

AN LC/MS ORTHOGONAL TOF (TIME OF FLIGHT) MASS SPECTROMETER WITH INCREASED TRANSMISSION, RESOLUTION, AND DYNAMIC RANGE

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Presented at ASMS, Montréal, Canada, 8th-12th June 2003

OVERVIEW

Exact mass LC/MS analysis using an orthogonal acceleration time of flight (oa-TOF) mass spectrometer is a well-established technique with a broad range of applications. These include elemental composition confirmation, metabolite ID, impurity profiling and natural product screening. The requirement for low detection limits, high mass accuracy and the ability to produce quantitative and qualitative data over a wide range of sample concentrations puts high demands on the instrumentation employed. In this paper we describe an LC/MS oa-TOF mass spectrometer with enhanced ion transmission, the option of increased resolution and the option of increased dynamic range by a method of 'transmission switching'.

EXPERIMENTAL

All results were obtained on a Waters® Micromass® LCT Premier™ Mass Spectrometer (Figure 1). The LCT Premier is a benchtop LC/MS orthogonal TOF instrument, with an integrated LockSpray™ source. Data acquisition is via a 4 GHz time to digital converter (TDC). All experiments were carried out by electrospray ionization. Samples were introduced via infusion from an integrated syringe pump, or by injection from a HPLC system.



Figure 1: The Waters LCT Premier LC/MS orthogonal TOF mass spectrometer.

RESULTS AND DISCUSSION

Resolution

The TOF analyzer of the LCT Premier incorporates W-Optics™, a technique that effectively doubles the path length of the analyzer, giving twice the resolution. The instrument can be switched from V to W mode by changing the energy of the beam that enters the pusher. In V-mode the ion beam from the pusher is reflected by the reflectron directly onto the detector, passing twice through the drift region (Figure 2). In W-mode, the ion beam axial energy is reduced, and the orthogonally accelerated ions approach the reflectron at a different angle, reflecting into the W-mirror (a second, smaller reflectron). The result is that the ion beam passes through the drift region a total of four times (Figure 3).

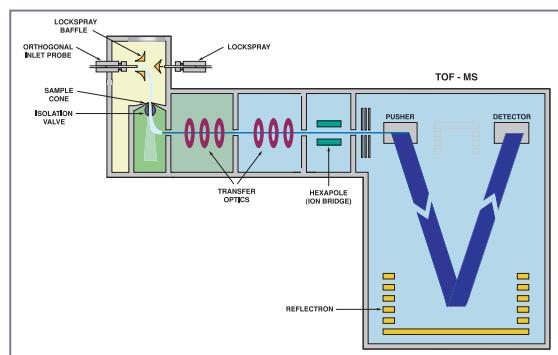


Figure 2: Instrument Schematic with Analyzer in V-mode.

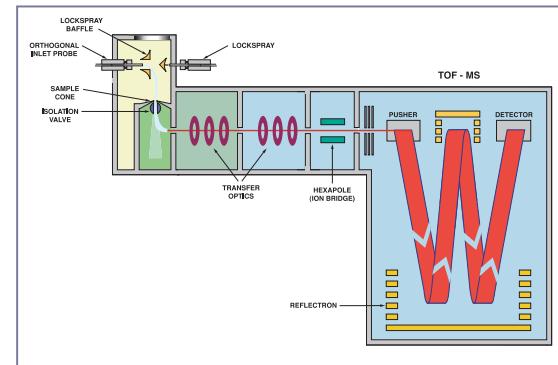


Figure 3: Instrument Schematic with Analyzer in W-mode.

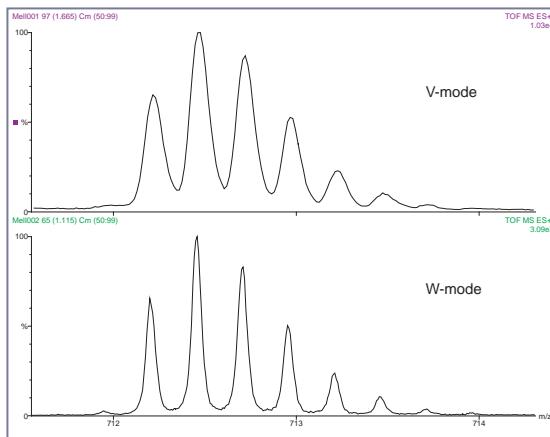


Figure 4: The Effect of W-Optics on Resolution and Transmission.

