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High Efficiency Tandem Mass Spectrometry Analysis Using Dual Linear Ion Traps

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Abstract

Tandem mass spectrometry (MS/MS) plays an essential role in modern chemical analysis. It is used for differentiating isomers and isobars and suppressing chemical noises, which allows high precision quantitation. The MS/MS analysis has been typically applied by isolating the target precursor ions, while wasting other ions, followed by a fragmentation that produces the product ions. In this study, configurations of dual linear ion traps were explored to develop high efficiency MS/MS analysis. The ions trapped in the first linear ion trap were axially, mass-selectively transferred to the second linear ion trap for MS/MS analysis. Ions from multiple compounds simultaneously introduced into the mass spectrometer could be sequentially analyzed. This development enables a highly efficient use of the sample. For miniature ion trap mass spectrometers with discontinuous atmospheric pressure interfaces, the analysis speed and the quantitation precision can be significantly improved.

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3 Tandem mass spectrometry (MS/MS)¹ has been widely used for analysis of
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5 chemical and biological compounds in samples with complex matrices. The precursor ions
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7 are isolated and fragmented and the product ions are then mass analyzed. Ion trap is a
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9 popular mass analyzer that can perform multiple-stage MS/MS analysis. In the current
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11 commercial mass spectrometers, the MS/MS analysis can be performed fast with a scan
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13 time of about 100ms for each compound; however, the sample usage is of low efficiency
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15 since the ions other than the precursors are wasted during the isolation process. For
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17 miniature mass spectrometry (MS) systems,² the MS/MS plays an even more essential role
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19 and the efficiency improvement for the MS/MS process may also have more significant
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21 impacts. The sample preparation and chromatographic separation for in-situ or in-field
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23 analysis need to be highly simplified or completely eliminated, as demonstrated with the
24
25 recent development of miniature MS systems^{3, 4} with ambient ionization^{5, 6} sources.
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27 However, it is necessary to use MS/MS to differentiate isomers and isobars. The limit of
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29 detection (LOD) and limit of quantitation (LOQ) can also be significantly improved⁴ with
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31 the chemical noise removed through the MS/MS process.⁷ Using the characteristic
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33 fragmentation pattern for confirmation of the chemical identification, high specificity
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35 potentially could also be retained for miniature mass spectrometers without ultra-high mass
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37 accuracy or resolution.
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46 Currently, discontinuous atmospheric pressure interface (DAPI)⁸⁻¹⁰ has been used
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48 to enable the coupling of atmospheric pressure ionization and ambient ionization sources
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50 with miniature linear ion trap (LIT) mass spectrometers.^{3, 4, 9} The ions are introduced in a
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52 pulsed fashion with about 20 ms opening of DAPI in about every 1.5s. The ions injected
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54 are trapped in the LIT at an elevated pressure and then analyzed after the pressure drops
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3 back to millitorrs level in 500-800 ms (Figure 1a). The required pumping capacity is highly
4 reduced for DAPI-LIT systems, while the efficiency and scan speed for MS and MS/MS
5 analysis are also reduced. The waste of samples due to the low duty cycle, e.g. 20 ms ion
6 introduction in every 1s or longer, could be minimized by using a pulsed ion source
7 synchronized with the DAPI operation.¹⁰ However, for MS/MS analysis of multiple
8 analytes in a sample, the entire process could take a significantly long time.
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18 In this study, we explore a solution for this problem using a dual LIT configuration
19 to execute multiple MS/MS analyses with a single ion introduction (Figure 1a). The ions
20 in a broad mass-to-charge ratio (m/z) range were trapped in the first LIT and the precursor
21 ions within different m/z ranges could be sequentially, mass selectively transferred to the
22 second LIT for MS/MS analysis. Dual LIT configurations^{11, 12} have been previously used
23 in commercial instruments such as the LTQ Velos (Thermo Scientific, San Jose, CA),¹³
24 but the precursor ions were typically isolated and fragmented in same (first) LIT without
25 saving the ions from other analytes; the fragmented ions were transferred with no mass
26 selection to the second trap at a lower pressure for mass analysis at a better resolution. In
27 a recent study,¹⁴ ions trapped in a first LIT were scanned to a second trap, trapped for a
28 short period and then scanned out for spectrum recording, which minimized the space
29 charge effect and hence helped to retain a high resolution with high populations of ions. In
30 the current study, the precursor ions of a m/z value was axially, mass selectively transferred
31 to the second LIT for MS/MS analysis, while other ions were still trapped in the first LIT
32 for subsequent mass-selective transfers and MS/MS analyses. For implementation of the
33 concept, we also combined the axial¹⁵ and radial¹⁶ mass-selective ion ejection methods,
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3 previously developed for two popular commercial ion trap instruments, to demonstrate the
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5 high efficiency MS/MS analysis.
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10 **Instrumentation, Results and Discussions**

