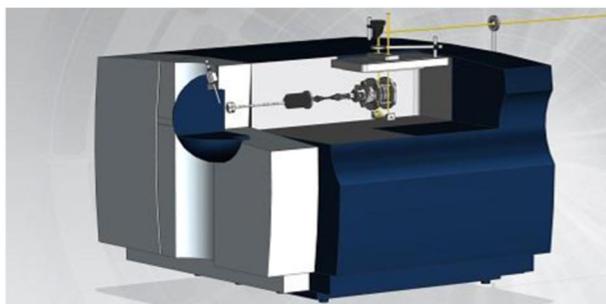


UV–Vis Photodissociation Action Spectroscopy on Thermo LTQ-XL ETD and Bruker amaZon Ion Trap Mass Spectrometers: a Practical Guide

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Abstract. We report automated procedures for multiple tandem mass spectra acquisition allowing UV–Vis photodissociation action spectroscopy measurements of ions and radicals. The procedures were developed for two commercial ion trap mass spectrometers and applied to collision-induced and electron–transfer dissociation tandem mass spectrometry modes of ion generation.

Keywords: Linear ion trap, 3-D ion trap, Photodissociation, UV–vis action spectroscopy, LabVIEW software, Laser alignment

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Introduction

Action spectroscopy refers to tandem mass spectrometry methods in which wavelength-dependent photodissociative formation of fragment ions is used to determine the absorption properties of the precursor ion [1–3]. This approach to ion structural analysis was pioneered by Dunbar [4], Beauchamp [5], Freiser [6], and Gaumann [7], using ion-cyclotron resonance in Penning ion traps. Photodissociation and ion spectroscopy have also been performed on beam instruments [8, 9] that are less readily adaptable to experiments for multistage ion preparation and analysis (MS^n). The different types of mass spectrometers used to obtain action spectra have been reviewed [3]. More recently, special ion traps have been designed and implemented for various types of action spectroscopy [10–13]. The Thermo linear ion trap (LTQ) has been adapted to allow photodissociation [14–17] and action spectroscopy measurements [18, 19]. Recently, the Bruker amaZon

3-D ion trap has been adapted for infrared multiphoton action spectroscopy (IRMPD) measurements using photons from a free-electron laser [20]. We have adapted both an LTQ-XL and an amaZon speed ion traps, which are both equipped with auxiliary ion sources for electron-transfer dissociation, to allow measurements of UV–visible action spectra of radical ions in the MS^n format where the precursor ions to be studied are generated by CID- MS^2 [21, 22] or electron-transfer dissociation (ETD)- MS^2 [23, 24]. We have developed automated data acquisition protocols that greatly facilitate action spectroscopy measurements and increase safety when handling the excitation lasers. In this Application Note, we wish to summarize our experience with operating both instruments in the action spectroscopy mode and also provide detailed protocols for UV–Vis action spectra measurements.

Design

Laser An Nd-YAG EKSPLA NL301G laser (Altos Photonics, Bozeman, MT, USA) was used to generate a beam of photon pulses at 20-Hz frequency and 3- to 6-ns pulse width. The photon pulses were directed into a PG142C unit (Altos Photonics, Bozeman, MT, USA) which incorporated a third harmonic generator and optical parametric oscillator coupled

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with an optional second harmonic generator to enable wavelength tuning between 210 and 700 nm. The laser beam (6-mm diameter) exiting the PG142C unit was aligned and focused into the ion trap of the modified mass spectrometer by a series of mirrors, optical posts, and telescopic lenses (Thorlabs, Newton, NJ, USA) to achieve photodissociation of isolated ions. A fast steering mirror (Newport Corporation, Irvine, CA, USA) was eventually implemented in the Bruker optical setup to improve beam alignment and ultraviolet photodissociation (UVPD) reproducibility. The laser pulse energies, typically ranging from 0.2–4.0 mJ, were measured at each experimental UVPD wavelength using an EnergyMax-USB J-10 MB energy sensor (Coherent Inc., Santa Clara, CA, USA) to calibrate the action spectra. The optical setups also enabled the option of UVPD using a higher-powered (~12–15 mJ) single-wavelength 355-nm beam, which exited the PG142C unit from a different output beam path and hence required a separate set of optical alignment components (i.e., optical flip mount, mirrors with higher damage thresholds). All mentioned laser components and mass spectrometers were set on optical tables to maximize laser alignment and experimental reproducibility.

