

Identification and Distribution of a Reserpine Metabolite in Whole-Body Rat Tissue using MS and MS/MS Mass Spectrometric Imaging

High Confidence Imaging using the AB SCIEX TOF/TOF™ 5800 System

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MALDI mass spectrometric imaging (MSI) is now a widely-used technique for determining the spatial distribution of various compounds within biological tissues¹. The applications for MSI include profiling of endogenous biomolecules within tissues/organs, comparisons of analyte abundances between samples from different states, as well as analysis of the distribution and metabolism of dosed drugs. Regardless of the application, it is advantageous to have the ability to perform survey/profiling scans followed by subsequent MS/MS experiments to confirm the structural/chemical composition of the analytes of interest on the same platform.

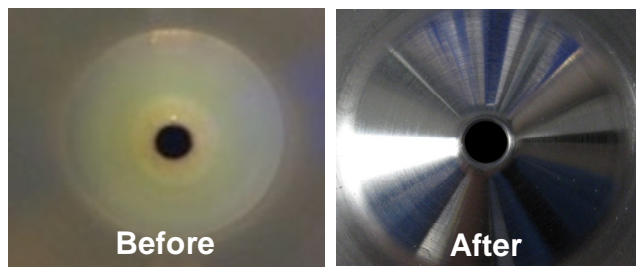
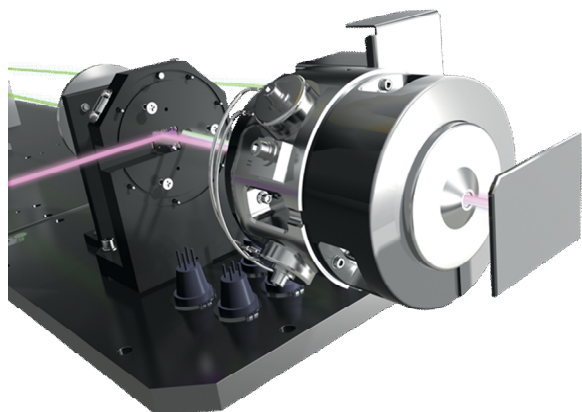
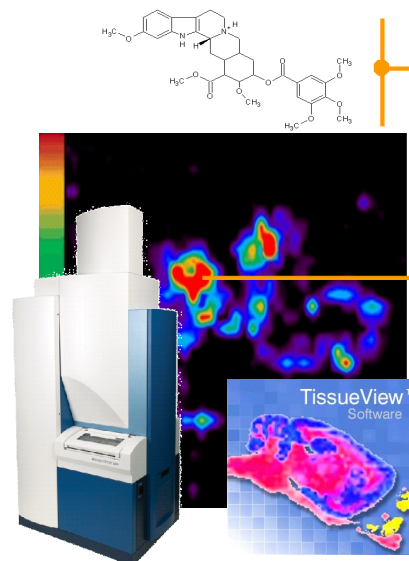


Figure 1. Self-Cleaning MALDI Source for Extended Operation. After extensive MS Imaging experiments, matrix can build up on the source (Bottom Left). Regular source cleaning keeps the instrument clean and operating at the highest sensitivity (Bottom Right).



The AB SCIEX TOF/TOF™ 5800 System is ideally suited for the MSI of various analytes of a wide range of masses. Its superior sensitivity, mass resolution and mass accuracy in both MS and MS/MS modes, as well as its high repetition-rate laser, allows for the acquisition of high-quality data with high speed. This application note describes the imaging analysis of reserpine-dosed rat tissues using a combined MS and MS/MS workflow.

Key Features of AB SCIEX TOF/TOF™ 5800 System for MS Imaging

- Unsurpassed sensitivity and resolution in both MS and MS/MS modes with broad mass range.
- High laser repetition rate (up to 1 kHz) minimizes data acquisition time.
- Superior robustness obtained with self-cleaning source and laser-mirror heating – instrument downtime is drastically reduced (Figure 1).
- Powerful and easy-to-use TissueView™ software allows for imaging data visualization and processing.

Methods

Sample Preparation: Wild-type Sprague Dawley rats were orally dosed with reserpine (20 mg/kg body weight). After two hours, the rats were sacrificed and whole body sections of 12 μ m thickness were generated using a cryostat. Tissue slices were mounted onto stainless-steel 3"x5" MALDI plates using two-sided conductive tape (3M, USA). MALDI plates were spray-coated with MALDI matrix (20mg/mL α -cyano-4-hydroxycinnamic acid in 50% acetonitrile/30% methanol/0.1% trifluoroacetic acid).

