall size was 15 in. × 15 in. × 15 in. (38 cm × 38 cm × 38 cm), and the outside walls were 0.5 in. (13 mm) in thickness. The elongated cross that divides the cube into four quadrants was 0.25 in. (6.4 mm) in thickness and welded in place. The cross piece was not used for mounting or alignment. Typical background pressure was ~1.2 × 10^{-6} Torr. (All reported pressures are uncorrected.)

Rectilinear Ion Traps. The half-distance between the *x* electrodes (x_o) was 5.0 mm, while that between the *y* electrodes (y_o) was 4.0 mm. The four rectangular (*x* and *y*) electrodes were each 50 mm in length. The *x* electrodes had centered slits 29 mm in length and 1.0 mm in width for radial ion ejection. The closest edges of the *x* and *y* electrodes were separated by 1.6 mm. A planar (*z*) electrode, 0.79-mm thickness with a centered hole (3.0 mm in diameter) for axial ion injection along the *z* axis of the mass analyzer, was positioned 1.6 mm from each end of the rectangular electrodes. All electrodes were precision machined from type 316 stainless steel.

The drive rf (1.49 MHz) was applied to the four rectangular (*x* and *y*) electrodes of each RIT, 180° out of phase between the *x* and *y* pairs, to trap ions in the *xy* plane in an approximately quadrupolar field. In addition, an auxiliary resonance ejection waveform was applied 180° out of phase between the two *x* electrodes of each RIT, with no particular relation to the phase of the trapping rf. The maximum rf amplitude was calculated to be 3610 V_{0-p} (See the Results and Discussion subsection entitled Mass Range and Mass Resolution); that is, 1805 V_{0-p} was applied to each electrode pair in a dipolar fashion at the upper limit of the linear rf ramp. A dc potential was applied to each pair of planar end cap electrodes to create a potential well in the axial (*z*) direction.

The exact RIT geometry chosen for the mass analyzer array in this instrument was the result of prior experiments and multipole expansion coefficients calculated with CreatePot, a program in the ITSIM suite[26,27] developed in this laboratory. For the RIT geometry used here, the quadrupole coefficient was only 0.654. According to field calculations, the relative multipole contribution (reported as % A_n/A_2, where A_2 corresponds to the quadrupole and $n = 4$ or 6 correspond respectively to the octapole and dodecapole field coefficients in the expansion of the trapping potential), was 7.9% for A_4/A_2 and −17.2% for A_6/A_2.

Electron Ionization Sources. An array of four identical EI source assemblies was coupled to the set of RITs. These commercial sources were designed for use in the GCQ Plus quadrupole ion trap mass spectrometer produced by Thermo Electron Corp. (Part 119720-60047, Austin, TX). The experiments reported herein utilized electron ionization source volumes (Part 119710-20061, GCQ Plus) in which the ion exit aperture was approximately equal to the diameter of the cylindrical volume, viz. an open ion source. For each channel of mass analysis, the source and adjacent Einzel lens were aligned with the ion trap by means of the mounting bracket used in the commercial instrument (Part 96000-20111).

The sample introduction system permitted the delivery of four separate analytes directly into the four ionization sources through four separate 13-gauge (1.9-mm inner diameter) poly(tetrafluoroethylene) tubes press fit onto lengths of ~0.8-mm-inner diameter stainless steel tubing, which were welded to the gas inlet adapter (part 119715-60075, GCQ Plus, Thermo Electron Corp.) on the source assembly. There was an opening in the opposite side of the commercial source assembly designed for a gas chromatography column. This aperture was unused in the multiplexed instrument and was O-ring sealed using a custom stainless steel plate.

For the experiments described here, analytes were introduced as neat headspace vapors via four leak valves (series 203 Granville-Phillips, Helix Technology Co., Longmont, CO) after three freeze−pump−thaw cycles to an indicated pressure between 1.3 × 10^{-6}

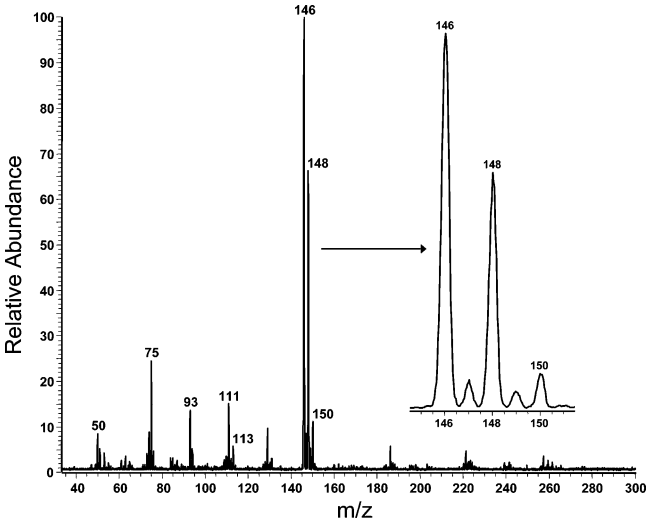

Figure 3. The 9:6:1 Cl_2 isotopic distribution characteristic of 1,3-dichlorobenzene (MW 147.00) used to establish the resolution of the instrument. The analyte was introduced into channel 4, and the spectrum was acquired in the normal scan mode at a scan rate of 3300 mass/charge units/s. Peak width (fwhm) for the ion m/z 146 is <0.3 mass/charge units, corresponding to a resolution ($m/\Delta m$) of ~500.

