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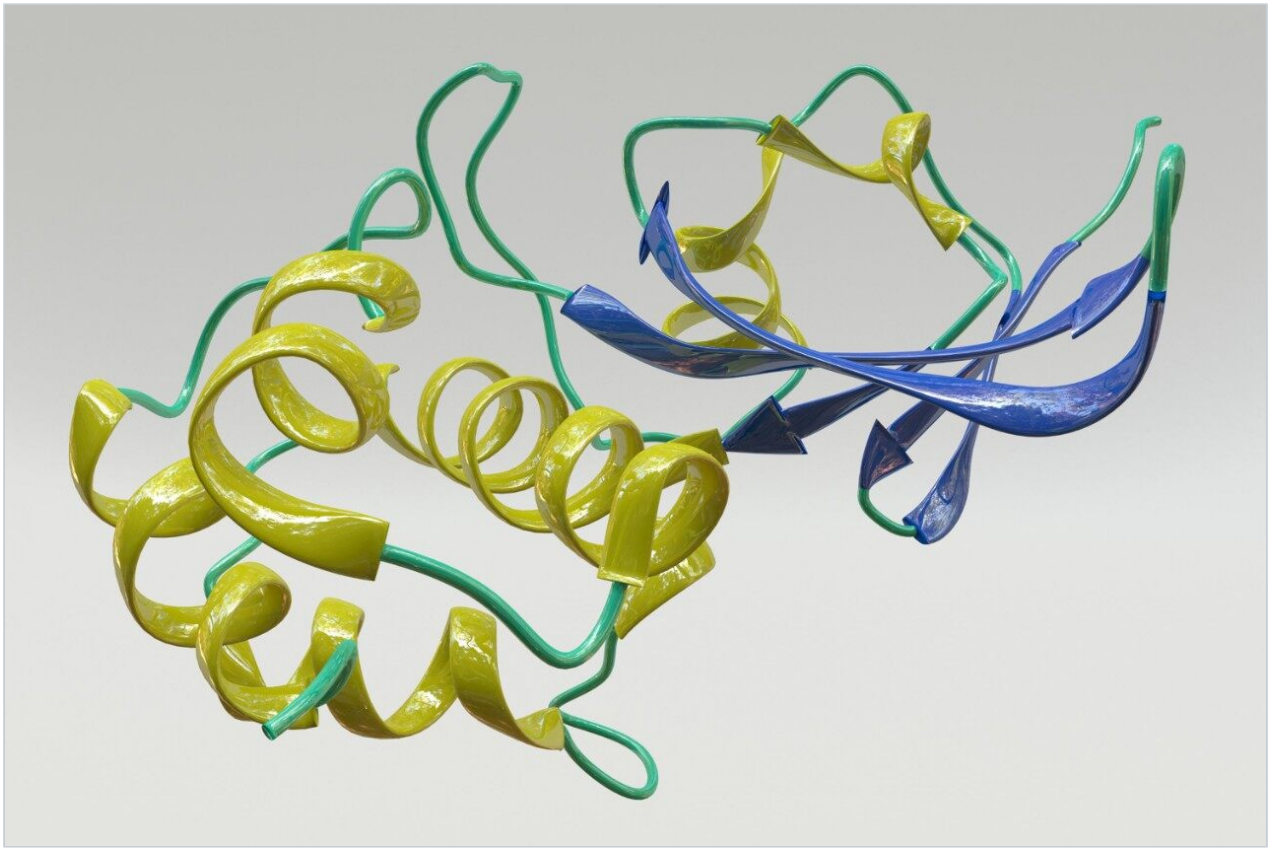
Application Note

Analysis of Complex Tryptic Digests with the Q-Tof Premier

Chris Hughes, Therese McKenna, Iain Campuzano, James I. Langridge

Waters Corporation



This is an Application Brief and does not contain a detailed Experimental section.

Abstract

In this application brief, we have studied the stability of the Q-Tof Premier analyzer, over extended time periods, and also the effect of mass spectrometer resolution and the mass measurement accuracy, on the number of peptide species that may be confidently assigned.