Mass Spectrometry: MS imaging was performed on the AB SCIEX TOF/TOF™ 5800 System at a pixel resolution of 300 μ m \times 300 μ m. Survey scans were performed using an MS Reflector Positive mode scan surveying a mass range of 400-700amu, firing 50 shots per pixel at a laser repetition rate of 400Hz. MS/MS imaging experiments were done using an MS/MS 1kV Positive method, firing 100 shots per pixel using a laser repetition rate of 1000Hz. All imaging data was processed using TissueView™ software.

Distribution of Reserpine Within the Whole-Body Rat Tissue

Figure 2A shows the intensity map of the ion at the expected mass of reserpine (m/z 609.28) throughout the entire rat body. When overlaid onto the optical image of the rat body (Figure 2B), it is clear that this ion displays strong localization within the rat stomach, and to a lesser extent the colon. This was expected given that the animals were orally dosed with reserpine and that harvesting of the animal occurred relatively quickly after dosing (after two hours). Interestingly, the MS spectrum at the region within the stomach (Figure 2C) shows that there is an ion signal observed with a 2 Da mass shift from the expected mass of reserpine (the major peak was observed to have an m/z 607.26). This could be due to degradation of reserpine occurring either before ingestion, or metabolism of the compound post-ingestion. It is also possible that the species at m/z 609.28 is in fact not reserpine. Conclusive determination of the compound structures detected required MS/MS analysis.