LTQ Hardware

To provide optical access into the linear ion trap of the LTQ-XL ETD mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA), a 1-mm diameter hole was drilled into the insert block of the auxiliary chemical ionization source used for anion production, and the backside vacuum gate to the chemical ionization source was replaced by an aluminum plate with a sealed quartz window in position of the incoming beam path (Figure 1). The sample solution was electro-sprayed into the instrument's front-end inlet; the desired ions generated by MSⁿ-ETD and/or CID were isolated in the linear ion trap; then, synchronized pulses of photons were sent into the mass spectrometer through the backside quartz window and past the chemical ionization source to induce MSⁿ photodissociation of isolated ions in the linear ion trap.

LTQ Software

Tandem MSⁿ-UVPD on the LTQ-XL ETD was achieved by interfacing the tunable laser system to the mass spectrometer using LabVIEW software (National Instruments, Austin, TX, USA) and by exploiting auxiliary features of the LTQ console. Specifically, pin-14 of the J1 connector on the LTQ was wired to a digital I/O device (National Instruments, Austin, TX, USA) connected to the operating PC. Additionally, the NL301G laser and PG142C unit were linked to the operating PC via USB-to-CAN connections. For every MSⁿ-activation (i.e., ETD, CID, UVPD) during an MSⁿ-UVPD sequence scan, a transistor-transistor logic (TTL) pulse from pin-14 was sent to the digital I/O device which relayed the signal to a LabVIEW code to synchronize the pump laser to pulse (or not pulse) photons at that activation. This LabVIEW code ("LV1"; Figure S1) used LabVIEW drivers provided by EKSPLA to control the photon

pulses at specified settings (i.e., pulse energy, number of pulses per activation, number of MSⁿ activations). For ETD and/or CID (i.e., "non-UVPD") activations in the MSⁿ sequence, a "0%" pulse energy was implemented via LV1. Instrumental MSⁿ sequence scan conditions including mass-to-charge ratios of desired ions, isolation widths, MSⁿ activation times, and sample flow rate were set using the Thermo Tune Plus program; for a UVPD activation, a CID activation was set in its place at "0" collision energy. The experimental UVPD wavelength was manually set via the laser system's control pad.

Action spectroscopy involving automated MSⁿ photoactivation sequence scans at incremental wavelengths was achieved via Xcalibur software (Thermo Electron Fisher, San Jose, CA, USA), LV1, and additional LabVIEW codes, and by exploiting the built-in contact closure connection on the LTQ console (for HPLC autosampler interfacing) along with feedback from the laser. In particular, the "Start In" and "Ready Out" ports on the LTQ's "Peripheral Control" interface were wired to a switchbox and digital I/O device (mentioned above) connected to the operating PC. After completion of MSⁿ-UVPD sequence scans (with photon settings specified using LV1) at the first desired wavelength of an action spectroscopy experiment, the LTQ's contact closure would trigger a "Ready" signal that was received by a second LabVIEW code (LV2; Figure S2) which stepped the laser wavelength, waited, then changed the status of the contact closure to trigger the LTQ to start the MSⁿ-UVPD sequence scans at the next wavelength in the action spectrum. These steps were continuously repeated until the MSⁿ-UVPD scans for the last specified wavelength were completed. Automated photoactivation settings including starting/ending wavelength and step size were controlled via LV2, which utilized drivers provided by EKSPLA. Automated MSⁿ sequence scan conditions such as mass-to-charge ratios of desired ions, MSⁿ activation times, and sample flow rate were set using Xcalibur software which was also used to facilitate the automated data acquisition process and contact closure status (Figure S3). A third LabVIEW code (LV3; Figure S4) incorporating the EnergyMax-USB J-10 MB energy sensor utilized drivers provided by Coherent and EKSPLA to perform automated power scans for more efficient spectra calibration. The automated MSⁿ-UVPD raw data was extracted from Xcalibur output files and exported to Excel, along with automated power scan measurements, to plot experimental action spectra.