and 1.6×10^{-6} Torr. They included perfluorotributylamine (PFTBA; Scientific Instrument Services, Ringoes, NJ), bromobenzene (Fisher Scientific Co., Fair Lawn, NJ), aniline (Mallinckrodt Baker, Inc., Phillipsburg, NJ), and D-camphor (Eastman Chemical Co., Kingsport, TN); as well as 1,3-dichlorobenzene, acetophenone, L-carvone, 1,4-difluorobenzene, 3-fluoroanisole, 4-fluoroanisole, 2,6-dimethylcyclohexanone, 2-fluorobenzyl alcohol, *m*-chloroanisole, and *p*-chloroanisole (all purchased from Aldrich Chemical Co., Inc., Milwaukee, WI). After the introduction of analytes, helium buffer gas was added simultaneously into each of the four RITs by means of a single variable leak valve (series 203 Granville-Phillips, Helix Technology Co.) to an indicated pressure of $\sim 1 \times 10^{-5}$ Torr. Unless otherwise stated, the ionization time was 0.2 ms, the minimum value permitted by the control system.

Ion Detection and Multichannel Data Acquisition Systems

The electron multiplier associated with each RIT mass analyzer was used to detect ions ejected radially from that particular RIT. The shielded dynode/multiplier assembly (model 397, Detector Technologies, Inc., Palmer, MA) was positioned 5.0 mm from the outer face of one of the x electrodes. The multipliers were gated so that they were only biased to allow operation during the mass analysis period of the ion trap scan function. A multiplier bias of −1000 V was used for all channels in the particular experiments reported here.

Simultaneous data acquisition from four parallel channels of mass analysis was achieved with a custom program written in-house using LabVIEW software (version 7.1, National Instruments Corp.). The program allowed the display of all four spectra in real time. The four spectra also could be saved as four separate files for subsequent calibration and display. For the experiments described, a sampling frequency of 200 kHz/channel was used, and the acquired data were the average of five consecutive scans, each scan requiring less than 160 ms. Features of the four-channel data acquisition program include averaging (continuous or a fixed number of scans), calibration of the displayed mass scale, and multiple ion monitoring of as many as nine ions with different m/z values. Four current amplifiers (428-PROG, Keithley Instruments, Inc., Cleveland, OH), operated using a gain of 1×10^7 and a rise time of 10 μs, converted the low-level electron current from the detector anodes into a voltage that was subsequently amplified. The data acquisition card (PCI-6071E, National Instruments Corp.) was coupled to a 1-m noise-rejecting, shielded cable (SH10001000, National Instruments Corp.) and connector block (SCB-100, National Instruments Corp.). Four NI-DAQ mx global channels (±10 V, analog input) were operated in the reference single-ended configuration for acquisition, and the shielding of the coaxial cables coming from the inverting output of the current amplifiers was connected to the analog ground of the connector block. Acquisition was triggered by a transistor transistor logic pulse provided by the control electronics at the beginning of the scan out period in the scan function.

For the differential experiment (described in the final subsection, Results and Discussion), preacquisition subtraction of the amplified ion signals from two channels of the multiplexed mass spectrometer was achieved using a differential operational amplifier (low-noise preamplifier model SR560, Stanford Research Systems, Inc., Sunnyvale, CA) with unit gain.

Control Electronics. Primary control electronics for the multiplexed RIT instrument were slightly adapted versions of that used in the commercial LCQ Classic mass spectrometer (Thermo Electron Corp., San Jose, CA). Xcalibur 1.3 (Thermo Electron Corp.) was run on the instrument's control computer (Precision Workstation 360 with Pentium 4 processor 3.00 GHz and 2 GB of SDRAM memory, Dell Inc., Round Rock, TX), which communicated with the LCQ's internal 486 CPU board by means of a network card and ethernet cable. The LCQ electronics controlled the scan function, which consisted of periods for prescan (2 ms), ionization (0.2 ms), cooling (2 ms), and mass analysis (duration determined by the scan range and scan rate). For tandem and multistage mass spectrometry experiments, appropriate isolation and activation periods of user-specified duration were added between the ionization and mass analysis periods.

For the experiments described herein, nonlinear resonance ejection at $q_x = 0.81$ ($\beta_x = 0.70$) was utilized to achieve optimal resolution. The amplitude of the auxiliary waveform applied to each set of x electrodes was held constant at $V_{ac} = 2.78$ V_{pp} during the scan, unless otherwise stated. The rf scan rate used was ~3300 mass/charge units per second for initial experiments (described in first subsection, Results and Discussion). However, following two-point calibration of the LCQ electronics system, the scan rate used was 2885 mass/charge units per second. The LCQ electronics could be operated in the "low" (m/z 15−200) or "normal" (m/z 50−2000) scan mode for mass analysis or tandem mass spectrometry. The upper mass limit in the normal scan mode was determined by the maximum rf voltage achieved when the frequency was optimally tuned.

