Application of External Customized Waveforms to a Commercial Quadrupole Ion Trap

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The Finnigan LCQ quadrupole ion trap has recently become part of the repertoire of instruments for many analytical laboratories. The LCQ commercial design, while employing complex waveforms to manipulate ions, does not allow the application of many state-of-the-art user-defined waveforms that enable one to perform other complex ion manipulations. The work presented here describes the simple modifications made to the LCQ electronics to allow the application of external customized waveforms. Results show that externally generated waveforms can be applied to the endcap electrodes while still working within the context of the commercial software and hardware. Double resonance, multiple ion isolation, and multiple ion excitation experiments are demonstrated to reveal the effectiveness of these modifications. (J Am Soc Mass Spectrom 1999, 10, 355–359) © 1999 American Society for Mass Spectrometry

Quadrupole ion traps have been used for a wide range of investigations from fundamental ion chemistry to peptide/protein sequencing [1]. The potential realized in quadrupole ion traps arises largely from its ability to store and manipulate a range of ions. Trapped ions can be controlled by changing the main rf drive voltage on the ring electrode, applying external dc voltages to the ring electrode, supplying auxiliary ac signals to the endcap electrodes, or employing any combination of these signals simultaneously. The most common means of affecting ion motion in the quadrupole ion trap is by applying supplementary waveforms to the endcap electrodes to resonantly increase the oscillations of one or more mass-to-charge ratios (m/z). Complex waveforms can also be applied to the endcap electrodes that allow complicated manipulations of ions to be performed. Stored waveform inverse Fourier transform (SWIFT) [2] has been used in quadrupole ion traps to selectively accumulate, isolate, and excite ions of interest [3–6]. Other methods of selective ion storage include filtered-noise fields [7–9], tailored waveforms (used by the Finnigan LCQ) [10, 11] and field-modulated constructed waveforms [12, 13].

A particular instrument that has seen recent commercial success is the LCQ manufactured by Finnigan (San Jose, CA). This instrument allows the user to easily perform MS/MS and MS^n experiments in which selected parent and product ions can be isolated and resonantly excited. Although the LCQ allows the user many choices for manipulating analyte ions, user-defined waveforms cannot be applied with the available software. As a result, we desired to modify the LCQ to allow the application of user-defined arbitrary waveforms within the context of the existing software and hardware. The following results show that these modifications can be easily made, and as examples of the capability of applying arbitrary waveforms, multiple-notch broadband waveforms, multiple excitation waveforms, and double resonance experiments have been demonstrated.

Experimental

A Finnigan LCQ quadrupole ion trap was used in these studies. Ions were generated by electrospraying solutions containing the analyte(s) of interest. The advanced scan definition page used in some of the experiments was obtained from Finnigan. The external waveforms used in these experiments were generated using an Odyssey data system (Finnigan FT/MS, Madison, WI) developed for Fourier transform ion cyclotron resonance mass spectrometers. SWIFT waveforms were created in the SWIFT editor of the Odyssey software and were applied to the LCQ after being initiated by a trigger pulse from the LCQ electronics. Although all of the experiments described here were performed using waveforms from the Odyssey data system, the same experiments could also be easily done using any arbitrary waveform generator that has the ability to generate complex multifrequency waveforms.

The application of user-defined arbitrary waveforms

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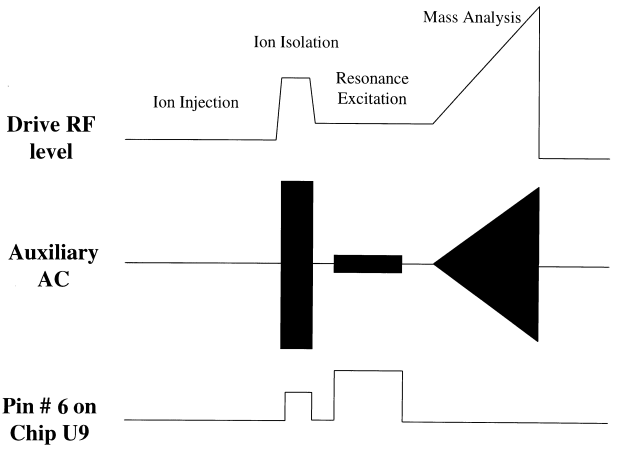