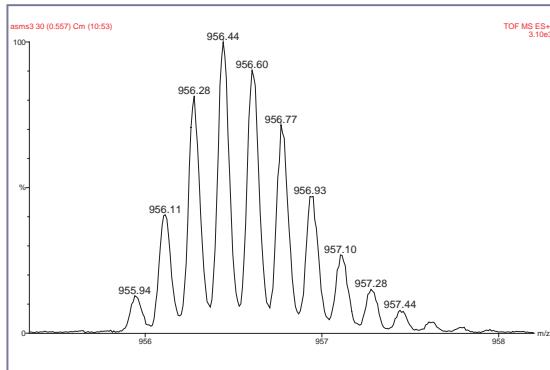


Figure 5: Bovine Insulin in W-mode.

The increased flight time in W-mode doubles the resolution, as illustrated in Figure 4. This shows the $[M+4H]^{4+}$ isotope cluster from melittin at m/z 712.2. The resolution in V-mode is 5880 FWHM, and switching to W-mode increases this to 12070 FWHM. Another consequence of switching to W-mode is a reduction in transmission. This is due to the increased number of passes through reflectron grids, and losses due to the lower axial ion energy. In the example shown, the peak intensity in W-mode is 30% of that in V-mode.

The increased resolution of W-mode can reduce mass errors due to mass interferences. It can also be useful in the analysis of compounds of higher mass. Figure 5 shows the fully resolved $[M + 6H]^{6+}$ isotope cluster from bovine insulin (molecular mass 5729.6 Da). In this example a resolution of 12900 FWHM was achieved.

Transmission

High ion transmission has been achieved through the use of an improved ZSpray™ source, dual ion guides and high transmission grids.

The new ZSpray source sampling cone aperture cross-sectional area is increased by a factor of 3.5 compared with the LCT™. The higher throughput of this source requires the use of an extra pumping stage, consisting of an ion guide pumped by a 28 m³/hr rotary pump. A second ion guide is pumped by the third stage of a split-flow turbomolecular pump.

The LCT Premier uses higher transmission grids, to give a factor of three increase in ion transmission over that of the LCT. These new grids have a 10:1 aspect ratio for optimum transmission without a reduction in resolution (1).

The transmission of the LCT Premier has been compared with the LCT. A solution of 50 pg/ μ L of leucine enkephalin was continuously infused at 5 μ L/min into each instrument. Both instruments were tuned to 5000 FWHM resolution. A typical result is shown in Figure 6. The peaks are labelled with ion counts, allowing easy comparison of transmissions. These data show that the transmission of the LCT Premier is more than 10 times that of the LCT.

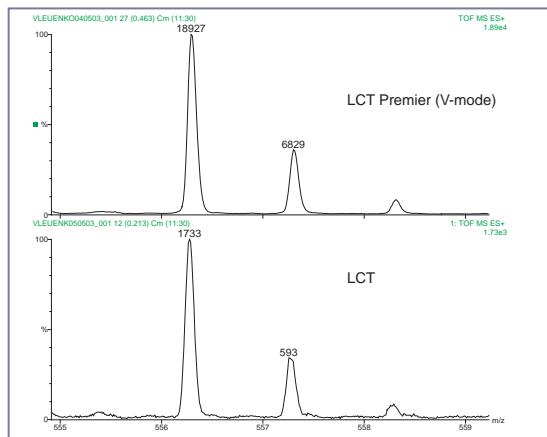


Figure 6: The Ion Transmission of the LCT Premier compared with the LCT.

Dynamic Range

All TDC-based detection systems suffer from saturation effects at high ion currents. The effective dead-time of the detector restricts the number of ions detected to one per peak per push. This causes a mass shift and intensity error at high sample concentrations. The use of mathematical algorithms to correct for this effect has proved quite successful, but as the ion currents increase, a point is reached at which a mathematical correction is no longer possible. To get beyond this limit a new strategy is required.

The dynamic range of the LCT Premier has been increased by using the method of transmission switching. A Z-focus lens has been added to the transfer optics, giving the capability of diverging the ion beam before the pusher, resulting in a reduced ion transmission. This is illustrated in Figure 7. High speed electronics allow rapid switching between high and low transmission states. As the ion beam is defocused in the Z-direction, orthogonal to the plane of the analyzer, the effect on resolution and mass measurement is minimized.

The transmission of the instrument is typically reduced by a factor of about 40 in the low transmission state. During an acquisition, the Z-focus lens is switched rapidly between two predetermined values. Data from the TDC is tagged, to show whether it is high or low transmission data.

