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Characterization of harmonic signal acquisition with parallel dipole and multipole detectors

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Abstract

Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS) is a powerful instrument for the study of complex biological samples because of its high resolution and mass measurement accuracy. However, the relatively long signal acquisition periods needed to achieve high resolution can serve to limit applications of FTICR-MS. The use of multiple pairs of detector electrodes enables detection of harmonic frequencies present at integer multiples of the fundamental cyclotron frequency and the obtained resolving power for a given acquisition period increases linearly with the order of harmonic signal. However, harmonic signal detection also increases spectral complexity and presents challenges for interpretation. In the present work, ICR cells with independent dipole and harmonic detection electrodes and preamplifiers are demonstrated. A benefit of this approach is the ability to independently acquire fundamental and multiple harmonic signals in parallel using the same ions under identical conditions, enabling direct comparison of achieved performance as parameters are varied. Spectra from harmonic signals showed generally higher resolving power than spectra acquired with fundamental signals and equal signal duration. In addition, the maximum observed signal-to-noise (S/N) ratio from harmonic signals exceeded that of fundamental signals by 50 to 100%. Finally, parallel detection of fundamental and harmonic signals enables deconvolution of overlapping harmonic signals since observed fundamental frequencies can be used to unambiguously calculate all possible harmonic frequencies. Thus, the present application of parallel fundamental and harmonic signal acquisition offers a general approach to improve utilization of harmonic signals to yield high resolution spectra with decreased acquisition time.

Introduction

FTICR-MS offers unique advantages in many research fields including proteomics[1–3], metabolomics[3, 4], and others[3], due to its high resolving power and mass accuracy for improved structural analysis. However, a limitation of the technique is the relatively longer signal acquisition time to obtain higher resolving power and mass accuracy. One approach to decrease required signal acquisition time is to use stronger magnetic fields.[5–9] Increased magnetic field results in increased detected frequencies and therefore, increase the achieved

Supporting Information

Supporting Information materials available include: Supplemental Figures 1–3.

resolving power for signal acquisition of the same duration. In recent years, FTICR-MS instruments equipped with 21 T have been developed at National Labs.[5, 6] Mass resolving power of 150,000 ($m/m_{50\%}$) was achieved for bovine serum albumin (66 kDa) for a 0.38 s detection period, and greater than 2,000,000 resolving power is achieved for a 12 s detection period. In addition, the use of multiple high resolution mass analyzers where parallel detection of several independent ion populations in multiple ICR cells was recently introduced as a complementary approach to help address this limitation.[10, 11] This has been accomplished both with linear arrays[10] with ICR cells position along the central magnetic field axis and cells position orthogonal to this axis using crossed-magnetic field drift[11]. Once the array was fully populated, all trapped ions were excited and detected at the same time to obtain parallel spectra during the time required for only a single acquisition event. This approach enabled simultaneous high resolution analysis of biomolecules across a wide mass range or with multiple stages of MS with improved analysis speed directly proportional to the number of analyzers.

As originally shown by Nikolaev et al.[12] the use of multiple detection electrodes to improve detection of harmonic signals is another development being pursued to obtain high resolving power and mass measurement accuracy with decreased acquisition time.[13–20] Achieved resolving power can increase linearly with order of the detected harmonic signal. In recent years, ICR cell designs with multiple harmonic detector have been demonstrated to enhance harmonic signals and improve acquisition rates. Harmonic signal acquisition is complementary to both efforts to increase magnetic fields and analyzer arrays mentioned above. However, a complication resultant from multiple harmonic detectors is that complex overlapping multiple harmonic signals can make data interpretation more difficult and lead to identification errors as compared with the signals from a dipole detector.[19] Here, we demonstrate a new ICR cell concept that enables parallel acquisition of dipole and multiple harmonic signals from the same ions. This configuration allows direct comparison of both signals and optimization of experimental conditions for either signal. Moreover, since all harmonic signals are resultant from the same ions whose fundamental frequencies are detected with dipole detectors, these fundamental frequencies can be used to calculate and deconvolute all observed overlapping harmonic signals. Thus, parallel detection of fundamental and harmonic signals can serve as a general, unambiguous method to deconvolute multiple harmonic signals, characterize S/N differences between both modes, and improve utility of harmonic signals for higher speed acquisition of high resolution spectra.

Experimental Section

LTQ FT-ICR MS.