Bruker Hardware

To provide optical access into the 3-D ion trap of the Bruker amaZon speed mass spectrometer (Bruker Daltonics, Billerica, MA, USA), two 1-mm holes were drilled into the ring electrode (i.e., one in the top-center, another in the bottom-center), and the vacuum housing lid directly above the modified ring electrode was replaced with an optical breadboard containing a mounted mirror, lens, and two sealed UV windows; two mirrors were also fixed beneath the modified ring electrode (Figure 2). The sample solution was electro-sprayed into the

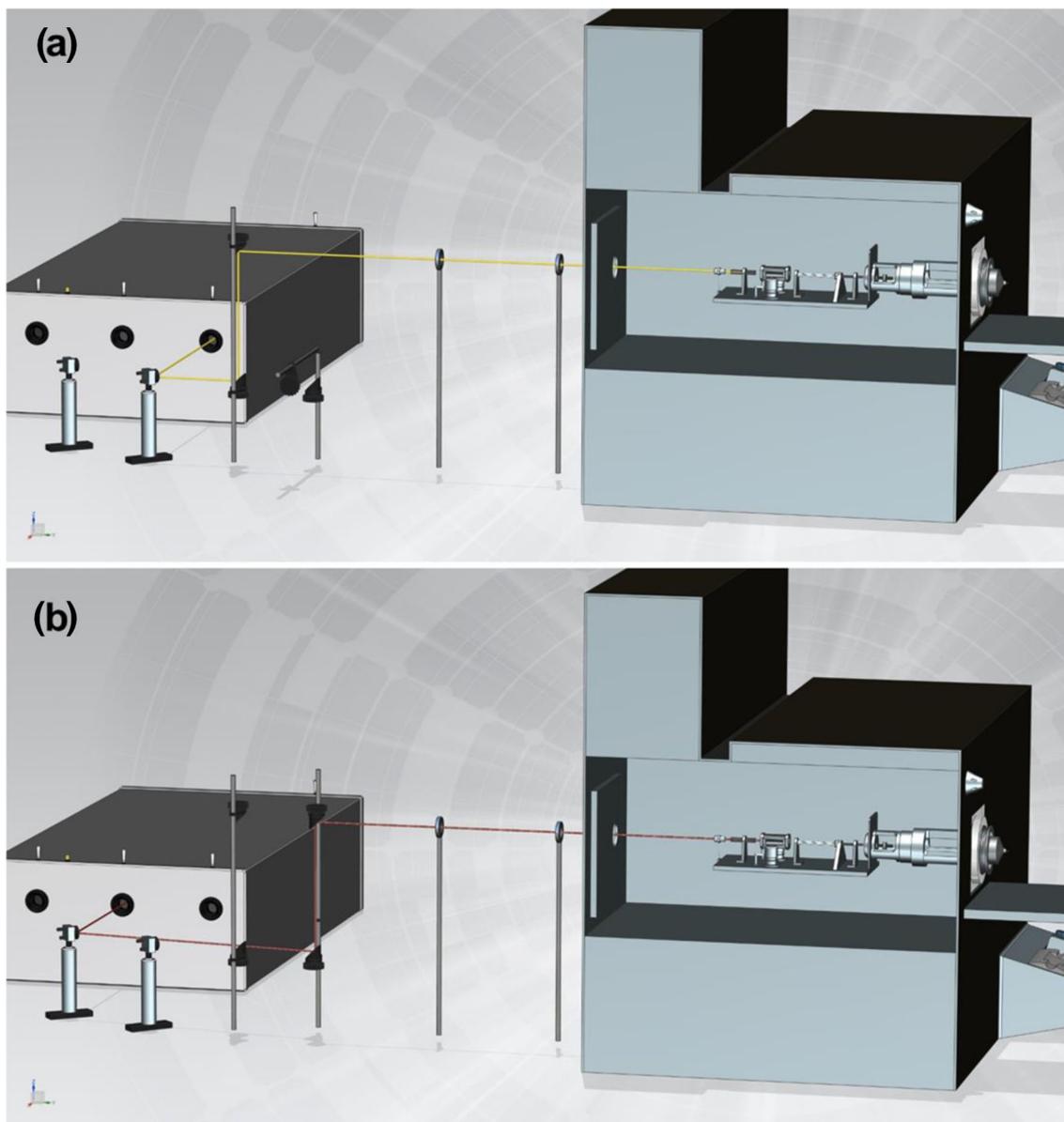


Figure 1. Optical setup and modifications of Thermo LTQ mass spectrometer for MS^n -UVPD and automated action spectroscopy, displaying (a) tunable 210–700 nm and (b) single-wavelength 355 nm capabilities using optical flip-mount

instrument's front-end inlet; desired ions generated by MS^n -ETD and/or CID were isolated in the 3-D ion trap; then, synchronized pulses of photons were sent into the ion trap through the top of the instrument to achieve MS^n photodissociation of ions. Residual photons exited the modified ring electrode through the bottom-centered 1-mm hole and reflected off the two mounted mirrors beneath the 3-D ion trap to exit the mass spectrometer vertically through the second UV window.

Bruker Software

Tandem MS^n -UVPD and automated action spectroscopy on the Bruker amaZon were achieved by interfacing the tunable laser system to the amaZon via LabVIEW software, by the XML scripting interface in the Bruker trapControl program,

and by exploiting auxiliary features of the amaZon console. Specifically, pin-17, 26, 29, and 34 from the amaZon's auxiliary interface were wired to a multifunction I/O device (National Instruments, Austin, TX, USA) connected to the operating PC. The NL301G laser and PG142C unit were also linked to the operating PC via USB-to-CAN connections. A LabVIEW code (LV4; Figure S5) incorporating drivers from EKSPILA and XML commands (provided by Bruker) first set the mass spectrometer to "slave mode," causing it to wait for an external hardware trigger before starting specified MS^n activations; LV4 then activated the multifunction I/O device to send a TTL pulse to pin-17 to initiate the desired MS^n -UVPD/automated action spectroscopy activation sequence(s). For every MS^n -activation (i.e., ETD, CID, UVPD) during an MS^n -UVPD sequence scan, LV4 ordered the amaZon to trigger a TTL pulse

