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Multiplexed Post Source Decay (PSD MX): A Novel Technique Explained

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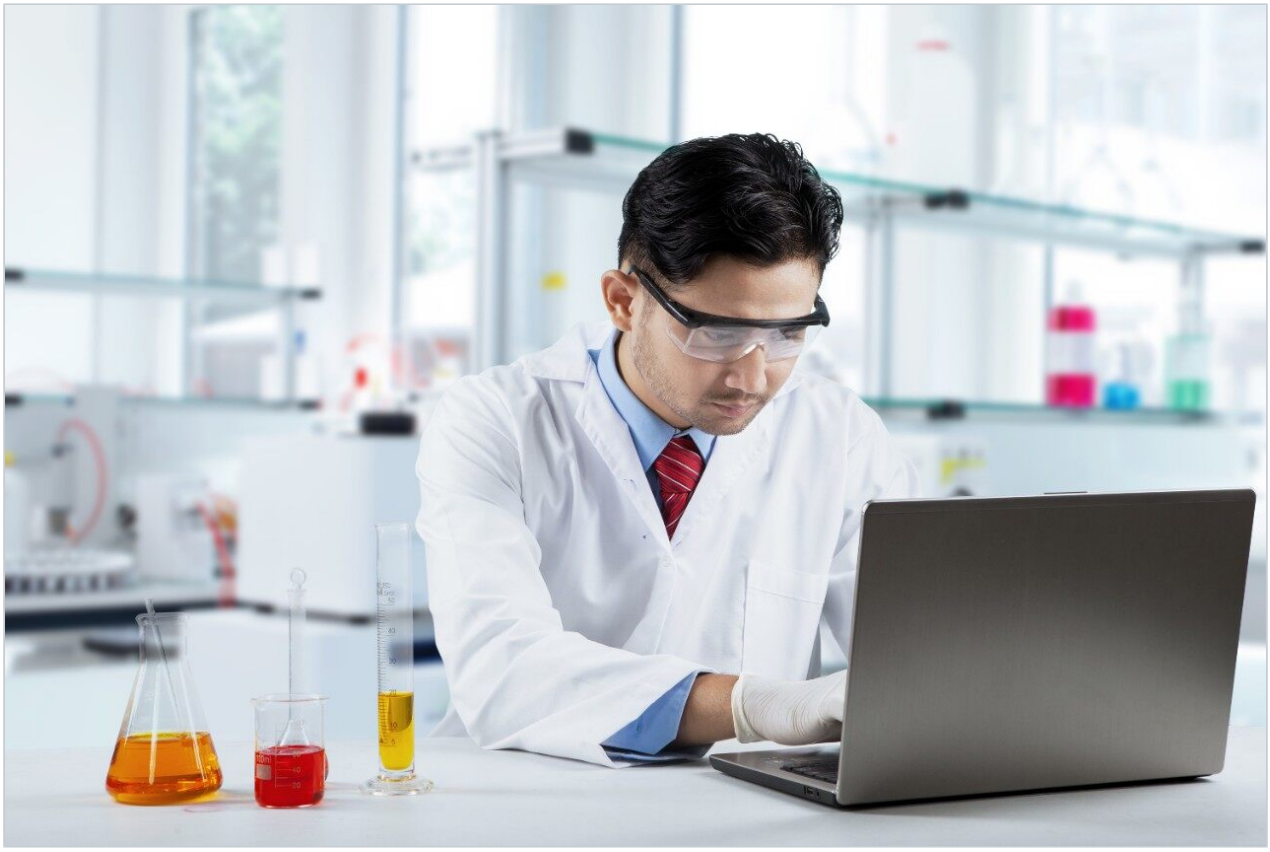


Abstract

This application note demonstrates a novel technique using multiplexed post source decay (PSD MX).

Benefits

- The benefits of PSD MX are a significant reduction in both analysis time and sample consumption in comparison with conventional PSD.
- Additionally, with PSD MX there are no typical side effects from using an ion gate, such as loss of transmission (and hence sensitivity) and resolution

Introduction

Post source decay (PSD) MALDI-Tof analysis is an established technique capable of providing complementary structural information. A conventional PSD experiment requires each precursor ion of interest to be isolated using a selecting device (such as an ion gate for example) so that all fragment ions may be associated with their correct precursor.

We present a novel axial geometry MALDI-Tof Mass Spectrometer, which enhances PSD analysis by the simultaneous acquisition of PSD fragments from a mixture of precursors. This new, patented technique of parallel or multiplexed PSD (PSD MX) removes the need for an ion gate and is available on the Waters MALDI micro MX Mass Spectrometer.