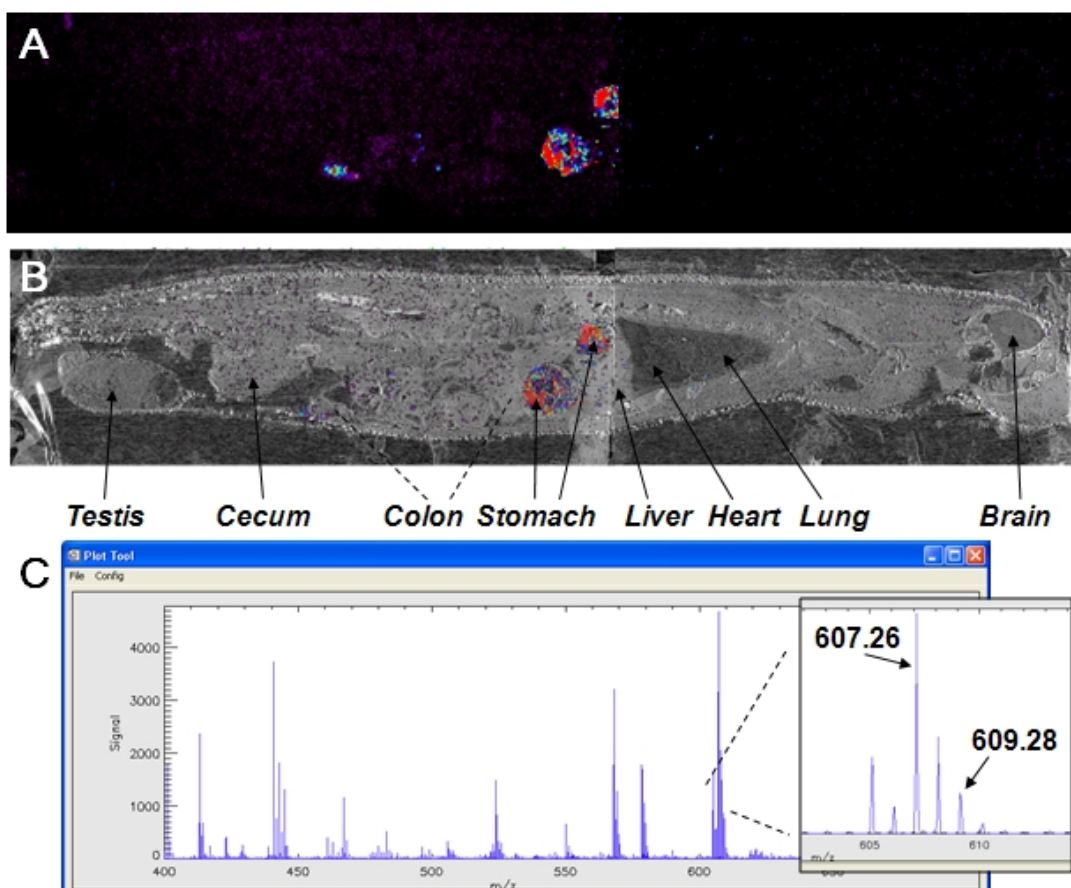


Figure 2. MS Imaging Analysis (MSI) of Reserpine in an Entire Rat Body Tissue Slice. The ion intensity map for reserpine (m/z 609.28) is shown in (A), along with the ion intensity map overlaid onto the optical image of the rat body slice (B), indicating that the ion signal is present predominantly in the stomach. The mass spectrum at the stomach region (C) indicates that there is a 2 Da mass shift from the expected mass of reserpine.

MS/MS Analysis

Reserpine is an extensively-studied compound that exhibits characteristic fragmentation products when analyzed in MS/MS mode. Major fragment ion signals include m/z 174.091 ($C_{11}H_{12}NO$), m/z 195.065 ($C_{10}H_{11}O_4$), m/z 397.212 ($C_{23}H_{29}N_2O_4$), m/z 448.197 ($C_{23}H_{30}NO_8$), and m/z 577.254 ($C_{32}H_{37}N_2O_8$). The rat body slice was analyzed in MS/MS mode looking for the fragmentation products of the precursor at m/z 607.26. Several of the known reserpine MS/MS fragment ion signals were detected in the MS/MS scan. The distribution of one major fragment ion signal (m/z 195.07) is shown in Figure 3A. This fragment ion shows distinct localization within the stomach and colon, thereby colocalizing with the reserpine parent ion signal (Figure 3B).

Analysis of the MS/MS spectrum (Figure 4A) shows that while several of the expected MS/MS fragment ions for reserpine are present (m/z 174.09, 195.07 and 448.04), two of the other fragment ions show a 2 Da mass shift similar to that observed with the parent ion signal (m/z 395.20 and 575.24). It has been documented in previous studies that the loss of 2 Da in reserpine occurs due to a dehydration reaction taking place at the piperidine ring nitrogen through a hydroxylation intermediate, resulting in the formation of a double bond at this position to yield 3,4-dehydroreserpine² (Figure 4B). The MS/MS fragment ion signals shown in Figure 4A exhibiting the 2 Da mass shift therefore correspond to fragments containing the piperidine ring nitrogen, while the other fragments exhibiting the expected masses do not contain this nitrogen. In fact, it is likely that the peak at m/z 609.28 that was detected in the MS imaging scan in Figure 2 is actually an isotope of 3,4-dehydroreserpine and not reserpine itself.