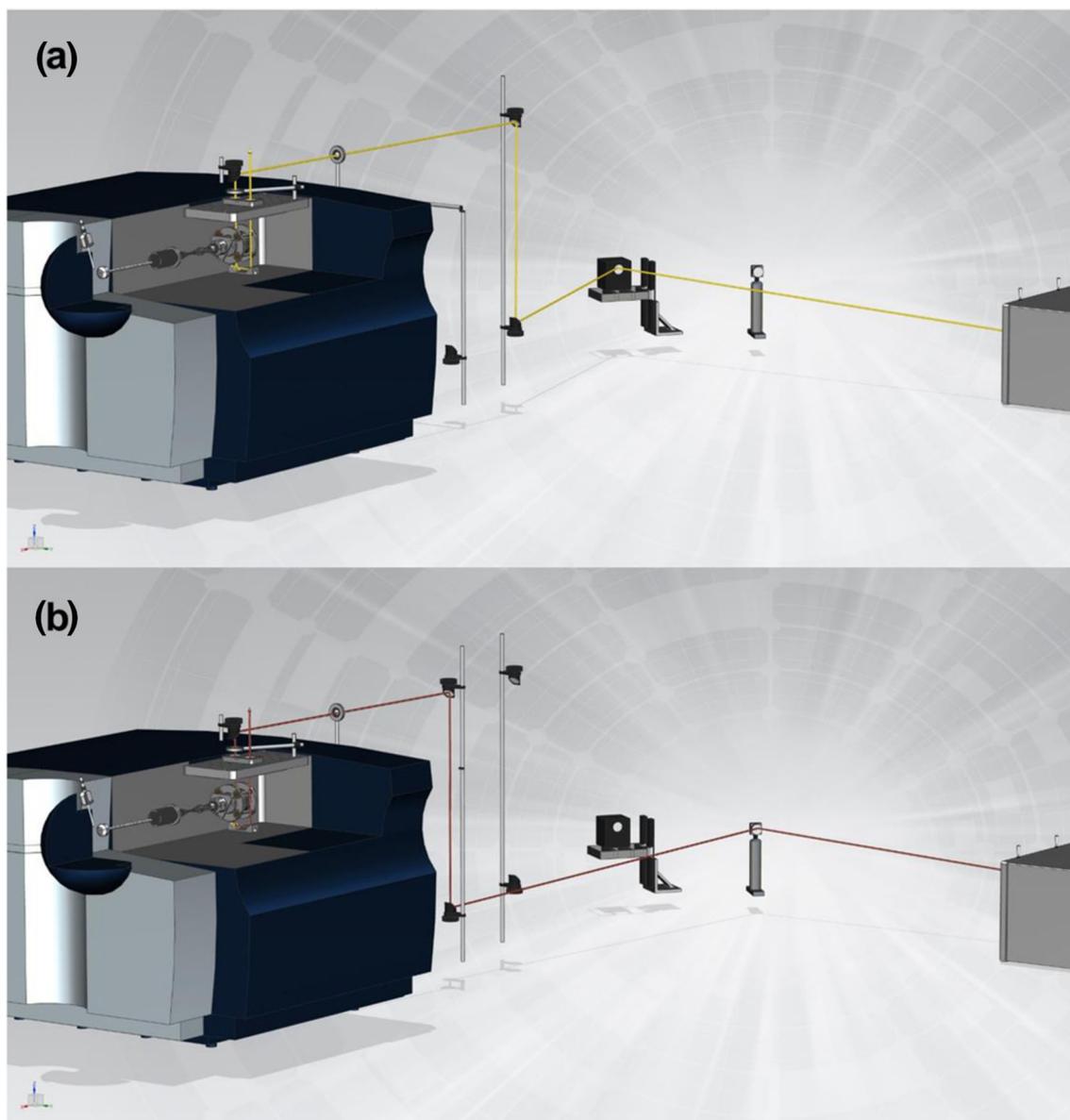


Figure 2. Optical setup and modifications of Bruker amaZon mass spectrometer for MS^n -UVPD and automated action spectroscopy, displaying (a) tunable 210–700 nm and (b) single-wavelength 355-nm capabilities using optical flip mount. The (a) tunable setup incorporated a fast steering mirror to improve beam alignment and UVPD reproducibility

from pin-26 to the multifunction I/O device which relayed the signal back to LV4 in order to synchronize the pump laser to pulse (or not pulse) photons at that activation. If performing action spectroscopy, LV4 would step the laser wavelength after completion of a specified set of MS^n scans (while the mass spectrometer would wait in slave mode), then trigger pin-17 to start the MS^n -UVPD sequence scans at the next desired wavelength in the action spectrum. These commands were repeated until the last specified wavelength was reached. The XML commands from LV4 were sent to a specified directory linked to the Bruker trapControl program in order to automate the mass spectrometer at desired settings (i.e., slave mode, MS^n activation times, external trigger configurations). Tandem MS^n -UVPD/automated action spectroscopy settings such as