Data is processed in real time on an embedded PC. If any saturated peaks appear in the high transmission data, the affected peaks are replaced by data from the adjacent low transmission data. The ratio of instrument transmission between the two states can be determined; the low intensity data is multiplied by this factor to give unsaturated high intensity data.

The result is a composite spectrum, which includes high intensity peaks that would normally be expected to be saturated.

Only one mass calibration is needed, which is acquired in the high transmission state. The small mass shift associated with the change in the Z-focus lens voltage when switching to the low transmission state, is automatically corrected using a lockmass.

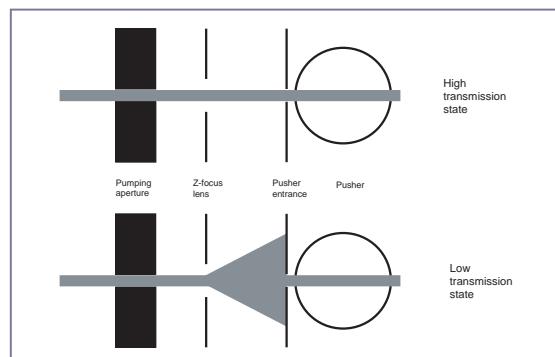


Figure 7: The Z-focus Lens.

Linear Quantitation over an Increased Dynamic Range

Sulfadimethoxine ($C_{12}H_{14}N_4O_4S$, $M+H = 311.0814$) has been analyzed by LC/MS over the concentration range 0.5 - 5000 ng/mL. Figure 8 shows results obtained without the use of transmission switching. The linear portion of the calibration curve extends over approximately 2.5 orders of magnitude.

Figure 9 shows results obtained with the use of transmission switching. In this case the linear dynamic range extends to four orders of magnitude.

LC Conditions

Isocratic Analysis (65/35 H₂O/MeCN+0.1% formic acid)

Flow Rate = 0.3 mL/min

Injection Volume = 10 μ L

Column = Waters® Symmetry® 2.1 x 50 mm, 3.5 μ

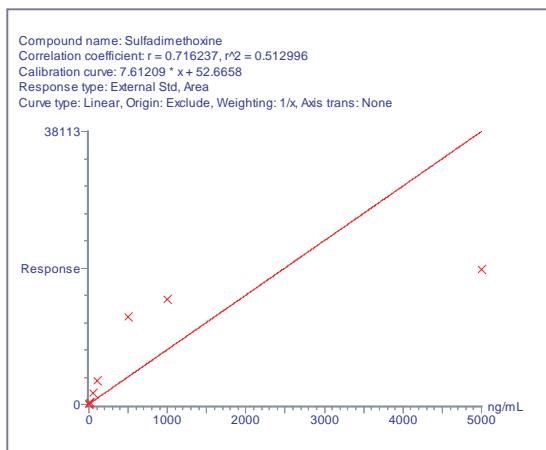


Figure 8: Quantitation without transmission switching.

Compound name: Sulfadimethoxine
Correlation coefficient: r = 0.999466, r² = 0.998933
Calibration curve: 32.1113 * x + 38.1674
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None

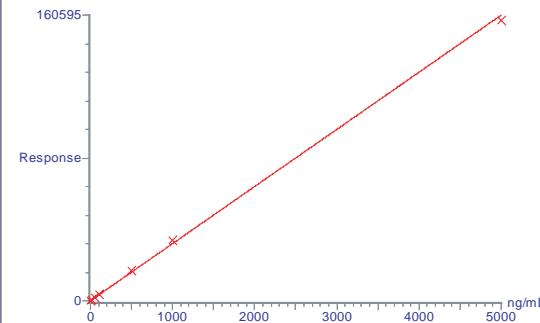


Figure 9: Quantitation with transmission switching.

Mass Measurement over an Increased Dynamic Range

The sulfadimethoxine experiments previously described have also provided mass accuracy data, measured as a function of concentration of analyte. Figure 10 shows the results obtained without the use of transmission switching. The increased mass error at higher concentrations restricts the usable dynamic range to about 2 orders of magnitude. In contrast, when transmission switching is used, the mass measurement accuracy is maintained over 4 orders of magnitude of analyte concentration. This is shown in Figure 11.