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12 Rectilinear ion traps (RIT),¹⁷ previously used in development of a series of
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14 miniature mass spectrometers,^{3, 4, 18, 19} were used for an initial test of this dual-LIT concept
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16 (Figure 1b and S1a). Each of the two RITs has a stretched geometry with an inter-electrode
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18 distance of 5.0 mm in the x direction and 4.0 mm in the y direction. A stainless steel mesh
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20 was used as the common end electrode between these RITs. A home built testing system
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22 previously reported¹⁰ was modified for the experimental characterization. Single phase
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24 RFs of 1015 kHz and 995 kHz were applied on the y electrodes of the RIT-1 and RIT-2,
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26 respectively. The ions were trapped in RIT-1 during the DAPI opening period and then an
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28 axial mass selective ejection toward RIT-2 was attempted using two methods, an RF scan
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30 with a resonance ejection by a dipolar AC,¹⁵ or an AC excitation with a steady RF.
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32 Adjustments in wide ranges were performed for the RF voltages, AC excitation frequency
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34 (q value) and amplitude, as well as the DC voltage on the common mesh end electrode.
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36 However, no ions were found to be trapped in the RIT-2.
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44 The axial mass-selective ion ejection from RIT-1 was then characterized using the
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46 setup shown in Figure S2a. Efficient axial ion ejection with AC excitation was observed
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48 and MS spectra were recorded with one example shown in Figure S2b for amitriptyline
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50 (3 μ g/mL) and amitriptyline-d6 (2 μ g/mL) in methanol ionized by nanoESI (electrospray
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52 ionization).²⁰ This led to a hypothesis that the kinetic energy (KE) of the ions ejected from
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54 RIT-1 might have been too high to be trapped in RIT-2. A mesh electrode was then added
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between the mesh end electrode of the RIT and the ion detector assembly to apply a repelling voltage, as shown in Figure S2c). The signal intensity of amitriptyline m/z 277 was measured as a function of the repelling voltage, reflecting a wide KE distribution up to 70 eV (Figure 1c).