starting/ending wavelength, number of wavelengths (i.e., step size), number of scans per wavelength, number of MS^n activations, MS^n activation times, photon pulse energy, and number of photon pulses per MS^n activation were set using LV4. For ETD and/or CID (i.e., non-UVPD) activations in the MS^n sequence, a 0% pulse energy was implemented via LV4. Additional MS^n sequence scan conditions such as mass-to-charge ratios of desired ions, MS^n isolation/activation settings, and sample flow rate were set using the Bruker trapControl program; for a UVPD activation, a CID activation was set in its place at 0 collision energy. A fast steering mirror controlled using another LabVIEW code (LV5; Figure S6) was implemented in the optical setup to improve beam alignment and UVPD reproducibility. Like with the LTQ, LV3 (described

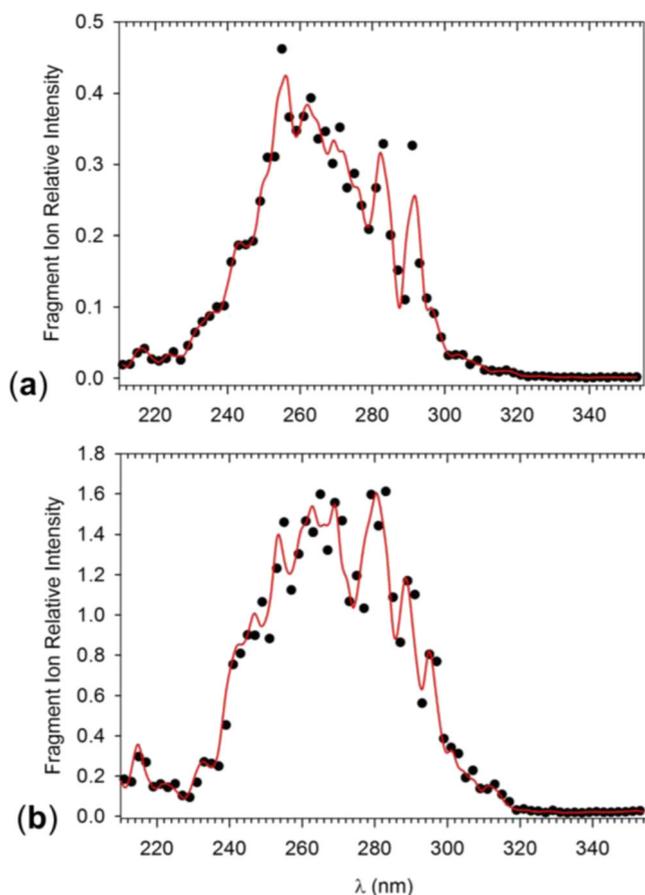


Figure 3. UVPD-MS² action spectra of (dGG + 2H)²⁺ *m/z* 299 ions obtained at (a) Bruker amaZon, and (b) Thermo-LTQ

above) was again used to perform automated power scans for more efficient spectra calibration. The automated MSⁿ-UVPD raw data was extracted from Bruker's Compass Data Analysis program using a data-extraction XML method (provided by Bruker), and was exported to Excel along with automated power scan measurements to generate experimental action spectra. Although the present application has been developed

to obtain UV-Vis action spectra, the authors believe that this software interface can be applied to any tunable laser system that has the drivers and USB/PC connections needed to communicate and be controlled by LabVIEW.