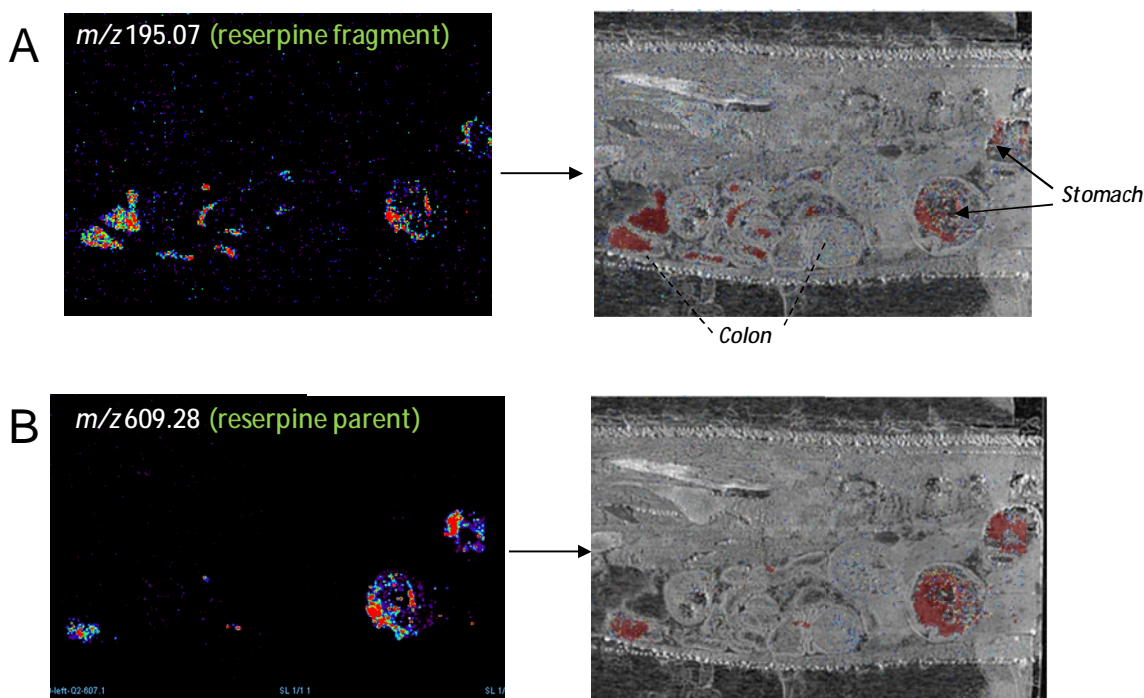


Figure 3. MS/MS Analysis of Reserpine. The species with m/z 607.26 observed in the MS survey scan was subjected to MS/MS analysis and the ion intensity map (left) for a major fragment (m/z 195.07) is shown (A), along with the ion intensity map overlaid onto the optical image (right). This is compared to the ion intensity map of the m/z value expected for the reserpine parent (B).

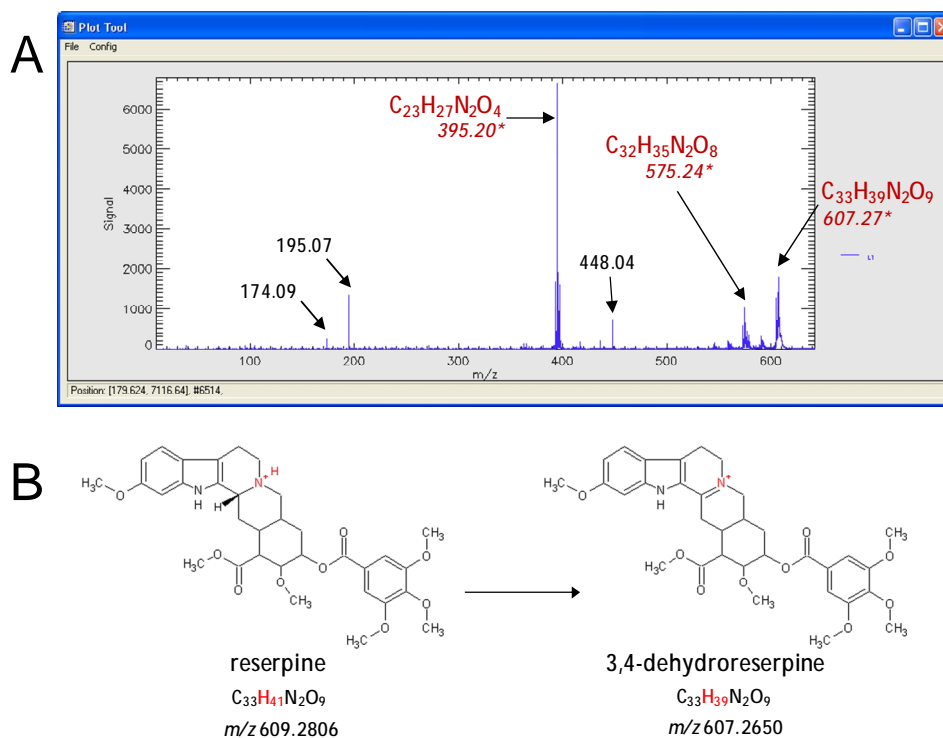


Figure 4. Analysis of the MS/MS spectrum obtained from fragmentation of the parent ion at m/z 607.26. Several of the MS/MS fragment ions match those expected for reserpine (m/z 174.09, 195.07 and 448.04), although others exhibit a 2 Da mass shift as with the parent ion (m/z 395.20 and 575.24) (A). The 2 Da mass shift is due to a degradation reaction occurring at the piperidine ring which converts reserpine to 3,4-dehydroreserpine (B).

This was confirmed by performing MS/MS analysis on the precursor at m/z 609.28. The fragment ions in the spectrum in Figure 5 match those observed for 3,4-dehydroreserpine in Figure 4. If the peak at 609.28 was in fact reserpine then one would expect to see fragment ions without the 2 Da mass shift as generated by the dehydration reaction.

Conclusions

This work demonstrates the imaging of an orally-dosed drug compound (reserpine) in rats using MSI. The parent ion m/z 609.28 was found to be distributed in the stomach and colon of the rat sacrificed 2 hours after drug administration. However, a significant ion signal in the MS spectrum with a mass shift of 2 Da from the parent ion mass was observed (m/z 607.26).