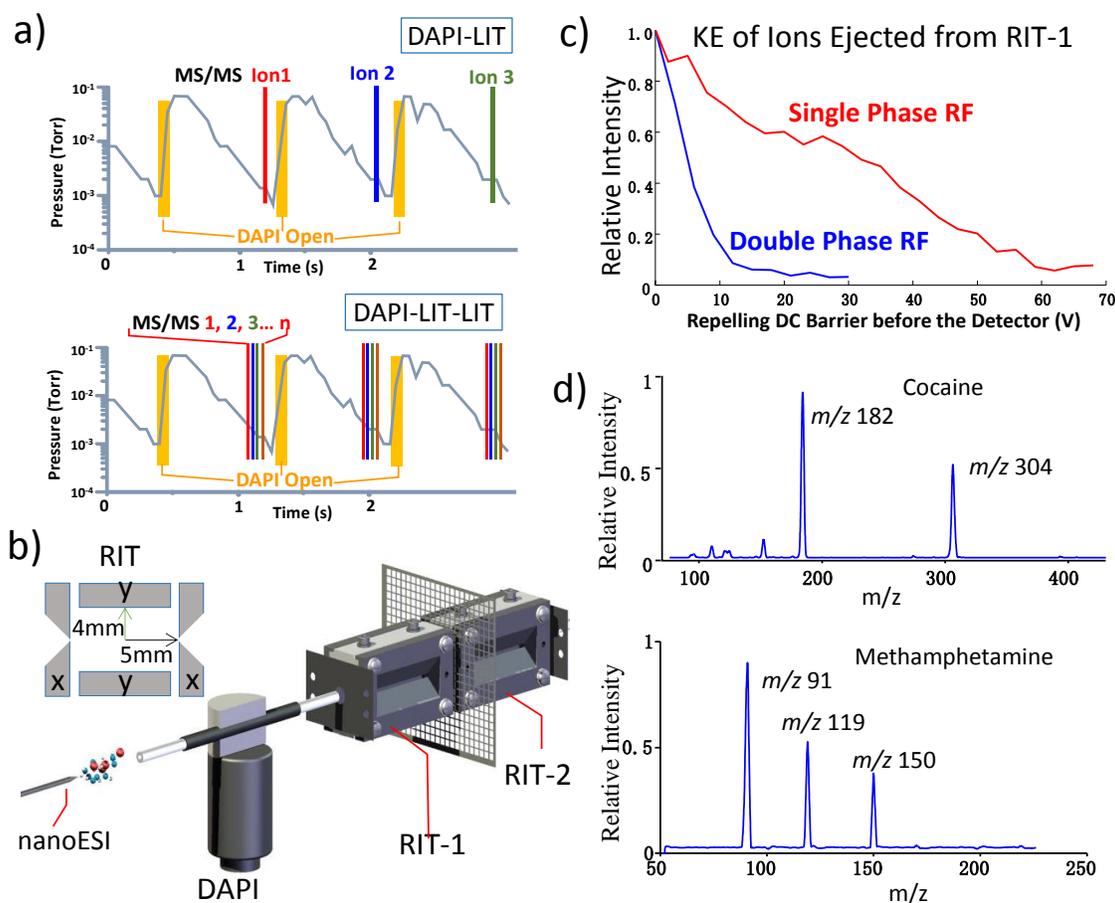


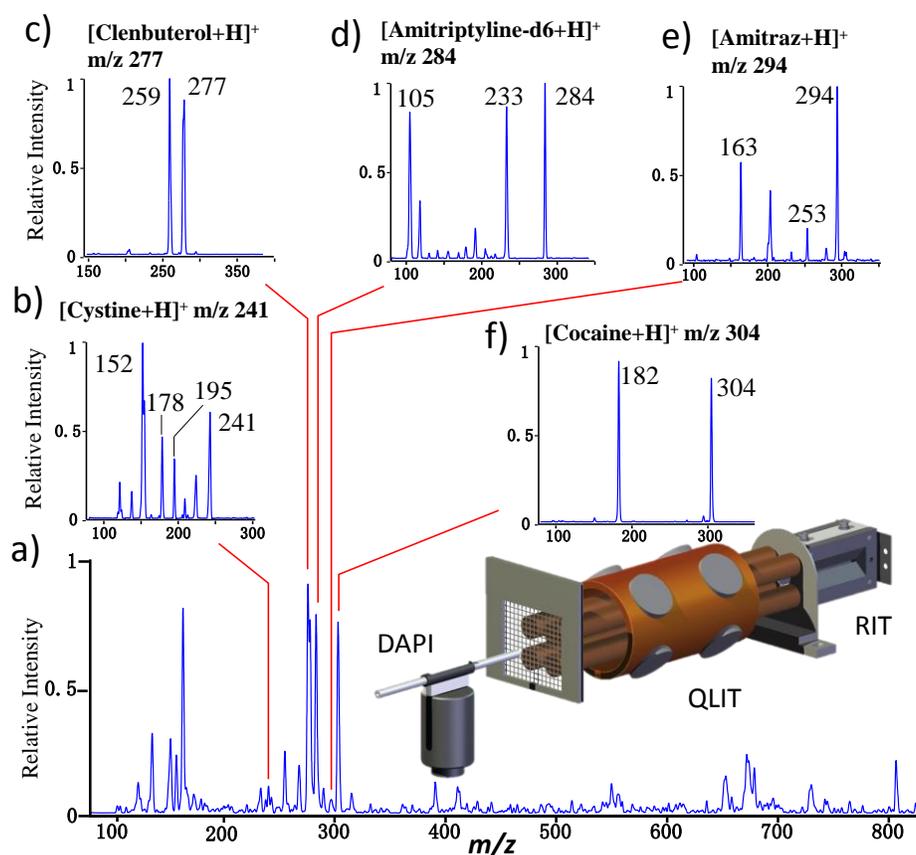
Figure 1. a) Comparison of MS/MS analysis for DAPI MS instruments with one LIT and dual LITs. b) DAPI-RIT-RIT configuration for MS/MS analysis. c) Signal intensities measured for amitriptyline ions m/z 278 reaching the ion detectors as a function of the repelling voltage (see Figure S2c for experimental setup). d) MS spectra recorded by RIT-2 for protonated cocaine m/z 304 and methamphetamine m/z 150, each mass-selectively transferred from RIT-1 to RIT-2, ions generated by ESI of methanol solutions containing only 1 $\mu\text{g/mL}$ cocaine or only 500 ng/mL methamphetamine, AC excitation of 80 mV and 168 kHz, $q = 0.45$ in RIT-1, low mass cutoff = 67 for trapping the ions in RIT-2

It is known that the field at the center axis of the LIT oscillates with unbalanced RF voltages applied on the LIT, which was responsible for the wide distribution of the KE for

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2
3 the axially ejected ions. A double phase RF was then applied on the RIT-1. The
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5 characterization of KE showed a much narrower distribution below 10 eV for the axially
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7 ejected ions (Figure 1c). However, when the double phase RFs were applied on both RITs
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9 for testing, the ions ejected from RIT-1 still could not be trapped in the RIT-2. As an
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11 attempt of increasing the collisional cooling, a second DAPI was then used to introduce a
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13 gas flow for 10 ms immediately before the ion transfer to increase the manifold pressure
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15 from 3 mtorr to 10 mTorr. An AC excitation of 80 mV at 148 kHz ($q = 0.4$) was used to
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17 mass selectively eject the ions from the RIT-1, followed by an MS analysis in RIT-2 after
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19 the pressure decrease to 3 mtorr in about 100 ms. The trapping of transferred ions in RIT-
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21 2 was observed; however, intense fragmentations was also observed, as shown by the MS
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23 spectra recorded for the protonated cocaine m/z 304 and methamphetamine m/z 150
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25 (Figure 1d). The collisions helped to reduce the kinetic energies of the ions but also