Figure 1. A timing diagram of a normal MS/MS experiment on the LCQ showing the drive rf level, the auxiliary ac signal applied to the endcaps, and the output of pin #6 on chip U9 of the waveform amplifier board.

in these experiments required the removal of the LCQ system waveforms in certain situations. The LCQ can apply four different waveforms to the endcap electrodes: an ion injection waveform, an ion isolation waveform, a resonance excitation waveform, and an axial modulation (resonance ejection) waveform. These waveforms are applied to the endcap electrodes through the waveform amplifier board, which has two inputs. One input (labeled AUX RF IN) is used to send a single frequency sinewave to the waveform amplifier board where its amplitude is then set accordingly to produce the resonance excitation or axial modulation (resonance ejection) signal. The other input (labeled WAVEFORM) allows the multifrequency isolation and injection waveforms to be sent through the amplifier board to the endcap electrodes. In the experiments described below, one or both inputs were removed and replaced with signals generated from an arbitrary waveform generator.

Precise timing for the external waveform application was achieved using a trigger pulse from the waveform amplifier board at the beginning of the ion isolation period during an MS/MS experiment. Figure 1 is a timing diagram of a normal MS/MS experiment showing the drive rf level, the auxiliary ac signal applied to the endcaps, and the output of pin #6 on chip U9 of the waveform amplifier board as a function of time. The importance of the output of pin #6 on chip U9 will become apparent as this discussion continues. During a normal MS/MS experiment, the LCQ isolates the ions of m/z chosen in the software by increasing the main rf drive voltage to a level that brings the ions of interest to a q_z value of 0.83. While these ions are stored at this rf level, an isolation waveform is applied to the endcap electrodes for 16 ms which isolates the specified m/z range around the ions of interest. After the application of the ion isolation waveform the rf drive voltage is decreased to a level that stores the isolated ions at a q_z value of 0.25. After a 6 ms delay a low-voltage single frequency sinewave is then applied to the endcaps for 30 ms to resonantly excite the isolated ions. This 30 ms resonance excitation time is followed by a 3 ms delay and an rf voltage ramp for mass analysis. In order to apply external signals during either the isolation or the resonance excitation portion of the scan, the timing of these signals must be very precise. Such precise timing can be achieved by using the first pulse from pin #6 on chip U9 of the waveform amplifier board as the trigger to initiate external waveform application. The external waveform generator is set to recognize the level of the first pulse and the second pulse is ignored while the waveform generator produces the desired string of waveforms and delays. The first pulse from pin #6 of chip U9 is timed with the beginning of the LCQ isolation waveform. Timing from this pulse allows an external waveform to be applied instead of the LCQ isolation waveform. Also, with the proper delay, an external excitation signal can be applied instead of the LCQ resonance excitation signal.

Results and Discussion

Double Resonance

Excitation of a parent ion, while simultaneously ejecting one of its product ions, is an experiment referred to as double resonance. The double resonance experiment was chosen to show the ability of the above described modifications on the analysis of the peptide leucine enkephalin (YGGFL). During this experiment the protonated species of YGGFL (m/z 556) was resonantly excited while the $\mathbf{b_4}$ product ion (m/z 425) was resonantly ejected. Table 1 shows the connections made to the inputs AUX RF IN and WAVEFORM and the details of the waveforms applied. One concern was that the direct coupling of the external single frequency signal with the LCQ signal would distort one or both signals. Very little signal distortion, however, was observed (i.e., high frequency noise $\sim$ 10 mV, $\sim$ 15% attenuation of external signal, and no attenuation of LCQ signal). Figure 2 shows a block diagram of the setup used for this experiment. In the LCQ software an MS/MS experiment was setup in which m/z 425 was chosen as the parent mass and the relative collision energy was chosen to be 100%. Because the normal WAVEFORM connection was removed, an external waveform was needed to isolate m/z 556 (see Table 1). The pulse from pin #6 on chip U9 was used to trigger the application of a 16 ms SWIFT waveform. The generation of the SWIFT waveform took into account the fact that m/z 556 has a q_z value of 0.63 when m/z 425 has a q_z value of 0.83, and the frequency range to isolate m/z 556 was calculated accordingly. The protonated species of YGGFL was then dissociated by applying an external single frequency signal (see Table 1) to the AUX RF IN input of the waveform amplifier board 22 ms after the trigger pulse. Simultaneously the LCQ supplied a single fre-