Introduction

It has become increasingly clear that relative quantitation of protein expression changes is important in proteomics. We have recently described an LC-MS strategy[1] where quantitation is achieved via normalization of the LC-MS datasets and comparison of the peptide intensities across samples is performed.

In this type of experiment, it is desirable to perform replicate injections and this places a requirement upon good chromatography and mass spectrometer analyzer performance. The ability to measure the mass to charge ratios of ions accurately, between injections and across samples, increases the confidence that the same ions have been matched from each sample injection.

Experimental

Nanoscale HPLC

A Waters nanoACQUITY UPLC System was configured in trapping mode with a C_{18} pre-column (320 µm ID x 5 mm, Waters Symmetry C_{18}) and a nanoscale analytical column (Waters Atlantis dC18, 3 µm, 75 µm x 100 mm) The flow through the columns was set to 300 nL/min and the output attached to a nanoLC sprayer. The solvents used were A, 95% water/5% acetonitrile + 0.1% formic acid, B, 95% acetonitrile / 5% water + 0.1% formic acid and C, aqueous 0.1% formic acid. After a three minute loading / washing period, a gradient was started. The gradient changed from 2%B to 40%B in 90-minutes, then ramped to 90%B.

The sample used in this study was a tryptic digest of an *E. Coli* cytosolic cell fraction (Waters, Milford, MA), with Yeast Enolase added in at the level of 50 fmol.

Mass Spectrometry

The Q-Tof Premier mass spectrometer was equipped with a NanoLockSpray source to provide exact mass measurements on all eluting peptide species. The reference probe of the NanoLockSpray source was set up to continually infuse a solution containing Glu- Fibrinopeptide B $[M+H]^{2+}$=785.8426 amu; The reference mass was sampled every 30 seconds. Data was acquired using an alternating low (8eV) and elevated (15 to 40eV) collision energy function and an integration time of 1.5 seconds was used for each scan. The oa-Tof was operated in either, V-Optics mode of operation at a mass resolution of >10000 FWHM, or in the W-Optics mode with a mass resolution of >17500 FWHM.

Results and Discussion

Mass Analyzer Stability

An important factor in the matching of ions across multiple runs and over extended time periods is the stability of the mass analyzer, both in terms of resolution and mass measurement accuracy.

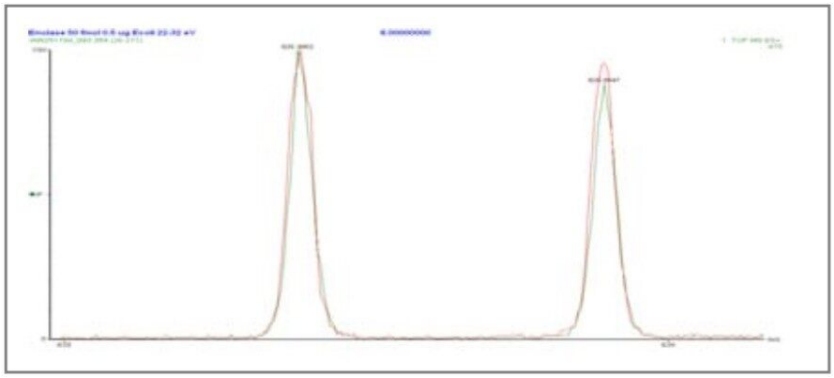


Figure 1. Shows the stability of Tof MS resolution. Displayed is a doubly charged ion (FWHM = 19500) from a tryptic peptide originating from an E.coli protein. This data was obtained from repeat injections of the sample, 28 hours apart.