A hybrid linear ion trap Fourier transform ion cyclotron resonance mass spectrometry (LTQ FT-ICR MS; Thermo Scientific, Bremen, Germany) equipped with a 7 T actively shielded superconducting magnet (Japan Superconductor Technology, Tokyo, Japan) was used to acquire all experimental data. The LTQ FT-ICR MS was modified with an ICR cell that supports parallel dipole and multiple detectors, an in-vacuum preamplifier array, a custom multi-pin feedthrough flange on the source side of the vacuum system and wiring after the

removal of a cylindrical Ultra ICR cell that was originally equipped with the system. After installation, the system was pumped and baked out overnight. Electrospray ionization (ESI) was used to generate ions with a syringe pump and direct infusion of samples at a rate of 3.0 $\mu\text{L}/\text{min}$. An ESI spray voltage of 4.5 kV was applied to a sample solution through a metal union for ionization. The ions were accumulated in the LTQ and were then transferred to the ICR cell through the original equipment octapole ion guide. Ion populations inside the LTQ were accumulated with automatic gain control (AGC) on and set to 1.0×10^5 . The pressure in the cell region during all experiments was approximately 0.3×10^{-10} Torr as indicated by the ion gauge on this chamber.

Design of an ICR cell with parallel dipole and multiple harmonic detectors

ICR cells with parallel dipole and multiple harmonic detectors were constructed with 6 printed circuit board (PCB) plates with FR4 substrate, top/bottom, two side and entrance/exit lens plates. On the PCB plates, all electrodes including excitation, detection, and front/back lens electrodes were constructed using gold-coated copper. Figure 1 shows images and a schematic diagram of the components of an ICR cell with dipole and 4-plate detectors used here for characterization of fundamental and 2nd harmonic signal acquisition. In this ICR cell, the top/bottom plates were 1.0" in width and were segmented into three sections as shown in Fig. 1a. The middle sections (0.18" in width) were used for dipole detection electrodes designated as "dipole detectors" and the electrodes on these plates (0.4" in width) were used for excitation electrodes. The length (along the magnetic field axis) of all electrodes was 3.44". In all cells, the segments next to the detection/excitation electrodes on all plates with width of 0.1" were used as trapping electrodes and segments next to the trapping electrodes (0.3" in width) were held at ground potential in all experiments. Fig. 1b shows side plates. In these plates, a middle section with 2.6" and 0.495" in length and width, respectively, was used as 2nd detection electrodes designated as a "4-plate detector" for 2nd harmonic signal detection. The 4-plate detection electrode with the same polarity on each side plate was connected together through the trace on the outer surface of the cell. Fig. 1c shows entrance and exit lens plates which contain a 0.2" diameter hole at the center of the electrode through which ions were transferred from the LTQ.

A similar ICR cell was constructed to enable parallel dipole and 8-plate detectors to allow characterization of fundamental and 4th harmonic signals. In this case, top/bottom and side plates were segmented into three sections similar to the top/bottom plates used for the ICR cell with dipole and 4-plate detectors shown in Fig. 1a. The middle section with 0.18" in width on the top/bottom and side plates were used for excitation and dipole detection electrodes, respectively, as shown in Figure S1a and b (Supporting Information). The other sections with 0.4" in width on the top/bottom and side plates were used as 8-plate detection electrodes for the detection of 4th harmonic signals. The 8-plate detection electrodes with the same polarity on the top/bottom and side plates were connected together through a trace on the cell outer surface. The length of all electrodes was 3.44". The entrance and exit lens plates as shown in Fig. S1c were identical to those used for the ICR cell with parallel dipole and 4-plate detectors as shown in Fig. 1c.

To assemble the ICR cell with parallel dipole and multiple harmonic detectors, the top/bottom and side plates were soldered to the entrance and exit lens plates. After that, the trapping electrode segments were electrically coupled together by soldering 22 AWG copper wires on the pads on the cell outer surface to connect all trapping electrodes. The assembled ICR cell shown in Fig. 1d was placed at the end of the octapole ion guide in the place of the ThermoFisher Ultra ICR cell. Each detection electrode pair was connected to a custom vacuum-compatible preamplifier array (GAA Custom Engineering) using Kapton-coated wires (22 AWG, Accu-Glass Products, Inc. Valencia, CA) to allow parallel ICR signal amplification.[10, 11] The preamplifier was mounted 0.6" away from the entrance lens plate of the ICR cell to reduce detection capacitance and noise and increase sensitivity. Fig. 1e and Fig. S1d show schematic diagrams and electric wirings for the ICR cell with parallel dipole and 4-plate detectors, and parallel dipole and 8-plate detectors, respectively.