The Waters MALDI micro MX Mass Spectrometer with PSD MX Technology.

Experimental

Conventional PSD Experiment

A schematic of a conventional PSD experiment is shown in Figure 1. In the example shown, ions of three different masses are formed and then accelerated out of the ion source at Time 1. At Time 2, the ion gate is opened to allow a specific precursor mass (red) to be transmitted whereas the lighter precursor ions (green) have been deflected. By Time 3 the ion gate has closed again and therefore deflects the heavier precursor ions (blue). While inside the field free region between the source and the reflectron, some of the selected (red) precursor ions undergo metastable decay, which leads to the formation of fragment ions. As shown at Time 4, the parent ion penetrates to the back of the reflectron and is well focused onto the detector. Fragment ions do not penetrate as deeply and consequently are not as well focused.

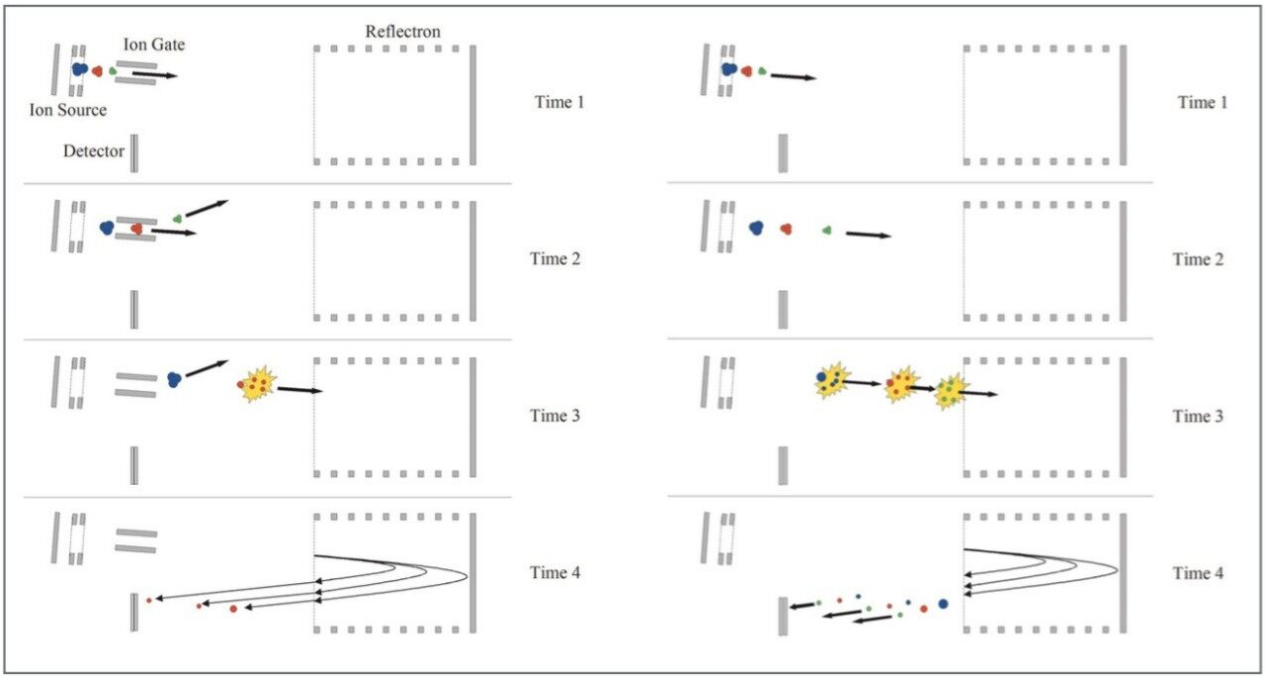


Figure 1. Schematics of a conventional PSD experiment (left) and a PSD MX experiment (right).

This is overcome by acquiring several spectra (known as segments) at reduced reflectron voltages.
The regions of each segment which are in focus are then stitched together to form a single MS/MS spectrum.

Results and Discussion

Post acquisition MS/MS deconvolution

To extract useful fragment information from several precursors simultaneously (a requirement for PSD MX), it is necessary to be able to match the fragments to their associated precursors. It is possible to make these assignments by exploiting a feature of PSD on an axial MALDI Tof instrument. Fragment ions formed in the field free region have a unique combination of mass and kinetic energy, which is related to the mass of the precursor. By acquiring two spectra at slightly different reflectron voltages for each segment, these properties can be determined.