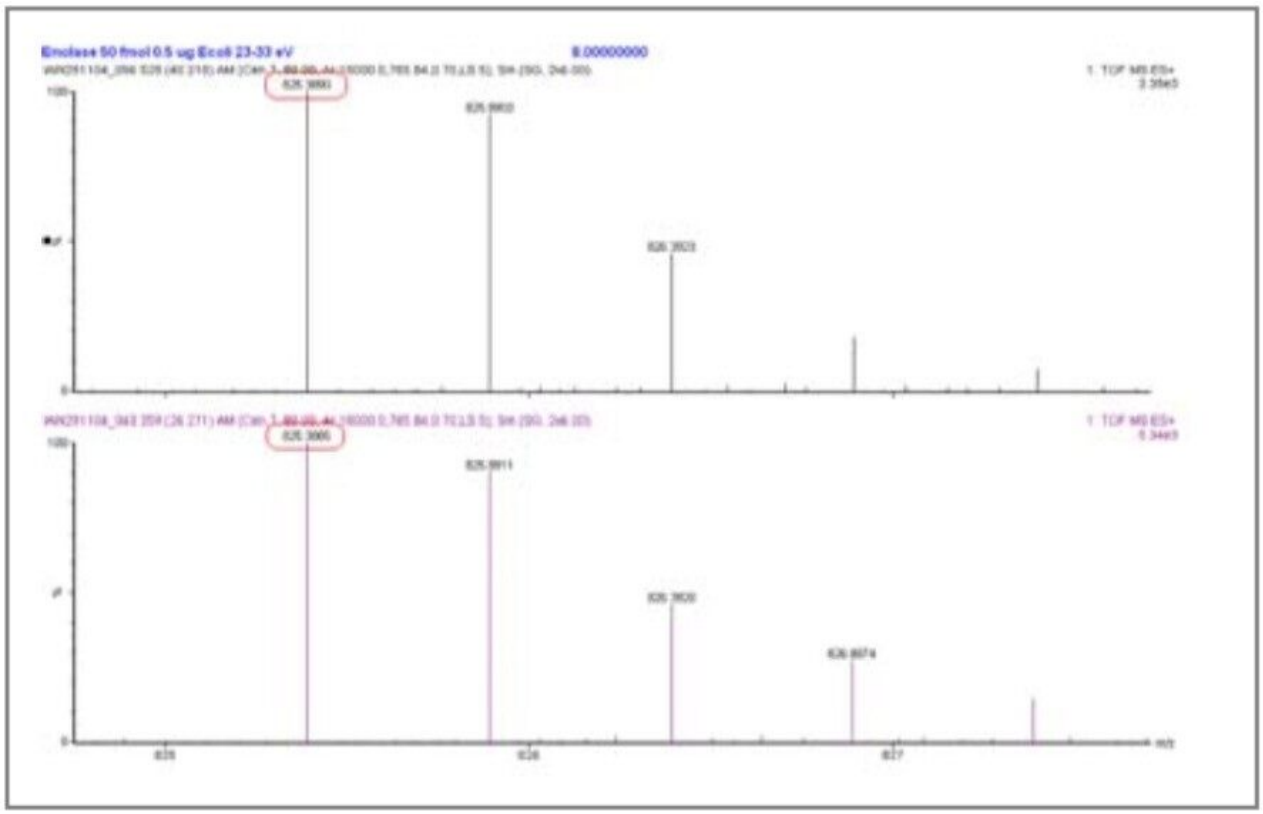


Figure 2. Displays the lockmass corrected doubly charged ion. The mass measurement difference observed over the 28-hour period is 0.5mDa or 0.6ppm.