All individual PCB components for preparing the ICR cells were designed using the circuit board layout program EAGLE ver. 7.3.0 (CadSoft Computer, Pembroke Pines, FL) and manufactured by OSH Park (Advanced Circuits, Aurora, CO). The solder used for the ICR cell was 99.3/0.7 Sn-Cu lead-free solder alloy.[11]

To transfer ions to the ICR cell, the voltages applied to each electrode for trapping ions were independently controlled with a multi-channel programmable DC power supply (Modular Intelligent Power Source (MIPS), GAA Custom Engineering, Benton City, WA, USA) to allow formation of trapping wells.[10, 11] After filling the cell, excitation of ion cyclotron motion was achieved using ThermoFisher excitation waveforms as normally used for ICR excitation with the Ultra Cell. The mass range of m/z 200–2000 covering frequency range of approximately 500 – 50 KHz with a duration of 10ms was employed for excitation of ion. After ion excitation, the parallel ICR signals from each detector were simultaneously amplified using the independent preamplifiers.[10, 11] Each amplifier output signal was connected with kapton-coated wire to a single vacuum feedthrough pin and then transferred to a Saleae digitizer (Logic 8, Saleae, South San Francisco, CA, USA).[11] The number of samples and sample rate were set to 2621440 and 6250000, respectively. DC power ($\pm 2.5V$) to operate the in-vacuum preamplifier was supplied by an external power supply. The digitized time-domain signals were transferred to a computer through a USB interface. Digitized time-domain signals were transferred to frequency-domain spectra with ICR-2LS (<http://omics.pnl.gov/software/icr-2ls>) without zero-filling or apodization.

Sample Preparation

Insulin and Ultramark 1621 (a mixture of fluorinated phosphazenes) were purchased from Sigma (St. Louis, MO, USA). HPLC grade methanol, acetic acid and dimethyl sulfoxide were obtained from Fisher Scientific (Pittsburgh, PA, USA). A 10 μM insulin standard solution was prepared by dissolving insulin in a 1:1 (v/v) water/methanol solvent mixture containing 0.1% (v/v) of acetic acid. An Ultramark 1621 stock solution was prepared by dissolving 10 μL of Ultramark 1621 in 10 mL of acetonitrile. A 10 μM solution of Ultramark 1621 was prepared by dissolving 100 μL of the stock solution of Ultramark 1621 in a solution of 1% acetic acid in 50:50 methanol:water.

Result and discussion

Characterization of fundamental and harmonic signals

The fundamental and harmonic signals observed with dipole and multiple harmonic detectors, respectively, were characterized with variation of excitation amplitude using a 10 μ M solution of Ultramark 1621. For this experiment, optimized trapping potentials of -9 V and $+4$ V were applied to front and back trapping electrodes, respectively, during injection of ions. After ion injection, the applied voltage to the front trapping electrode was switched to $+4$ V for trapping ions. At 8 ms after trapping ions, cyclotron motion of the trapped ions was excited, and then the ICR signals were simultaneously obtained using dipole and multiple harmonic detectors with a decreased trapping voltage ($+3$ V). For characterization of excitation amplitude for each detector, S/N for the base peaks observed in mass spectra simultaneously obtained from each detector was calculated as a function of excitation amplitude. To obtain ICR signals from each detector, the RF voltage applied to excitation electrodes for 10 ms was varied over the range from 20 – 85 Vpp (peak to peak) at optimum trapping conditions (4V and 3V during excitation and detection events, respectively). The obtained parallel ICR signals were transformed to mass spectra and with these, S/N and standard deviation of the S/N were calculated.