Static lenses were biased using a nine-channel, ±500 V variable power supply (TD9500 HV, Spectrum Solutions, Inc., Russellton, PA). These included the electron lens, the first element of the Einzel lens, and the third element of the Einzel lens. The biases for these lenses throughout the duty cycle were maintained at +15, −76, and −47 V, respectively. The nine-channel power supply

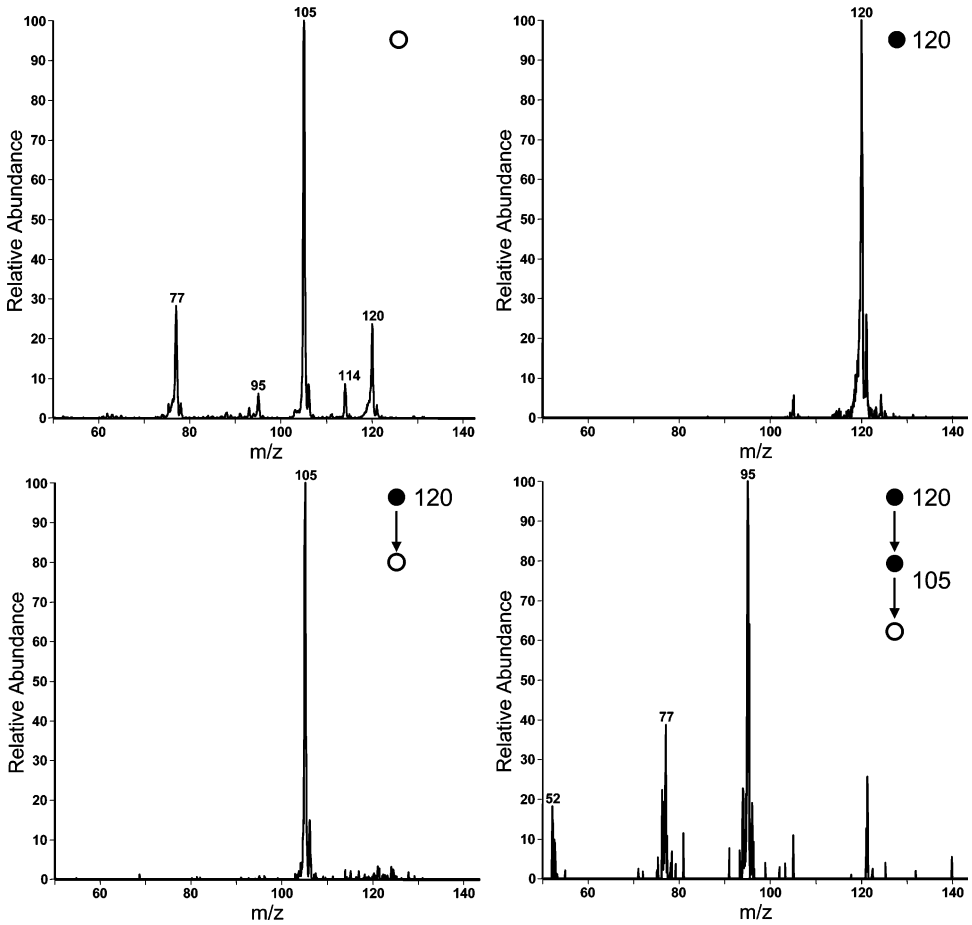

Figure 4. Mass spectrum of acetophenone, as shown in the upper left. In the nomenclature of MS/MS experiments, open and closed circles indicate mass analysis and isolation steps, respectively. The molecular radical cation of acetophenone at *m*/*z* 120 was isolated (upper right) and fragmented (lower left). Subsequently, the major fragment ion at *m*/*z* 105 was isolated and fragmented to give *m*/*z* 52, 77, and 95 (the product of addition of water to *m*/*z* 77), as seen in the lower right mass spectrum.

also provided the filament bias (−70 V) for each of the current supplies designed in-house to regulate electron emission current. The emission current for each channel was typically between 3 and 5 μA.

RESULTS AND DISCUSSION

Mass Range and Mass Resolution. The RIT mass analyzer utilized in the multiplexed mass spectrometer was developed as a geometrically simple alternative to linear ion traps. The mass range of a RIT with a quadrupole coefficient A_2 is given[24,28] by the following equation:

$$m/z_{\max} = A_2 4 V_{\max} (x_o^2 q_x \Omega^2)^{-1}$$

where m/z is the highest mass/charge ion (in kg·C^{-1}) that can be ejected at $V_{\max}$, the maximum rf amplitude (zero-to-peak), from an RIT with inner radius x_o (in m), operated with resonance ejection at q_x (dimensionless Mathieu parameter) and using an rf frequency of Ω (in rad·s^{-1}). The mass calibrant PFTBA (MW 671.09) was introduced into channel 4 of the multiplexed RIT mass spectrometer to determine the mass range and linearity in the normal mass scan mode. The amplitude of the resonance ejection waveform was ramped in parallel with the increase in rf amplitude, using a slope of 0.015 (V_{pp} per m/z) and an intercept of 1.66 V_{pp}. The resulting mass spectrum is shown in Figure S-2 in the Supporting Information. Fragment ions falling in the range from m/z 69 to 502 are present. Calculated from the equation above, the maximum rf amplitude is 3610 V_{0-p}. Calibration of the PFTBA spectrum (mass/charge versus time) exhibited excellent mass linearity ($R^2 = 0.999\,999$) and was later utilized for the two-point calibration routine. The low mass cutoff in the low mass scan mode was m/z ~15, which enabled water background ions to be seen (m/z 18). Other background peaks were routinely observed to be present at m/z 19 and 32.