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27 facilitated the collisional induced dissociation (CID) that is efficient at elevated pressures.
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29 A complete transfer of the ions could take as long as 80 ms. For practical implementation,
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31 the fragmentation of the precursor ion during the mass selective transfer represents an
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33 advantage since operation for CID is not required for RIT-2, which saves about 20 ms or
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35 more and simplifies the control procedure; however, an additional DAPI would be required
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37 and an additional 100 ms delay for pressure drop was needed for each MS/MS scan.
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46 To further reduce the KE of the axially ejected ions, an LIT of a symmetric
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48 configuration (QLIT) was used to replace the RIT-1 (Figure 2 and S1c). The QLIT was
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50 previously used in development of the QTrap instruments¹⁵ (AB Sciex, Ltd, Toronto, CA),
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52 constructed with round electrodes ($r = 4.17$) and has a symmetric configuration with $r_0 =$
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54 4.0 mm. Simulations were carried out for the comparison of the electric fields for the RIT-
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RIT and the QLIT-RIT configurations (Figure S3). The narrowest KE distribution was expected for the QLIT operated with a balanced, double phase RF. The mass selective transfer of the intact precursor ions was indeed achieved for the QLIT-RIT configuration without elevated pressure. For analysis of a mixture of clenbuterol (1 $\mu\text{g/mL}$), amitriptyline-d6 (1 $\mu\text{g/mL}$), amitraz (200 ng/mL), cysteine (500 ng/mL) and cocaine (500 ng/mL) in a methanol solution (Figure 2 and S4), each of the precursor ions from these compounds could be mass-selectively transferred with minimal fragmentation during the transfer (Figure S4) and then MS/MS analyzed in RIT (Figure 2b-e). The mass selective ion transfers could all be completed within 30 ms. In comparison with the non-selective transfer using DC potential (Figure 2a and S4a), the efficiency for mass-selective transfer of the ions is about 55%.