Experimental Results and Discussion

To compare the performance of the new automated action spectroscopy methods on the different modified mass spectrometers, we first present the MS²-UVPD spectra of doubly protonated dGG dinucleotides (*m/z* 299) that were generated on the Bruker amaZon and Thermo LTQ (Figure 3a and b, respectively). The Bruker spectrum was obtained for the *m/z* 152 photofragmentation channel, whereas the LTQ spectrum was expressed as a sum of photofragment intensities. The spectra are remarkably similar, showing the overlapping bands of $\pi \rightarrow \pi^*$ transitions in the guanine rings with a center at 270 nm [25, 26]. To illustrate a more sophisticated experiment, we present the MS³-ETD-UVPD action spectra of the dinucleotide cation radical (GG + 2H)^{+•} (*m/z* 598) that were obtained on the Thermo LTQ and the Bruker amaZon. In brief, a sample solution of dinucleotide GG (Integrated DNA Technologies, Coralville, IA) and dibenzo-18-crown-6-ether (Sigma-Aldrich, Milwaukee, WI) in acetonitrile:water:acetic acid (80:20:1) was electro-sprayed into the mass spectrometer, the doubly charged (GG + DBCE + 2H)²⁺ complex (*m/z* 479) was isolated and subjected to MS²-ETD; then, the desired (GG + 2H)^{+•} (*m/z* 598) cation radical was isolated and exposed to MS³-ETD-UVPD at incremental wavelengths (~210–700 nm); the relative intensities of the MS³ photofragments were calibrated with automated power scan measurements and plotted as a function of UVPD wavelength to generate the action spectra.

As seen from the MS³-ETD-UVPD mass spectra of the cation radical (GG + 2H)^{+•} (*m/z* 598) recorded using the LTQ (Figure 4a) and using the amaZon (Figure 4b) at ~220 nm with one photon pulse per UVPD activation, the same photofragments (*m/z* 348, 349, 446, 447) were produced; these fragment ions were also observed on MS³-ETD-CID. Although

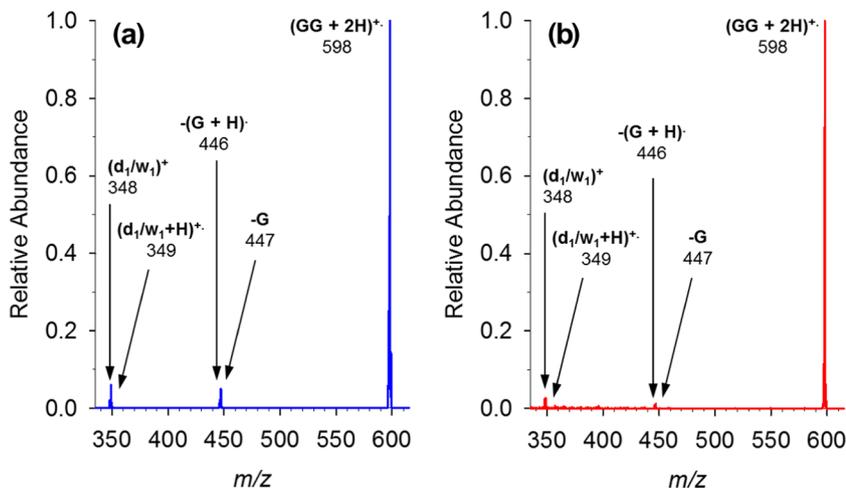


Figure 4. MS³-ETD-UVPD of dinucleotide cation-radical (GG + 2H)^{+•} *m/z* 598 obtained via automated action spectroscopy on (a) the LTQ (224 nm, one pulse) and (b) the amaZon (223 nm, one pulse)