The Cl_2 isotopic distribution characteristic of 1,3-dichlorobenzene (MW 147.00) was used to establish the resolving power of the instrument and to evaluate the accuracy with which ion abundances could be measured. This analyte was introduced into channel 4, and spectra were acquired in both the low and normal mass scan modes, the data for which are shown in Figures S-3 and 3, respectively. The ion abundances for the theoretical Cl_2 isotopic distribution (9:6:1) were measured to be 9.00:5.91:0.942. The peak width (fwhm, full width at half-maximum) for the m/z 146 ion is <0.3 mass/charge units, corresponding to a resolution ($m/\Delta m$, 50% valley) of ~500. The resolution of an ion trap mass

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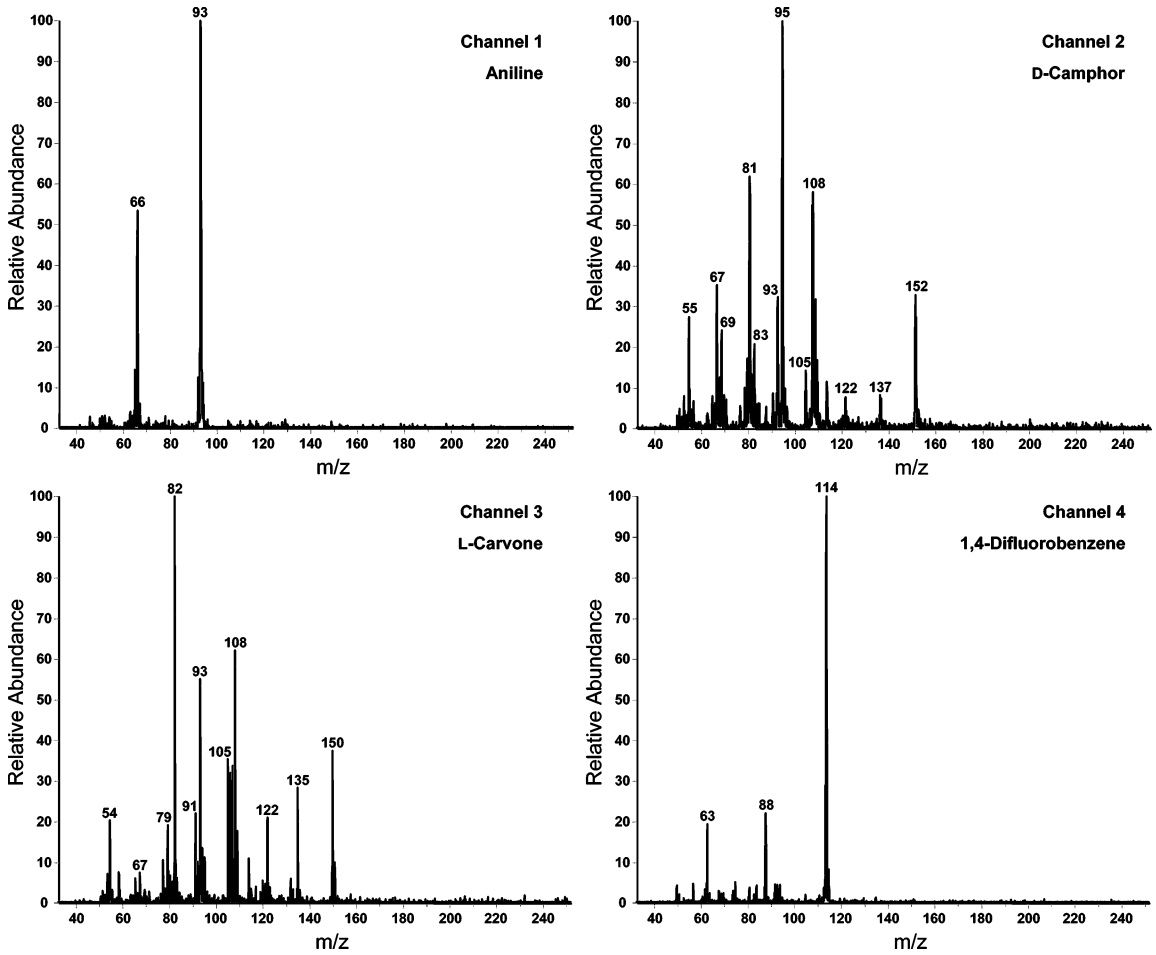

Figure 5. Mass spectra recorded by simultaneous analysis of aniline, D-camphor, L-carvone, and 1,4-difluorobenzene in channels 1, 2, 3, and 4, respectively.