MS/MS analysis of the m/z 607 peak confirmed this to be the result of a common degradation reaction occurring with reserpine. Additional MS/MS analysis indicated that the m/z 609 peak was in fact an isotope of the metabolized reserpine and not reserpine itself. This work highlights the importance of the ability to perform MS/MS analysis during MSI to have the highest confidence in the results obtained. The high quality data and fast acquisition speed of the AB SCIEX TOF/TOF™ 5800 system for both MS and MS/MS was critical for this experiment.

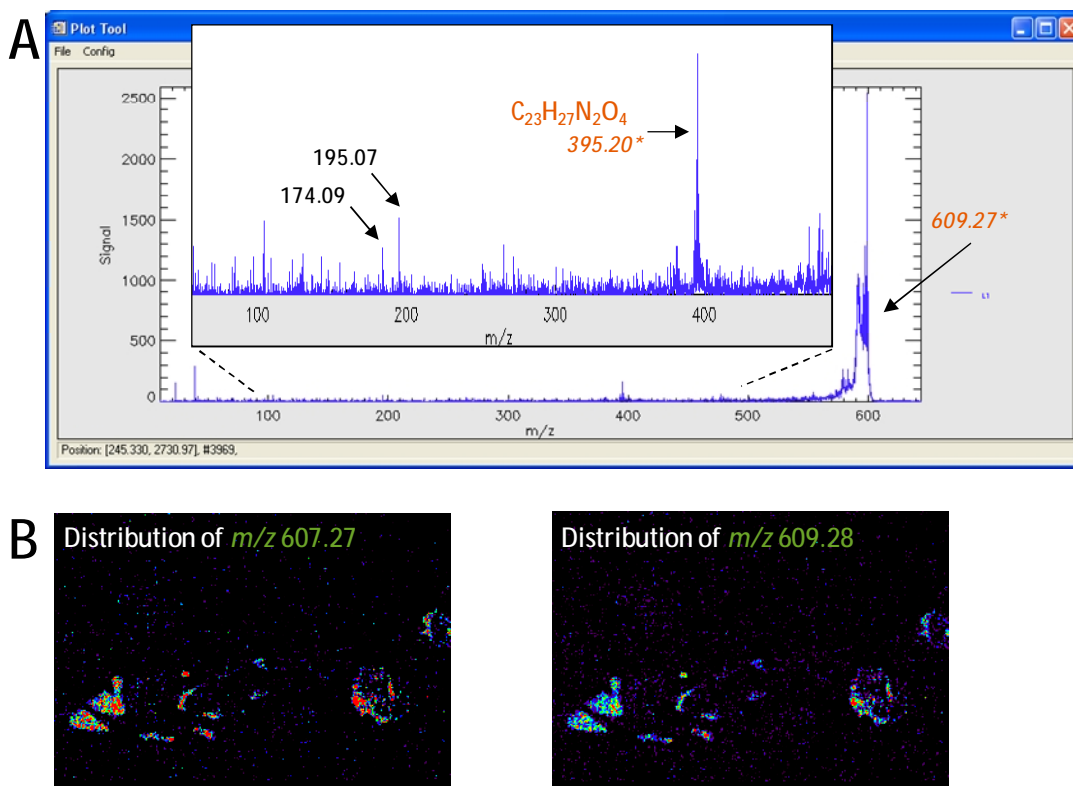


Figure 5. MS/MS Analysis of the Precursor Ion with m/z 609.28. The MS/MS spectrum (A) indicates that the precursor is in fact an isotope of 3,4-dehydroreserpine and not reserpine itself – this is indicated by the masses of the fragment ion signals, some of which exhibit the same 2 Da mass shift as observed in the MS/MS spectrum of 607.27. The distribution of the fragment ion signal of m/z 395.20 is shown for both the m/z 607.27 and the m/z 609.28 precursors (B), indicating that they both co-localize within the same regions of the tissue.

References

1. Reyzer ML and Caprioli R, (2007) MALDI-MS-based imaging of small molecules and proteins in tissues., *Current Opinions in Chemical Biology*, 11:29-35.
2. Pasilis SP, Kertesz V and Van Berkel GJ, (2008) Unexpected analyte oxidation during desorption electrospray ionization-mass spectrometry, *Analytical Chemistry*, 80:1208-14.

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