The first spectrum for each segment is acquired at the same reflectron voltage as for conventional PSD and is referred to here as the Major spectrum or segment. The second spectrum is acquired at a reflectron voltage approximately 4% lower and is referred to as the Minor spectrum or segment. The precursor of each fragment ion is determined by measuring the shift in time-of-flight of the fragment ions between the two spectra. The difference in the shift in time-offlight of fragment ions from different precursor ions is illustrated in Figure 2.

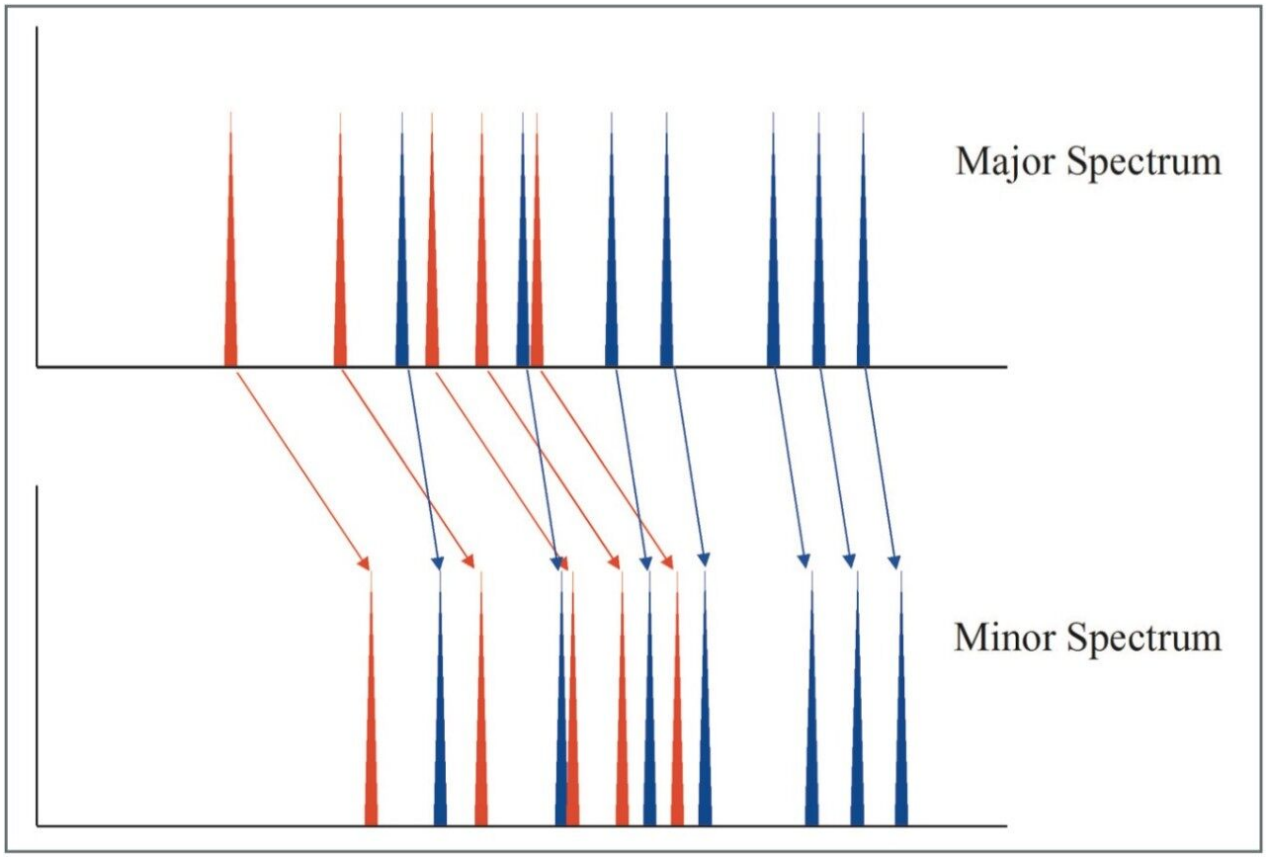
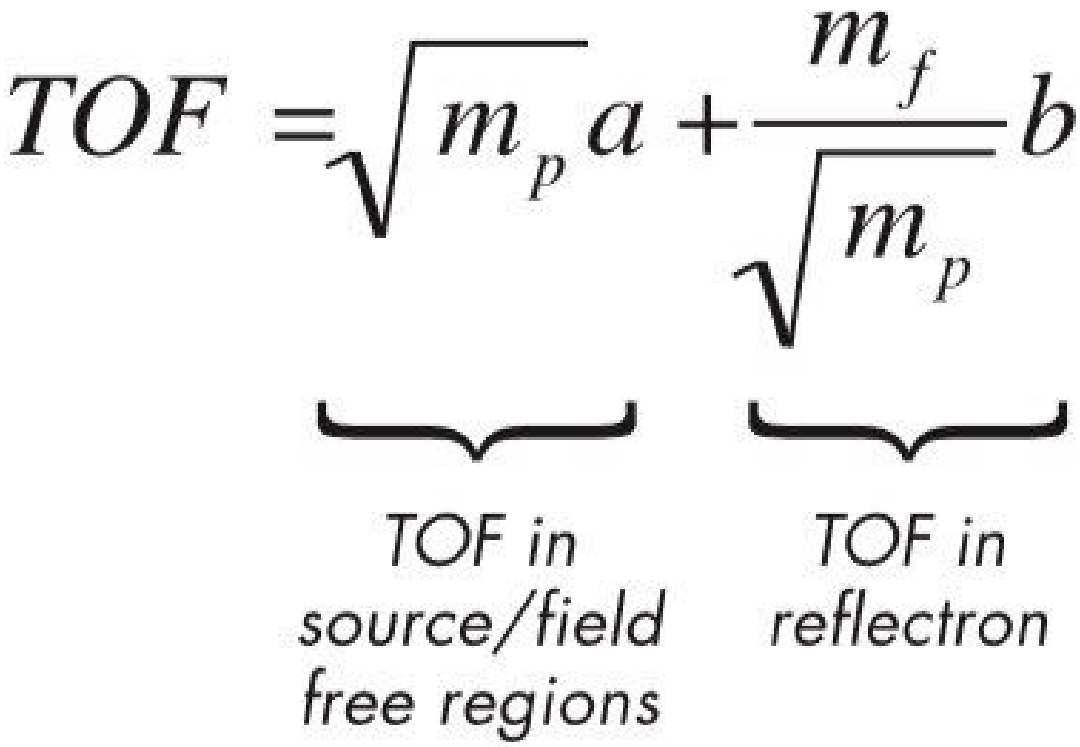