spectrometer is affected by the rf scan rate. The molecular radical cation peaks for 1,3-dichlorobenzene (m/z 146−150) were recorded for scan rate values of 5515 mass/charge units/s (default after calibration of the LCQ electronics), 3801, 2885, and 1491 mass/charge units/s, as shown in Figure S-4. The resulting resolution values measured for the m/z 146 ion were 310, 380, 380, and 510, respectively. The ^{13}C isotopic peaks are clearly resolved for all but the fastest scan rate. The signal intensity decreased when using the slowest scan rate (1491 mass/charge units/s), and the ionization time was increased to 10 ms to compensate. The scan rate used for the experiments described herein was chosen to be 2885 mass/charge units/s, which required 156 ms to scan from m/z 50 to 500 (normal mass scan mode) and 64 ms to scan from m/z 15 to 200 (low mass scan mode).

It should be noted that the data acquired from channel 4 in the multiplexed mass spectrometer are presented here as representative of the instrument. That is, all four RIT mass analyzers provided much better than unit mass resolution during the analysis of 1,3-dichlorobenzene under optimized operating conditions, which included nonlinear resonance ejection performed at $q_x = 0.81$ ($\beta_x = 0.70$) with $V_{ac} = 2.78$ V_{pp}. However, when the amplitude of the auxiliary waveform applied to the x electrodes was decreased, specifically to $V_{ac} = 1.32$ V_{pp}, two strong sets of signals were observed in the spectrum from channel 4 (not shown), one corresponding to nonlinear resonance ejection of the molecular radical cation distribution and the second to boundary ejection of the same ions. At the same time, using identical conditions, no boundary ejection peaks were visible in the spectra of channels 1−3. This experiment was suggested in a preliminary communication as a novel method of comparing ion trap mass analyzers,[29] one that is particularly effective when distinguishing nominally identical mass analyzers operated from a single power supply. The fact that the ejected ion signal associated with resonance energy uptake can be referenced to the amount of ejection due to normal boundary ejection makes this a sensitive measure of higher-order field contributions. Subtle mechanical dissimilarities between the RITs are believed to be the cause of the dramatic differences in the mass spectra recorded under the appropriately chosen operating conditions (resonance ejection frequency and amplitude). Thus, the RIT used as the mass analyzer in this novel multichannel instrument is simultaneously a device in which the quadrupole fields are of poor quality due to its simplified geometry and a high-performance ion trap (providing much better than unit mass/charge resolution).

Tandem Mass Spectrometry. The capability to perform MS^n experiments is an attribute of the multiplexed RIT mass spectrometer that affords increased selectivity in chemical analysis. Triple-stage (MS^3) experiments were demonstrated, and isolation and fragmentation efficiencies were measured for acetophenone

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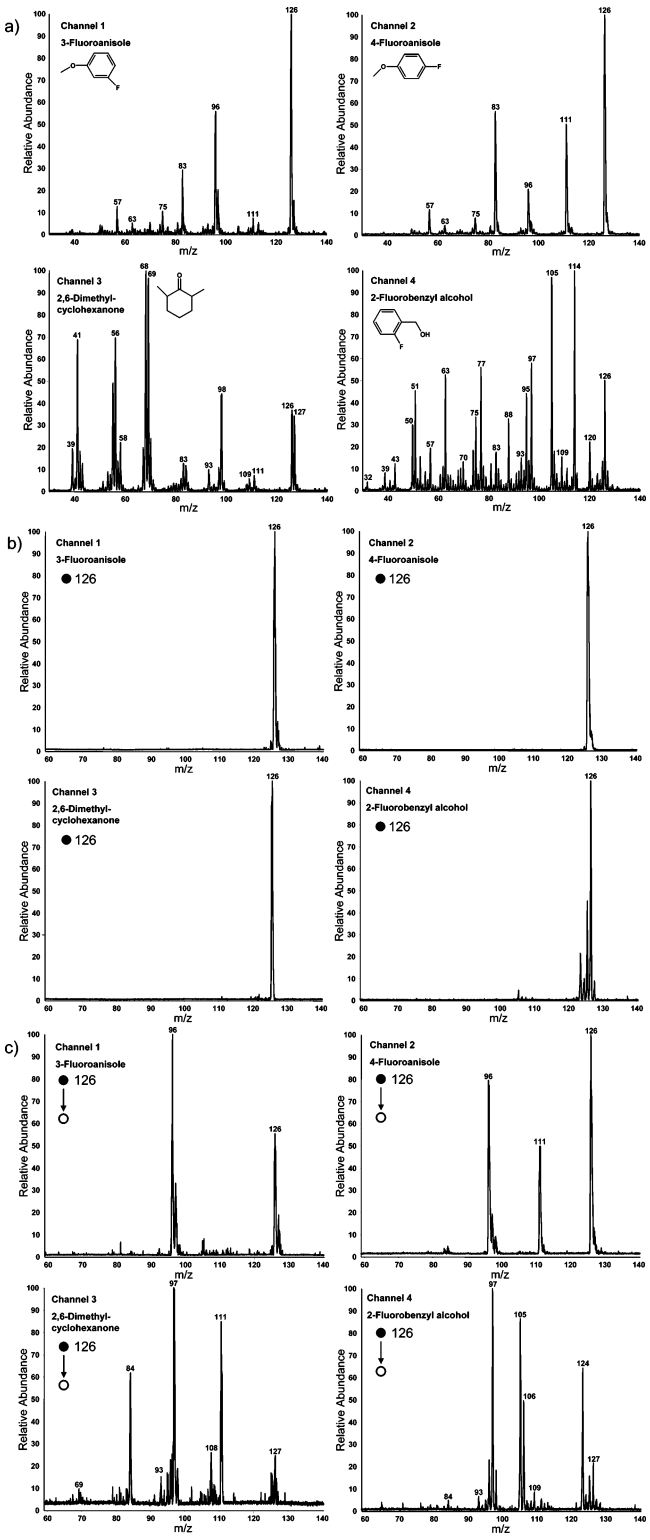