Table 1. Details of the waveforms applied to AUX RF IN and WAVEFORM during the double resonance, multiple ion isolation, and multiple ion excitation experiments

Experiment	AUX RF IN	WAVEFORM
Double resonance	**LCQ**: resonance ejection: 30 ms, 5.0 V_{p-p} for m/z 425 (q_z = 0.25, 67.56 kHz); 3 ms delay then axial modulation: 12 V_{p-p}, 348 kHz **External**: resonance excitation: 30 ms, 0.70 V_{p-p} for m/z 556 (q_z = 0.19, 51.72 kHz)	**External**: 16 ms SWIFT isolation − 12 V_{p-p} for m/z 556 (q_z = 0.63, 187.6–193.8 kHz)
Multiple ion isolation	**LCQ**: axial modulation: 12 V_{p-p}, 348 kHz	**External**: 16 ms SWIFT isolation − 12 V_{p-p} for m/z 495 (q_z = 0.64, 187.0–190.4 kHz) for m/z 611 (q_z = 0.51, 146.2–150.8 kHz) for m/z 727 (q_z = 0.43, 121.0–125.7 kHz)
Multiple ion excitation	**External**: 30 ms SWIFT excitation ~ 1 V_{p-p} for m/z 495 (q_z = 0.31, 83.84 ± 0.03 kHz) for m/z 611 (q_z = 0.25, 67.41 ± 0.03 kHz) for m/z 727 (q_z = 0.21, 56.43 ± 0.03 kHz) 3 ms delay then axial modulation: 160 ms, 12 V_{p-p}, 348 kHz	**External**: 16 ms SWIFT isolation − 12 V_{p-p} for m/z 495 (q_z = 0.64, 187.0–190.4 kHz) for m/z 611 (q_z = 0.51, 146.2–150.8 kHz) for m/z 727 (q_z = 0.43, 121.0–125.7 kHz)

quency signal corresponding to the secular frequency of the product ion at m/z 425. Because the LCQ stores ions subjected to CID at a q_z value of 0.25, the resonant excitation frequency for m/z 556 had to be chosen accordingly. In this case m/z 425 is stored at a q_z = 0.25, and an easy calculation determines that m/z 556 is stored at a q_z = 0.19.

The combined result of applying these waveforms is to dissociate m/z 556 while resonantly ejecting any product ions formed at m/z 425. Figure 3 shows the MS/MS spectra of YGGFL with (a) and without (b) resonantly ejecting the $\mathbf{b_4}$ product ion. A comparison, for example, of the $\mathbf{a_4}$ product ion (m/z 397) with and without the double resonance experiment shows that about 86% of the $\mathbf{a_4}$ product ion results from dissociation of the $\mathbf{b_4}$ product ion (compare the intensity of m/z 397 with m/z 538 in both spectra; the intensity of m/z 538 is not affected in the double resonance experiment). This result is the same as reported recently for double resonance experiments of YGGFL [14].

Multiple Ion Isolation and Excitation

Multiple ion isolation was applied to a mixture of vinylbenzene substituted triethylenetetramines using SWIFT. Table 1 shows the appropriate connections, q_z values, and waveform frequencies for this experiment. In this experiment ions at m/z 495, 611, and 727 were isolated. To accomplish this, an MS/MS experiment was set up in which the parent mass chosen in the

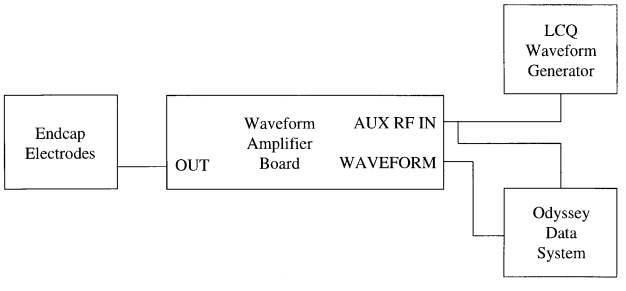

Figure 2. A block diagram showing the connections of the various components used to perform the double resonance experiment.