Figure 2 shows the S/N as a function of excitation amplitude for an ICR cell with dipole and 4-plate detectors. For fundamental peaks from the dipole and 4-plate detectors, S/N increases until excitation of 28Vpp were applied. Further increase of excitation amplitude resulted in decreased S/N. However, the maximal S/N for 2nd harmonic peaks from the 4-plate detector was observed with excitation at 40Vpp. It is difficult to say precisely what radius the ions achieved with the excitation voltage due to the effect of trapping electric potential harmonicity and excitation electric field homogeneity and phase coherence at larger radius. However, our best estimates on observed ion loss dependence on excitation amplitude, the excitation at 40Vpp corresponds to approximately 78% of the cell radius. The S/N for the fundamental peaks with the 4-plate detector was reduced to a level of less than 10% of that from the 2nd harmonic peaks. The S/N for 2nd harmonic peaks was 2 times higher than that for fundamental peaks from the dipole detector at each optimal excitation voltage. Figure S2 shows parallel mass spectra from dipole and 4-plate detectors in a single ICR cell with excitation at 40Vpp as example. Figure 3 shows the S/N as a function of excitation amplitude for an ICR cell with dipole and 8-plate detectors. For fundamental peaks from the dipole and 4-plate detectors, S/N increase until excitation of 56Vpp was applied. Further increase of excitation amplitude resulted in decreased S/N. The maximal S/N for 2nd, 3rd and 4th harmonic peaks from the 8-plate detector was observed with excitation at 72Vpp. The excitation at 72Vpp corresponds to approximately 87% of the cell radius. However, the 4th harmonic peak showed 27, 12 and 4 times higher S/N than fundamental, 2nd and 3rd harmonic peaks, respectively. Figure S3 shows parallel mass spectra from dipole and 8-plate detectors in a single ICR cell with excitation at 72Vpp.

Revolving power

To compare performance from dipole and multiple detectors in a single ICR cell, the achieved resolving power from each spectrum was evaluated with insulin. The mass spectra

with resolving power from dipole and 4-plate detectors are compared in Figure 4. The acquisition period was 0.3s. The 2nd harmonic signals (Fig. 4b) showed two times higher resolving power with $(M + 3H)^{3+}$ charge state insulin ions as compared with that from the fundamental signals (Fig. 4a). More importantly, ions within the insulin isotopic distribution of the +3 charge state were resolved in 2nd harmonic peaks (Fig. 4b) acquired with 4-plate detection electrode, but unresolved with conventional dipole detection electrode (Fig. 4a). The mass spectra with resolving power from dipole and 8-plate detectors are compared in Figure 5. The acquisition period was 0.09s. As shown “n” times higher resolving power was observed from “n” harmonic peaks (n = 2, 3 and 4) as compared with fundamental signals; the insulin isotopic distribution of the +3 charge state were unresolved in fundamental and 2nd harmonic peaks, but resolved in 3rd and 4th harmonic peaks. Similar resolving power (FWHM = 14,000) from the 4th harmonic peaks (Fig. 5d) were observed with 0.09s data acquisition time as compared with the resolving power (FWHM = 16,000) from the 2nd harmonic peaks with 0.3s data acquisition time (Fig. 4b)

Deconvolution of complex overlapping multiple harmonic signals with dipole signals.

To demonstrate deconvolution of complex overlapping harmonic signals from a multiple harmonic detector using the dipole signals from a dipole detector, parallel acquisition of dipole and multiple harmonic signals was obtained from a single ion population using a 10 μ M solution of Ultramark 1621. Figure 6 shows parallel mass spectra from dipole and 4-plate detectors in a single ICR cell with excitation at 26Vpp. With the dipole detector (Fig. 6a), fundamental peaks were observed like a conventional ICR cell with full side plate detection electrodes. The mass spectrum from the 4-plate detector (Fig. 6b) had more peaks as compared with that from the dipole detector due to fundamental and 2nd harmonic peaks. To identify the observed multiple harmonic peaks, the fundamental peaks from the dipole detector were divided by “n” (n = 1, 2, 3, 4, ...) to calculate multiple harmonic peaks. The m/z for the calculated multiple harmonic peaks and mass differences calculated by subtracting “calculated m/z” from “observed m/z” are shown in Table 1. The distribution of fundamental peaks from the 4-plate detector was similar to those from the dipole detector as shown in Fig. 6. The mass differences between calculated and observed m/z were $-0.066 \sim -0.073$ and $-0.041 \sim -0.051$ for the fundamental and 2nd harmonic peaks, respectively. For spectra from simple samples with only fundamental and second harmonic peaks such as those shown here, manual identification and assignment of harmonic peaks is straightforward. However, complex spectra from samples with wide disparity of molecular weights, charge states and multiple overlapping harmonic signals can benefit from parallel dipole and harmonic signal acquisition. Figure 7 shows parallel mass spectra from dipole and 8-plate detectors in a single ICR cell with excitation at 56Vpp. In this case, smaller excitation electrodes were used (as compared to the cell developed for 2nd harmonic signal detection shown above) and optimal dipole S/N was observed with excitation amplitude of 56V pp. Fundamental peaks were recorded using the dipole detector and are shown in Fig. 7a. Increased spectral complexity was observed from the 8-plate detector due to fundamental, presence of 2nd, 3rd and 4th harmonic peaks recorded in parallel with the multipole detector as shown in Fig. 7b. Observed multiple harmonic peaks can be unambiguously assigned using the observed fundamental peaks from the dipole detector by calculating all possible harmonic frequencies as shown in Table 1. In ICR cells where