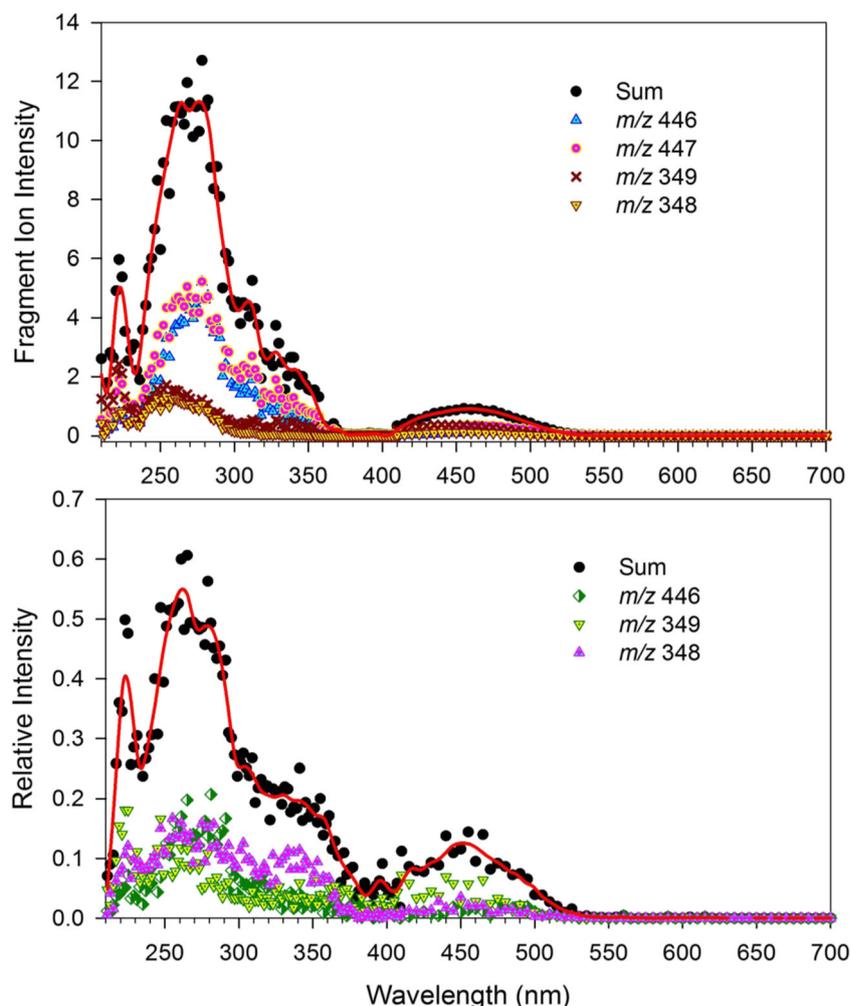


Figure 5. MS³-ETD-UVPD action spectra of dinucleotide cation radical (GG + 2H)^{+•} *m/z* 598 obtained via automated action spectroscopy on (a) the LTQ and (b) the amaZon

the intensities of the photofragments (with respect to the parent ion *m/z* 598) were somewhat higher when performing UVPD on the LTQ, the relative distributions of the photofragmentation from both mass spectrometers were comparable. That is, with both the LTQ and the amaZon, the four photofragments were represented by two doublet peaks in the MS³-ETD-UVPD mass spectra (at ~220 nm), with the *m/z* 348/349 doublet being slightly larger than the *m/z* 446/447 doublet. Furthermore, when comparing the MS³-ETD-UVPD action spectra of the *m/z* 598 cation radical using the LTQ (Figure 5a) and the amaZon (Figure 5b), virtually identical absorption trends are observed with the same major bands at ~220, 275, 330, and 450 nm. The experimentally obtained action spectra were interpreted with the help of TD-DFT calculations of excited states for structure elucidation of the isolated ions, as reported previously [27].

Conclusions

Automated MSⁿ-UVPD action spectroscopy can be achieved on modified LTQ and amaZon mass

spectrometers for a more efficient and reproducible analysis of gas-phase ions. These commercial mass spectrometers can readily be modified to allow optical access to the ion traps, and can be interfaced to a tunable laser system by exploiting various auxiliary features on the mass spectrometers with different LabVIEW codes. The cation radical (GG + 2H)^{+•} (*m/z* 598) analyzed via automated action spectroscopy on both mass spectrometer/laser systems exhibited comparable photofragmentation trends and MSⁿ photodissociation action spectra.

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