Figure 2. Fragment ions from different precursor ions, marked in different colors, display different shifts in time-of-flight when the reflectron voltage is altered.

Solving the time-of-flight equation

Solving the time-of-flight equation for conventional PSD (Equation 1) is trivial if the calibration parameters and the mass of the parent are known:

Equation 1



- *Tof is time-of-flight*
- *m_p is the mass of the parent*
- *m_f is the mass of the fragment*
- *a and b are instrument-specific constants determined during instrument calibration*

With PSD MX, both the mass of the fragment and the mass of the parent are unknown. By acquiring two sets of data, the Major and Minor, it is possible to solve the Tof equations (Equations 2 and 3) to find both unknowns (Equations 5 and 6):

Equation 2

$$TOF_{major} = \sqrt{m_p}\,a + \frac{m_f}{\sqrt{m_p}}\,b_{major}$$

Equation 3

$$TOF_{minor} = \sqrt{m_p}\,a + \frac{m_f}{\sqrt{m_p}}\,b_{minor}$$

Equation 4

$$\Delta TOF \equiv \frac{m_f}{\sqrt{m_p}} \Delta b$$

Equation 5

$$m_p = \left(\frac{TOF_{major}}{a} - \frac{b_{major}}{a} \cdot \frac{\Delta TOF}{\Delta b} \right)^2$$

Equation 6

$$m_f = \frac{\Delta TOF}{a \cdot \Delta b} \left(TOF_{major} - \frac{b_{major} \cdot \Delta TOF}{\Delta b} \right)$$

- Tof_{major} is time-of-flight of a fragment in the Major spectrum
- Tof_{minor} is time-of-flight of a fragment in the Minor spectrum
- Tof is $Tof_{minor} - Tof_{major}$
- $b = b_{minor} - b_{major}$
- a, b_{major} and b_{minor} are instrument-specific constants determined during instrument calibration

Conclusion

- By designing a novel PSD experiment, it is now possible to perform analysis on fragment ions from multiple precursors in parallel.
- This approach offers a performance increase over conventional PSD, decreasing acquisition time, eliminating the need for precursor ion selection, and increasing the number of precursors that can be studied per MALDI sample.

720000948, September 2004

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