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3 Figure 2. a) MS spectrum recorded by RIT for ions transferred from QLIT without mass
4 selection. MS/MS spectra of protonated b) cysteine m/z 241, c) clenbuterol m/z 277, d)
5 amitriptyline-d6 m/z 284, e) amitraz m/z 294 and f) cocaine m/z 304, precursor ions of
6 each compounds mass selectively transferred from QLIT to RIT, fragmented in RIT, and
7 MS analyzed. AC excitation of 80 mV and 169 kHz, $q = 0.45$ in QLIT; $q = \sim 0.2$ for the
8 trapping the ions in RIT, $q = \sim 0.2$ for CID in RIT.
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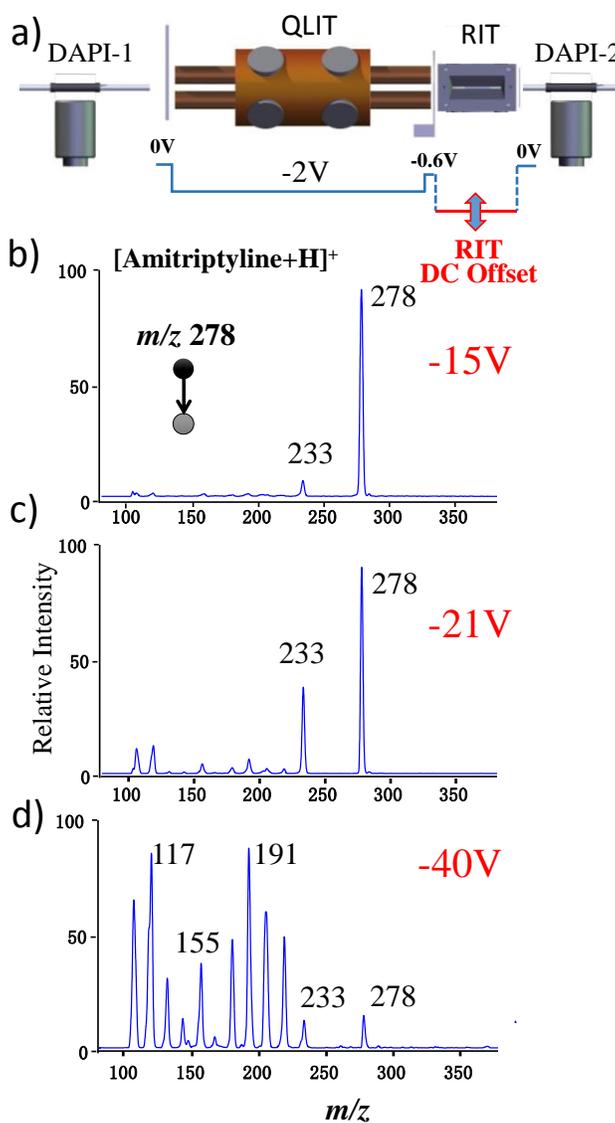
11 The ions were introduced through DAPI for 20 ms, trapped and cooled in LIT for
12 500 ms. The MS/MS analysis of the precursor ions from each compound took about 100
13 ms, including 30 ms for the mass-selective transfer from the QLIT to RIT, 20 ms for the
14 fragmentation by CID, and about 50 ms for MS analysis of the fragment ions. With five
15 MS/MS analysis executed for each scan cycle, the average analysis speed for each MS/MS
16 analysis is 0.2 s, which is comparable with commercial instruments operated with
17 continuous atmospheric pressure interface. The testing system used in this study had a
18 large manifold and also a pumping system for lab scale mass spectrometer, viz. a 30 m³/h
19 rotary vane pump (UNO-030M, Pfeiffer Vacuum Inc., New Hampshire) and a 345 L/s
20 turbo pump (TurboVac 361, Leybold Vacuum, Germany).¹⁰ The scan with DAPI opening
21 could be repeatedly run at a speed of 0.6s per scan. For miniature DAPI-RIT systems with
22 small pumping systems,^{4, 19} the scan period is typically 1.5s for one MS/MS analysis. With
23 a DAPI-LIT-LIT configuration implemented, the average time for MS/MS analysis of each
24 of the n analytes can be estimated by the following equation:
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$$\text{Average time per MS/MS} = \frac{1.5+0.1*n}{n} \text{ s} \quad \text{Equation 1}$$