Figure 6. Four isomeric and isobaric analytes (*m*/*z* 126) chosen for simultaneous MS and MS/MS analysis: 3-fluoroanisole, 4-fluoroanisole, 2,6-dimethylcyclohexanone, and 2-fluorobenzyl alcohol, introduced into channels 1−4, respectively. (a) Mass spectra and molecular structures; (b) Isolation of *m*/*z* 126 (±4) in each channel; and (c) resulting MS/MS product ion spectra when the *m*/*z* 126 ions with unique chemical identities were activated simultaneously. Major fragment peaks are labeled to illustrate the distinctive fragmentation patterns of these four species.

(MW 120.15) in channel 4 of the instrument. The molecular radical cation at m/z 120 (±9) was isolated and fragmented. Subsequently, the major fragment ion at m/z 105 (±5) was isolated and fragmented to m/z 52, 77, and 95 (the product of addition of water to m/z 77). Note that the parameters reported here are those entered into LCQ Tune, the control program for the LCQ electronics, to achieve the best performance. Isolation was optimized in the MS^n mode while holding the activation energy value at 0% and the activation time at 1 ms. Figure 4 depicts the MS^3 data in four frames. An isolation width of 18 mass/charge units was used for m/z 120; viz. the window passed ions of m/z 111 to 129, and activation was executed at $q = 0.3$ with a nominal activation energy of 50%, applied for 50 ms. Subsequently, ions at m/z 105 (±5) were isolated, and then activated at $q = 0.4$ with an energy of 95% for 60 ms. An isolation efficiency of ~25% was obtained for m/z 120 with a fragmentation efficiency of ~75%. The overall MS^3 efficiency was ~1%. Separate MS/MS product ion spectra (not shown here) of m/z 105 (±5) yielded ~90% isolation efficiency and ~20% fragmentation efficiency.

Simultaneous Multichannel Mass Analysis. The four-channel rectilinear ion trap instrument can be used to perform parallel mass analysis on four unique samples. This capability underscores the motivation for multiplexing an ion trap mass spectrometer while maintaining a common electronics system and vacuum system. Acetophenone, PFTBA, bromobenzene (MW 157.01), and 1,3-dichlorobenzene were introduced into the electron ionization source volumes of channels 1, 2, 3, and 4, respectively. Note that these are the same four analytes used in characterization of the multiplexed miniature CIT array instrument previously reported,[7] and they were chosen to allow direct comparisons of performance. Mass spectra of each of the four samples were acquired simultaneously, as shown in Figure S-5. Considerable improvement over the data obtained from the multiplexed CIT array mass spectrometer was seen, with less than ~5% cross-talk between adjacent channels in the current RIT instrument. Peaks attributed to cross-talk are typically due to the most abundant ions in an adjacent channel. Cross-talk is most accurately reported as a ratio of peak areas, namely, the sum of the areas of the cross-talk peaks divided by the total peak area of the entire spectrum. The cross-talk is 3.1, 5.1, and 3.0% for the spectra acquired from channels 1, 2, and 4 in this experiment, respectively. The remaining minor cross-talk contribution can be removed from spectra with postacquisition data processing. The mass spectrum of the species causing cross-talk is scaled to the relative intensity of the largest cross-talk peak present in the spectrum of interest and the spectra are subtracted.

The response of the multiplexed RIT mass spectrometer to other chemical species was tested by performing an additional high-throughput experiment. Figure 5 shows data obtained from the simultaneous mass analysis of aniline (MW 93.13), D-camphor (MW 152.23), L-carvone (MW 150.22), and 1,4-difluorobenzene (MW 114.09) in channels 1, 2, 3, and 4, respectively. The m/z 114 peak of 1,4-difluorobenzene (introduced into channel 4) is present as a cross-talk peak in the spectra of D-camphor in channel 2 and L-carvone in channel 3. The ratio of the peak area of m/z 114 to the total peak area in channel 2 is 1.8%, while that ratio in channel 3 was calculated to be 1.3%. The volatility of 1,4-difluorobenzene is nearly 2 orders of magnitude greater than that