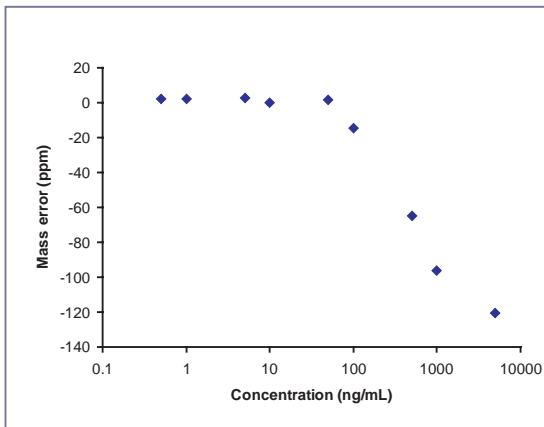


Figure 10: Mass Measurement without transmission switching.

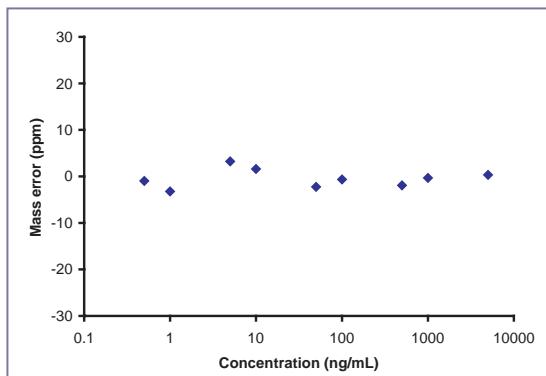


Figure 11: Mass Measurement with transmission switching.

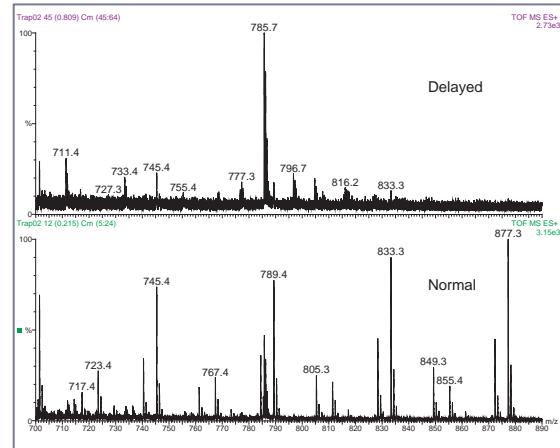


Figure 12: Charge State Discrimination.

Charge State Discrimination

The LCT Premier uses a newly developed ion guide². This enables a potential gradient to be created within the guide, and allows ions to be stored in this region for short periods of time. Storing ions in the first ion guide, at a pressure of ~ 2 mbar, allows the abundance of singly charged ions to be suppressed compared to that of multiply charged ions. This effect is illustrated in Figures 12 and 13. The lower traces show a mixture of singly charged and doubly charged ions. The upper traces show the spectrum that is obtained when ions are deliberately delayed in the ion guide. The effect is an almost complete elimination of singly charged peaks from the spectrum. The theory behind this effect is described in poster TPJ 183: Charge State Discrimination by means of Ion Storage at Intermediate Pressures.

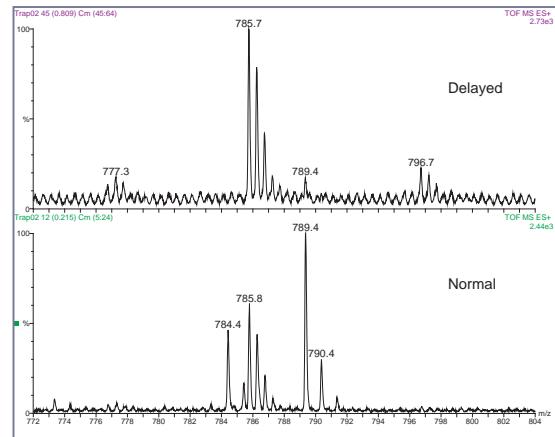


Figure 13: Charge State Discrimination.

CONCLUSIONS

1. Source, ion guide and grid changes have led to a ten-fold increase in ion transmission.
2. The incorporation of W-Optics has increased resolution to over 10000.
3. Dynamic range for both quantitation and accurate mass measurement has been extended to 4 orders of magnitude through the use of transmission switching.
4. The ability to store ions at high pressure allows suppression of singly charged ions in an electrospray spectrum, thereby enhancing the relative abundance of multiply charged ions.

REFERENCES

1. Lewin MJ, Guilhaus M, J. Wildgoose, Hoyes J, Bateman R. Ion dispersion near parallel wire grids in orthogonal acceleration time-of-flight mass spectrometers: predicting the effect of approach angle on resolution. *Rapid Comm. Mass Spectrom* 2002;16:609-615.
2. Patent Pending

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