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48 With 10 or more MS/MS scans implemented within each analysis cycle, the analysis
49 efficiency of a miniature instrument could be comparable to or significantly higher than
50 the current lab scale ion trap instruments.
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The fragmentation during mass selective transfer, as previously observed with the RIT-RIT configuration, was also explored with the QLIT-RIT in a controllable fashion. As shown in Figure 3a, a second DAPI was used and the manifold pressure was raised to 10 mtorr with a 10 ms opening immediately before the mass-selective axial transfer. As shown in Figure 3b-d for the protonated amitriptyline m/z 278, the degree of fragmentation during the mass-selective transfer could be well controlled by the difference in the DC float voltages of the QLIT and the RIT.



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3 Figure 3. a) Instrument setup for MS/MS with CID during mass selective ion transfer.
4 MS/MS spectra recorded for mass-selective transfer of protonated amitriptyline m/z 278 at
5 RIT DC offset at b) -15V, c) - 21V and d) -40V. AC excitation for axial ejection from
6 QLIT, 80 mV and 169 kHz, $q = 0.45$ in QLIT; $q = 0.2$ for trapping the ions in RIT.
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9 One additional significant advantage of performing multiple MS/MS analyses with
10 a single pulse of ion introduction is the potential improvement of the precision in
11 quantitative analysis. In the previous evaluation of the Mini 12 system of the DAPI-RIT
12 configuration,⁴ we have shown that the stability of the signal is critical for the quantitation
13 precision. Since the MS/MS analysis of the analyte and its internal standard (IS) are
14 performed in two completely separated scans with an interval of 1-2s, the fluctuation of
15 the ion current between the two ion introductions would result in large errors in the analyte-
16 to-IS ratios (A/IS) measured. Using the multi-MS/MS analysis in a single scan, the analyte
17 and IS ions can be collected at the same time and MS/MS analyzed sequentially with a
18 time interval as short as 0.1 s.
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34 To validate this concept, 40 μ L methanol solution containing amitriptyline and the
35 IS amitriptyline-d6, each at a concentration of 120 ng/mL, was dropped on a triangle paper
36 substrate for paper spray ionization. For each scan cycle, all the ions were trapped in the
37 QLIT; the protonated amitriptyline m/z 278 was first transferred to RIT and after 0.1 s the
38 protonated amitriptyline m/z 284 was also transferred to RIT. MS analysis was then
39 performed by the RIT and the intensities of m/z 278 and 284 were measured to calculate
40 the A/IS ratios for each scan cycle. As shown in Figure 4, the ion signal intensity could
41 vary significantly with the paper spray, due to the evaporation of the solvent.^{4,21} However,
42 the signal intensities of the analyte and IS were well tracing each other, since the analyte
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and IS ions were always sampled at the same time. A good precision in the A/IS ratio (RSD < 15%) were thereby obtained.