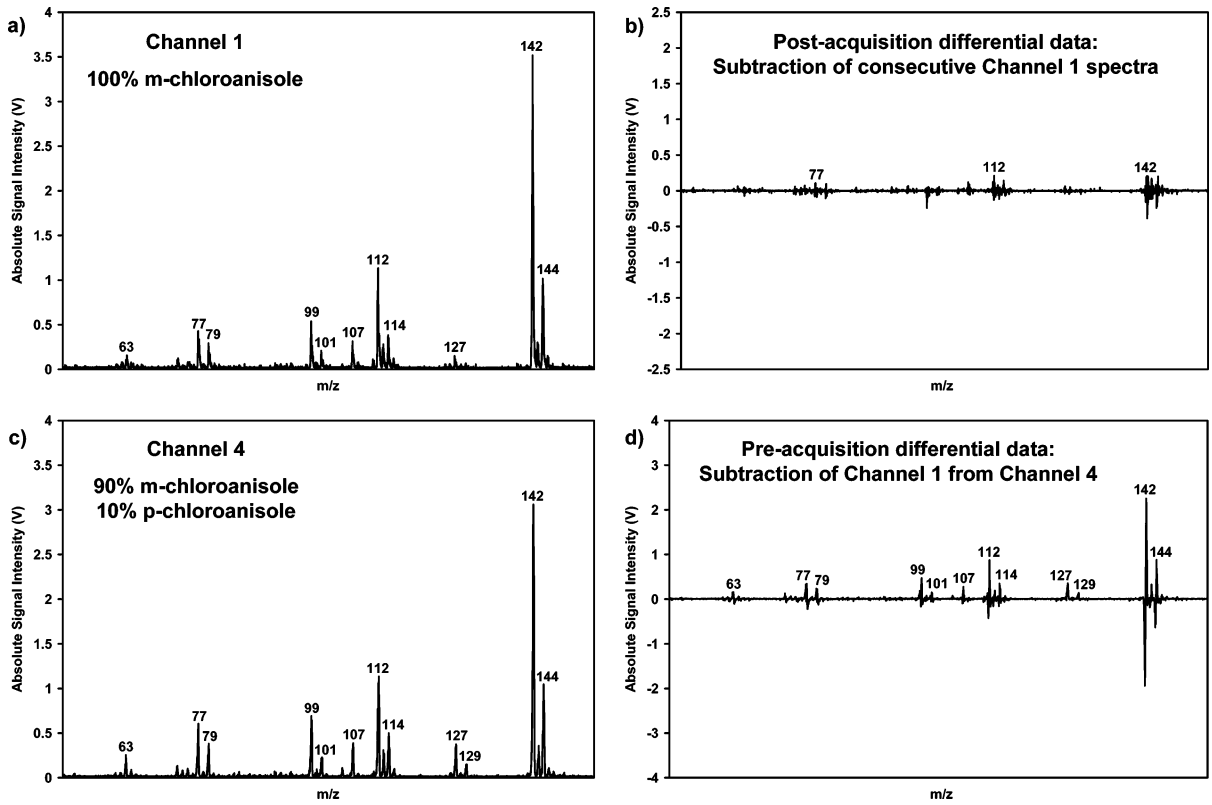

Figure 7. Novel differential experiment performed using the multichannel instrument. Mass spectra of (a) 100% *m*-chloroanisole and (c) a mixture consisting of 90% *m*-chloroanisole and 10% *p*-chloroanisole were recorded simultaneously in channels 1 and 4, respectively. Subtraction of consecutive spectra acquired from channel 1 resulted (b) only in low-level noise. By contrast, preacquisition subtraction of the analog ion signal of channel 1 from that of channel 4 using a differential operational amplifier allowed comparison of the spectra in real time and produced the spectrum in (d), which shows characteristic features of the minor (10%) component.

of the other analytes.[30] There is a correlation between high volatility and the probability of cross-talk. This provides evidence for the fact that cross-talk is the result of neutral analyte molecules migrating into the ionization volume or ion-transfer path of an adjacent channel, via the common region above the turbopump inlet. Using a more enclosed ionization volume or a directional baffle in the region above the turbopump would further reduce the likelihood of cross-talk ion signal. It is worth noting that other ionization mechanisms, especially ESI used in a previous multiplexed instrument,[6] give negligible cross-talk.

Multichannel MS/MS. The auxiliary ac applied to the x electrodes of the RIT mass analyzers for ion isolation and activation in tandem mass spectrometric experiments is coupled to the appropriate phase of the rf at the secondary winding of the coil. In this way, the same signals are applied to all four traps simultaneously, and ions of the same m/z range are isolated and activated.

Because the same ion(s) is (are) isolated and activated in all four channels simultaneously during a four-channel MS/MS experiment, it is imperative that the calibration of the RITs be the same. Calculation of the calibration curve for each of the channels and comparison of slope and intercept values indicated that the calibration is indeed very similar among the four channels, with three being nearly indistinguishable. Channel 3 proved to be the exception, exhibiting an offset of ~2 mass/charge units (later ejection) at m/z ~150 compared to the other three channels.