multiple overlapping harmonics signals are observed, this capability to assign peaks can allow unambiguous deconvolution, as illustrated in the inset of Fig. 7b.

As described in early consideration by Marshall and Hendrickson,[20] peaks that arise from multiple harmonic frequencies combined with charge state distributions can require additional information to distinguish. Parallel acquisition of dipole and multiple harmonic signals enables unambiguous identification of all peaks observed with multiple harmonic frequency detection. For instance, the parallel mass spectra shown in Figure 8 were obtained from dipole and 8-plate detectors with excitation at 56 Vpp using insulin ($M_w = 5733$). With the dipole detector, fundamental peaks at m/z 1912 and 1434 resultant from $(M + 3H)^{3+}$ and $(M + 4H)^{4+}$ charge state insulin ions were observed as shown in Fig. 8a. The mass spectrum from the 8-plate detector showed ions at m/z 1912, 1434, 956, 717, 637, 478 and 359 as shown in fig. 8b. As expected, the ions at m/z 1912 and 1434 are fundamental peaks from +3 and +4 charge state insulin ions. The peaks at m/z 956, 717, 637 and 478 could be 2nd, 3rd and 4th harmonic peaks for the +3 and +4 charge state insulin ions or might come from +6, +8, +9 and +12 charge state insulin ions. On a basis of information derived from the dipole detector which illustrates that only fundamental frequencies of +3 and +4 charge state insulin ions were observed, the peaks at m/z 956, 717, 637 and 478 can be unambiguously assigned as 2nd, 3rd and 4th harmonic peaks for the +3 and +4 charge state insulin ions as shown.

CONCLUSIONS

FT-MS is a powerful instrument for the study of complex biological samples due to its ability to acquire high resolution and mass measurement accuracy, but requires longer signal acquisition times to achieve high resolution. In ICR cells with multiple detectors, mass spectrum that yield equivalent high resolving power can be obtained with shorter signal acquisition times, or higher resolving power spectra can be obtained with equivalent acquisition periods. However, a challenge that remains is to optimize cell geometry so as to cleanly detect only a single set of harmonic peaks, such as the 4th harmonic peaks which are also observed among the 3rd, 2nd and fundamental frequencies as shown above. In this study, we demonstrated a new ICR cell with parallel dipole and multiple detectors. In this technique, fundamental peaks were obtained with a dipole detector like conventional ICR cell with a dipole detector, but higher harmonic peaks were obtained with a multiple detector from a single ion population. The observed higher harmonic peaks showed higher resolving power, better mass resolution and S/N as compared with fundamental peaks from the dipole detector. With 0.3s data acquisition periods, ions within the insulin isotopic distribution of the +3 charge state were resolved in 2nd harmonic peaks acquired with 4-plate detector, but unresolved with conventional dipole detector. Moreover, use of an ICR cell with parallel dipole and 8-plate detector and the 4th harmonic signal acquisition revealed isotopic resolution of insulin +3 ion ($R=14,000$) is 0.09 s which is a 3 fold reduction in acquisition time showed similar resolving power to 2nd harmonic signals from the 4-plate detector with 3 times faster scan times. These results shown in Fig. 4 and 5 showed that achievable resolving power for a given acquisition period appears linearly proportional to n , the order of the harmonic. However, harmonic detection can be limited due to increased spectral complexity due to the presence of multiple sets of harmonic peaks and charge state

distributions. Parallel signal acquisition with an ICR cell that includes dipole and multiple harmonic detectors can enable identification of all fundamental frequencies that can then be used to calibrate or deconvolve the complex overlapping multiple harmonic signals acquired with multipole detection. This combination can help advance harmonic signal detection as a general method for acquiring high resolution spectra with decreased signal acquisition duration.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Individual PCB components for an ICR cell with dipole and 4-plate detectors. Top/bottom (a) and side (b) plates showing excitation, detection and trapping electrodes printed on board. Entrance/exit lens plates (c) and the transverse cross section (magnetic field axis projects into the plane of this figure) with a wiring diagram (d) for the ICR cell.