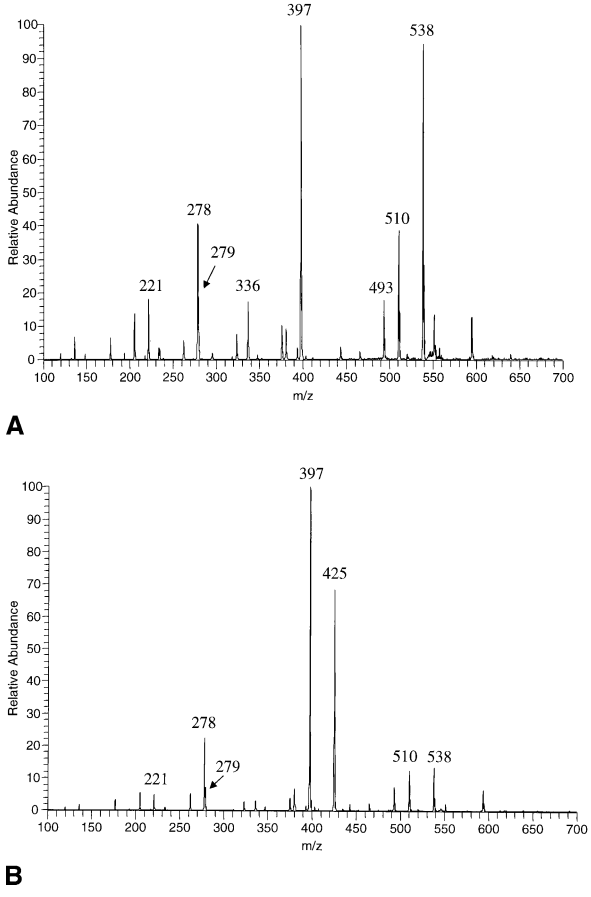

Figure 3. (**a**) Spectrum resulting from the resonance excitation of the protonated species of leucine enkephalin (YGGFL, m/z 556) while resonantly ejecting the $\mathbf{b_4}$ product ion (m/z 425). (**b**) Spectrum resulting from the resonance excitation of the protonated species of leucine enkephalin (YGGFL, m/z 556) without resonantly ejecting the $\mathbf{b_4}$ product ion (m/z 425).

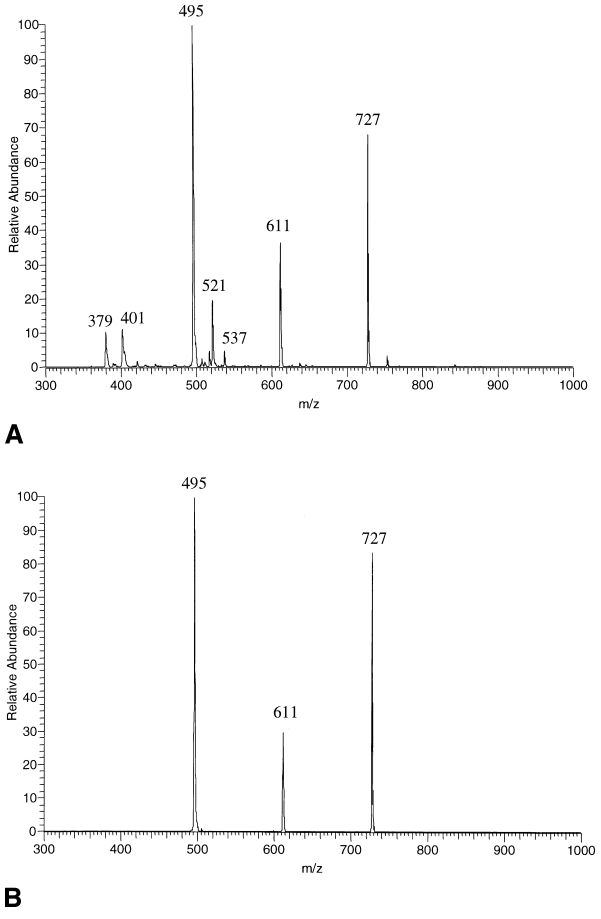

Figure 4. (**a**) Mass spectrum of a mixture of vinylbenzene substituted triethylenetetramines. (**b**) Mass spectrum of the same mixture of vinylbenzene substituted triethylenetetramines while applying a SWIFT pulse to isolate ions at m/z 495, 611, and 727.