To illustrate the multichannel tandem mass spectrometric ability of the multiplexed RIT mass spectrometer, four different analytes that gave ions of m/z 126 (isomers and isobars) were chosen for simultaneous MS and MS/MS analysis. Three of the four analytes are structural isomers (having a molecular mass of 126.13 Da), two of which are positional isomers with differing fragmentation patterns. The fourth analyte is an isobar (2,6-dimethylcyclohexanone, MW 126.20), which shares a nominal molecular mass of 126 Da with the other three. The species involved are 3-fluoroanisole, 4-fluoroanisole, 2,6-dimethylcyclohexanone, and 2-fluorobenzyl alcohol, and they are introduced into channels 1, 2, 3, and 4, respectively. The simultaneously acquired mass spectra and molecular structures are displayed in Figure 6a. Isolation of m/z 126 was performed using an isolation centered at m/z 124 ($\pm$4). The resulting spectra are shown in Figure 6b. Note that the slight difference in the calibration of channel 3 required a compromise in selecting isolation parameters in comparison to those that would be ideal for each channel separately. In channel 3, m/z 126 was successfully isolated from the peak due to the adjacent ion m/z 127, which had similar intensity. However in channel 4, the isolation is not clean because m/z 123−127 peaks are visible. The selected m/z 126 ions (with unique chemical identities) were activated simultaneously at q = 0.4 with 50% normalized activation energy for 50 ms. The resulting MS/MS spectra are displayed as Figure 6c. Major fragment peaks are labeled to illustrate the distinct fragmentation patterns and the increased selectivity of analysis afforded by tandem mass spectrometry in the multiplexed RIT instrument.

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Preacquisition Subtraction of Ion Signals. A novel multichannel experiment utilized a differential operational amplifier to subtract the signals of two channels prior to acquisition. In this way, analytical informatics could be accomplished in hardware rather than software, and the storage space required for high-throughput data could be reduced. An example of the potential utility of this novel multichannel experiment is high-throughput screening in which the presence or absence of minor components is to be assessed rapidly.

A proof-of-principle differential experiment was performed, the data from which are shown in Figure 7. Spectra are displayed as absolute signal intensity (voltage of the amplified ion signal) versus mass/charge. For proper prospective, the differential data are shown on the same scale as the two ion signals from which they were determined. Pure *m*-chloroanisole (MW 142.58) was introduced into channel 1 while a mixture consisting of 90% *m*-chloroanisole and 10% *p*-chloroanisole was introduced into channel 4. Panels a and c in Figure 7 show the spectra acquired from channels 1 and 4, respectively. Positional isomers *m*- and *p*-chloroanisole have unique fragmentation patterns. For *p*-chloroanisole, the loss of the methyl radical (m/z 127) or CH_3CO (m/z 99) is favored over the loss of CH_2O (m/z 112). However, for *m*-chloroanisole, the latter two mechanisms are predominant, and thus, m/z 127 is a minor component of its mass spectrum (Figure 7a). Preacquisition subtraction of the ion signal of channel 1 from that of channel 4 (Figure 7d) produced a real-time comparison of the two signals, highlighting the presence of the minor component (*p*-chloroanisole). The principal features that indicate the presence of *p*-chloroanisole, the peaks at m/z 127 and 129, do not have negative-going components in the differential spectrum. The appearance of both positive- and negative-going peaks at particular m/z values is ascribed to statistical variation in peak shapes as well as the slight difference in the mass calibration between channel 1 and channel 4. For routine use of the real-time differential experiment described here, it would be essential that channels of interest have identical mass calibration. However, postacquisition data processing using analytical informatics could correct for even minute differences in mass calibration, detector gain, etc., between parallel channels of the multiplexed mass spectrometer. It should be noted that postacquisition subtraction of the unaltered spectra produced data (not shown) that were indistinguishable from the preacquisition differential data.

By contrast to the differential data obtained by subtracting the ion signal of channel 1 from that of channel 4, postacquisition subtraction of consecutive spectra acquired from channel 1 yielded the spectrum in Figure 7b. Subtraction of spectra from the same channel ensured identical mass calibration. The presence of only low-level noise, mainly caused by statistical variation in peak shapes, is evidence for the excellent reproducibility of the instrument.

CONCLUSIONS

A mass spectrometer with four parallel channels allows simultaneous high-throughput mass analysis, or tandem mass analysis, of four unique analytes ionized using electron ionization. Increased specificity is provided by tandem mass spectrometry as demonstrated by the multichannel tandem mass analysis of four compounds. A differential multichannel experiment in which spectra are compared in real time, prior to acquisition, was demonstrated for future development of high-throughput screening applications using multiplexed mass spectrometers. In future multiplexed experiments, the large quantity of data generated will be analyzed with the algorithms of analytical informatics.

Future applications of this instrument will be directed toward characterization of four parallel gas streams flowing from separate catalyst beds. In parallel with this effort, a multichannel ESI RIT mass spectrometer is under construction, and engineering efforts to scale up beyond four mass analysis channels are in progress. The current rf electronics need to be significantly redesigned to apply unique auxiliary ac waveforms, either at the same time or in a rapid, sequential fashion, for the purpose of performing multichannel MS/MS on different and arbitrarily selected precursor m/z ions.

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SUPPORTING INFORMATION AVAILABLE

Additional experimental details and figures as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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