Figure 2.

Characterization of S/N for the base peaks (m/z 1422 (fundamental) and m/z 711 (2f)) observed in the parallel mass spectra from the dipole and 4-plate detectors. Blue square (■) is a fundamental signal from the dipole detector. Red square (■) and circle (●) are fundamental and 2nd harmonic signals from the 4-plate detector, respectively.

Figure 3.

Characterization of S/N as a function of excitation amplitude for a ICR cell with dipole and 8-plate detectors. Blue triangle (■) is a fundamental signal from the dipole detector. Red square (■), circle (●), diamond (◆) and triangle (▼) are fundamental, 2nd, 3rd and 4th harmonic signals from the 8-plate detector.

Figure 4.
Parallel mass spectra from dipole (a) and 4-plate (b) detectors for 0.3s acquisition period.

Figure 5. Mass spectra of +3 charged insulin ion with 0.09s data acquisition period. Fundamental signal (a) from the dipole detector, and 2nd (b), 3rd (c) and 4th (d) harmonic signals from 8-plate detector.

**Dipole
detector****a)** **4-plate
detector****b)** **Figure 6.**

Mass spectra obtained with parallel dipole and 4-plate detectors in a single ICR cell using Ultramark 1621. Dipole (a) and 4-plate (b) detectors with excitation at 26Vpp. Fundamental peaks (■) from the dipole detector. Fundamental (■) and 2nd harmonic (●) peaks from the 4-plate detector.

**Dipole
detector****a)** **8-plate
detector****b)** **Figure 7.**

Mass spectra obtained from parallel dipole and 8-plate detectors in a single ICR cell using Ultramark 1621. Dipole (**a**) and 8-plate (**b**) detectors with excitation at 56Vpp. Fundamental peaks (■) from the dipole detector. Fundamental (■), 2nd harmonic (●), 3rd harmonic (◆) and 4th harmonic (▼) peaks from the 8-plate detector.

**Dipole
detector****a)** **8-plate
detector****b)** **Figure 8.**

Mass spectra obtained from parallel dipole and 8-plate detectors in a single ICR cell using insulin. Dipole (a) and 8-plate (b) detectors with excitation at 62Vpp. Fundamental peaks (■) from the dipole detector. Fundamental (■), 2nd harmonic (●), 3rd harmonic (◆) and 4th harmonic (▼) peaks from the 8-plate detector.

Table 1. Calculated m/z for each harmonic signal with multiple harmonic detector using for Ultramark 1621.

Dipole detector	Multipole detector											
	1f (■)			2f (●)			3f (◆)			4f (▼)		
Obs. m/z (■)	Cal. m/z	Obs. m/z	m/z	Cal. m/z	Obs. m/z	m/z	Cal. m/z	Obs. m/z	m/z	Cal. m/z	Obs. m/z	m/z
1221.989	1221.989	1221.923	-0.066	610.995	610.954	-0.041	407.330	407.301	-0.029	305.497	305.474	-0.023
1321.986	1321.986	1321.920	-0.066	660.993	660.951	-0.042	440.662	440.632	-0.030	330.497	330.474	-0.023
1421.976	1421.976	1421.907	-0.069	710.988	710.943	-0.045	473.992	473.962	-0.030	355.494	355.470	-0.024
1521.973	1521.973	1521.903	-0.070	760.987	760.939	-0.048	507.324	507.289	-0.035	380.493	380.467	-0.026
1621.967	1621.967	1621.895	-0.072	810.984	810.933	-0.051	540.656	540.618	-0.038	405.492	405.464	-0.028
1721.961	1721.961	1721.888	-0.073	860.981	860.930	-0.051	573.987	573.949	-0.038	430.490	430.462	-0.028

• The calculate m/z were obtained through dividing the observed " m/z " from a dipole detector by " n " ($n = 1, 2, 3$ and 4).

• m/z : observed m/z - calculated m/z

• Cal. m/z : calculated m/z

• Obs. m/z : observed m/z