software was m/z 379. The widths of the SWIFT frequency notches were not narrow enough for single mass isolation, but instead were sufficient to isolate the isotopic cluster of each analyte. Again, the external signal was triggered from pin #6 on chip U9 and applied to the WAVEFORM input during the ion isolation portion of the LCQ scan. Figure 4 shows the mass spectra of these vinylbenzene substituted tetramines before (a) and after (b) application of the SWIFT pulse. Clearly, effective isolation of each is accomplished.

Simultaneous excitation of these isolated ions was performed using another SWIFT waveform that excited a small range of frequencies around the secular frequencies of each ion. Table 1 outlines the appropriate connections and waveform details. The SWIFT excitation pulse was applied 22 ms after the trigger pulse from pin #6 on chip U9 and lasted 30 ms. In this multiple ion excitation experiment, the parent mass again was chosen to be m/z 379 in the software, but in this case the storage q_z value during the resonance excitation portion of the scan was chosen to be 0.40 for m/z 379. (Although the advanced scan definition page was used in this experiment, the same experiment can very easily be done using the normal scan definition page.) Also, the

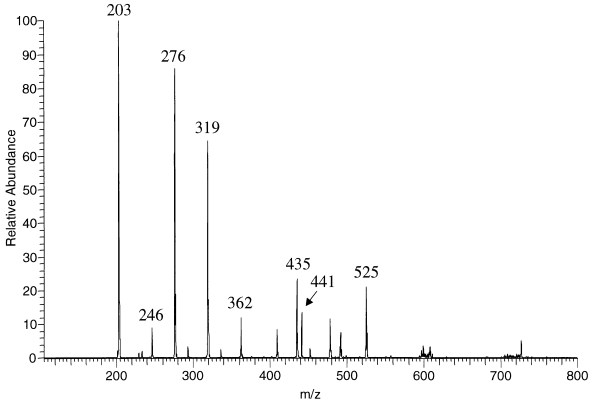

Figure 5. Spectrum resulting from the simultaneous resonance excitation of all three isolated vinylbenzene substituted triethylenetetramines (m/z 495, 611, and 727).

relative collision energy (or activation amplitude in the advanced scan definition page) was chosen to be 100% (or 5.0 V in the advanced scan definition page). The waveform amplifier board controls the voltage that is eventually applied to the endcaps, so by choosing the maximum voltage in the software, the full voltage of the incoming signal, in this case a SWIFT waveform of low voltage ($\sim$ 1 V_{p-p}), can be accessed. Because the LCQ's signal into the AUX RF IN input was removed, a signal for the axial modulation waveform was provided by the Odyssey data system (see Table 1). During the rf acquisition ramp, the waveform amplifier board controls the amplitude of the incoming waveform so that the 12 V_{p-p} external signal is actually attenuated when the rf drive level is low (i.e., low mass at instability point) and its voltage is linearly increased as the rf drive level is increased. This method of executing axial modulation (resonance ejection) has been shown to result in improved mass accuracy [15]. The modulation of the waveforms by the LCQ, including externally applied waveforms, is fortuitous because construction of a comparable arbitrary waveform is much more complicated. With all of the external waveforms properly timed and applied, the spectrum in Figure 5 was generated. This figure shows the spectrum that results from simultaneous resonance excitation of m/z 495, 611, and 727. Separate experiments in which the MS/MS spectra of these ions are individually taken (data not shown) reveal that Figure 5 is indeed a summation of each individual MS/MS spectrum.

Conclusions

It has been demonstrated that slight adjustments to the Finnigan LCQ quadrupole ion trap can be carried out that allow external arbitrary waveforms to be applied to the endcap electrodes. The ability to apply customized waveforms enables a variety of experiments to be performed on the LCQ that are not possible with the standard instrumental setup. In this paper examples of double resonance, multiple ion isolation, and multiple

ion excitation experiments are described. These types of experiments extend the research utility of the LCQ quadrupole ion trap. The ability to apply customized external waveforms will enable many more types of experiments to be performed on the LCQ.

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