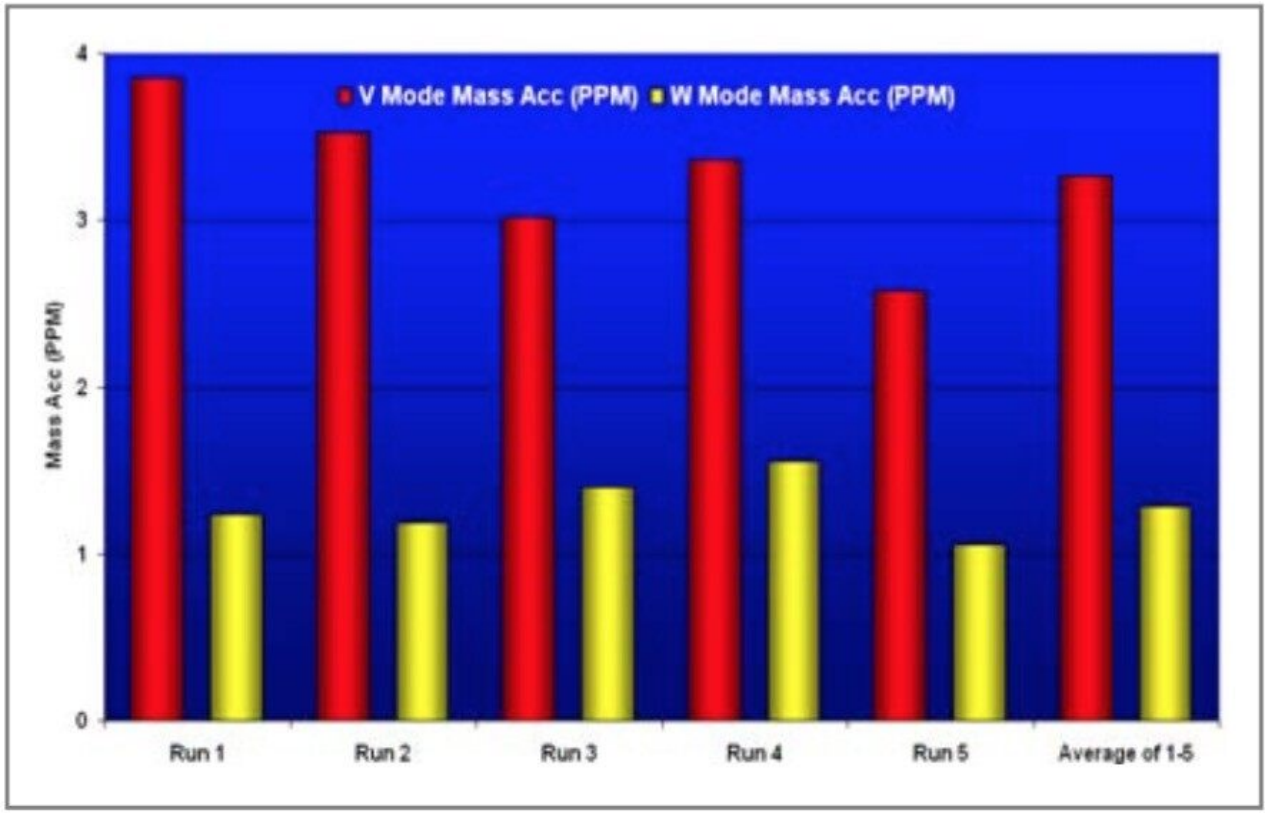


Figure 3. A comparison of the mass accuracies obtained in V-Optics mode vs W-Optics mode. Plotted is the mass accuracy for the two highest scoring peptides in the top ten proteins identified in each injection. The average error for V-Optics is 3.3ppm while W-Optics is 1.29ppm.

Comparison of V-Optics to W-Optics

Yeast Enolase spiked into an *E. Coli* tryptic digest was analysed five times in both V- and W-Optics, to compare the protein identifications obtained from the LC-MS system. Identifications were made by databank searching.

	V Mode		W Mode	
Injection	No. Peptides	Score	No. Peptides	Score
1	17	313	15	426
2	15	300	15	417
3	18	325	14	505
4	15	218	17	487
5	16	335	16	490
	Total = 81		Total = 77	

Figure 4. Databank search results obtained from the V-Optics and W-Optics data, from 5 repeat injections. Total number of peptides is slightly lower in the W-Optics mode, however, the improved mass measurement accuracy in W-Optics leads to considerably higher protein scores and hence more confident identification. The peptides not apparent in the W-Optics mode, but identified in V-Optics, are of low intensity and provide little fragment ion data.

Conclusion

The Q-Tof Premier mass analyzer exhibits excellent stability in terms of the resolution and mass measurement accuracy obtained, which is vital in analysing large sample sets over extended periods of time.

The W-Optics mode (mass spectrometer resolution of >17500 FWHM) leads to improved mass measurement accuracy, in comparison to the 'V-Optics' mode of operation leading to more confident protein identifications.

References

1. T. McKenna *et al.;* Proceedings of the 52nd Conference ASMS, Nashville, TN

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