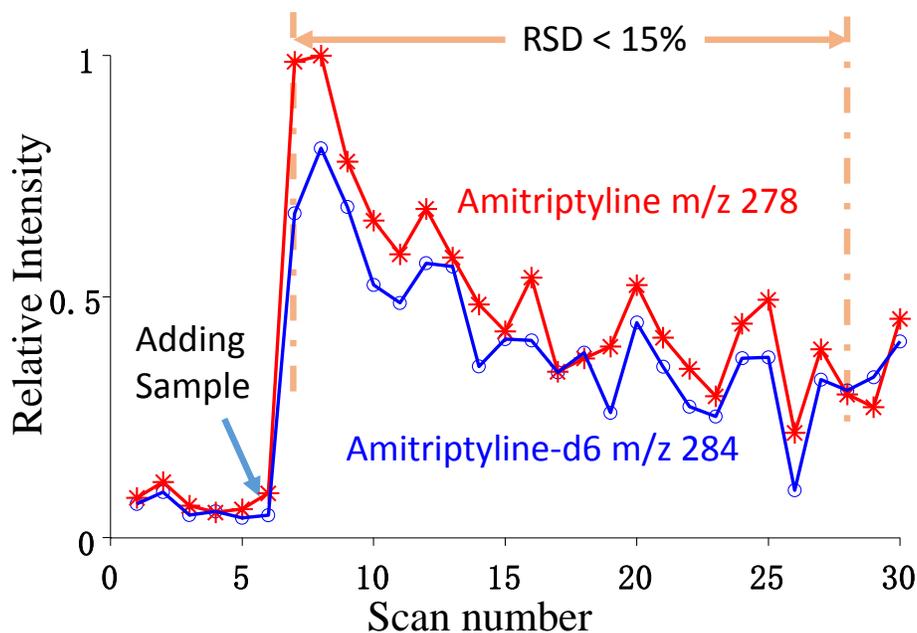


Figure 4. The signal intensities measure by RIT for sequentially mass-selectively transferred amitriptyline m/z 278 and amitriptyline-d6, m/z 284. Paper spray of methanol solution with amitriptyline and amitriptyline-d6 at 120 ng/mL, spray voltage of 4.3 kV. AC excitation of 80 mV 169 kHz, $q = 0.45$, for mass-selective transfer from QLIT. $q = 0.2$ for the trapping the ions in RIT.

Conclusions

In this study, dual LIT configurations have been explored to enable multiple MS/MS processes with a single ion introduction. This development has a direct impact on the analytical performance of the MS systems using DAPIs, especially for miniature instruments coupled with ambient or atmospheric pressure ionization sources. The scan speed and the quantitation precision could be significantly improved. Efficiency in sample consumption can also be highly increased, which should also be attractive for both miniature and lab scale MS systems performing analyses of complex mixtures. An ideal configuration for implementing this concept should have the first LIT with a large trapping

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3 capacity to overcome the space charge effect; and the second LIT can be of relatively small
4 size but with high resolution and mass accuracy for MS/MS analysis. In this study, the
5 balanced RF field for LIT-1 enforced with a double phase RF and the symmetric trap
6 geometry has been shown to be effective for improving the ion transfer efficiency. Further
7 optimization might be achieved by applying a balanced RF field for LIT-2, along with
8 locking the phases of the RFs applied on both traps and the AC for axial resonance ejection.
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TOC

Dual LIT mass spectrometer for high efficiency MS/MS analysis.

