Addressing the Issues of Very Sharp LC Peaks Width in Quantitative LC/MS/MS

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The advent of the electrospray interface for mass spectrometry in the late 1980's [1] had a dramatic impact on the field of analytical chemistry. The sensitivity and specificity that mass spectrometry confered on analytical detection could now be combined with the sample compatibility and selectivity of liquid chromatography to produce, perhaps, the most powerful and exploited hyphenated analytical technology, LC/MS(MS) [2]. The application of LC/MS(MS) has moved from the early adopters for structural elucidation to impurity measurement, metabolite profiling [2] and bioanalysis [3]. In the pharmaceutical industry, natural products analysis, proteomics [4] and metabonomics [5]. In academia water quality testing, environmental monitoring and food safety testing to name but a few. Indeed the doping control of the sports that we watch on TV relies heavily on LC/MS as well as GC/MS [6].

The hyphenation of mass spectrometry with LC and the subsequent increase in demand for this mode of sample analysis required better utilisation of expensive MS detector technology. Many scientists quickly realized that the increased specificity of MS and MS/MS technology reduced the need for complete analyte chromatographic resolution and analysis times could be significantly shortened from the typical 25-30 minutes to just 3-5 minutes. Indeed many scientists in the bioanalytical field tried to remove chromatography altogether from the analysis process - relying solely on the selectivity and specificity of the mass spectrometer for the analysis [7]. Whilst this approach had some success, factors such as ion suppression, specificity from metabolites and absolute sensitivity for low systemic exposure compounds still required some form of separation prior to mass analysis. The advent of a commercial sub 2 µm chromatographic system in the early 2000's gave both high throughput, with analysis times in the 1-2 minute range, and high resolving power (efficiency), typically 3-5 times greater than that of an equivalent length conventional column. Many bioanalytical scientists were quick to see the potential of this form of chromatography [8], as with the increase in chromatographic efficiency came sharper peaks and hence more sensitivity, many researchers quoting figures in the 3-8 fold range, with analysis times cut by a similar figure.

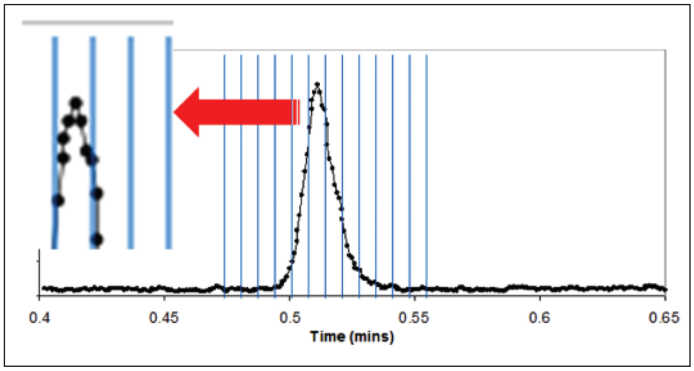

Figure 1: Illustration of the effect insufficient sampling rate on peak definition

Importance of data sampling

These narrow peaks however do have their drawbacks. To achieve accurate reliable quantification it is necessary to measure between 10 and 15 data points in order to accurately define the chromatography peak. Failure to employ the correct acquisition rate will result in the incorrect sampling of the peak and missing of the peak apex (Figure 1). As we can see in this example the lower data acquisition rate results in the apex of the peak falling between two data points. This would result in a flat toping of the peak and an underestimate of the peak area.

With peak widths from the sub 2 µm particles in the region of 1-2 seconds at the base, this means that data acquisition rates of less than 50 milliseconds (ms)are required for an analysis with an internal standard. If more than one analyte is required during analysis then the acquisition times are even lower. However these higher data acquisition rates can result in reduced data quality as the mass spectrometer must quickly switch the electronic setting between the two (or more) data channels reducing the inter-scan delay and resulting in a lower signal to noise value. This is due to the increase in noise rather than a reduction in signal. The major reason for this is that most triple quadrupole mass spectrometers are not designed with this fast switching in mind and hence suffer from increased electronic noise at these data acquisition rates. The rapid switching between analyte channels also requires that the collision cell be completely cleared of ions between data points as internal standards and related metabolites often have the same product ion as the analyte ion. Failure to completely remove these fragment/product ions will result in cross talk between channel monitoring and reduced data quality.

T-wave collision cell

Although many manufacturers have increased the speed of MS quadrupole scanning to compensate for the sharp sub 2 µm LC peaks, the increased scan speed still results in a

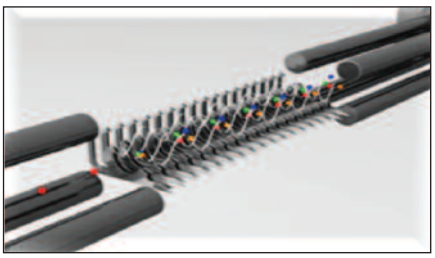

Figure 2. T-Wave ion guide

reduced signal to noise value. To address this problem a new form of collision cell has been designed which uses a set of stacked ring electrodes (approximately 100) rather than quadrupoles. The stacked ring ion guide (SRIG) comprises a series of planar ring electrodes arranged orthogonally to the ion transmission axis where opposite phases of an RF voltage are applied to adjacent electrodes to confine the ions. To propel ions through the ion guide a transient DC voltage is superimposed on the RF applied to a pair of adjacent electrodes in a repeating sequence along the length of the device, providing a continuous sequence of travelling waves (T-Wave – see Figure 2), similar to that described by Kirschner [9]. The ions within the device are carried through the device with the waves, minimising their transit time. The use of this approach allows ions to be ejected from the collision cell at any point in time allowing very low inter scan delay times to be employed with little or no cross talk. It also means the cell can be rapidly filled, therefore maximising signal in a short dwell time.

This new collision cell approach, along with faster electronics allow the use of ultra – low dwell and inter scan delay times with little or no loss in signal to noise values. The data displayed in Figure 3 compares the MRM signal for a typical small molecule compound, propranolol (m/z transition 260 ⇒ 116), with a dwell time of 100 ms and 10 ms. Here we can see that the signal to noise has only been reduced by 7% with the faster data collection rate. This fast data capture rate is critical if the true speed advantage of the sub 2 µm particles is to be exploited. Failing to do so will result in unnecessarily long chromatographic analysis times to compensate for the slow MS duty cycle.

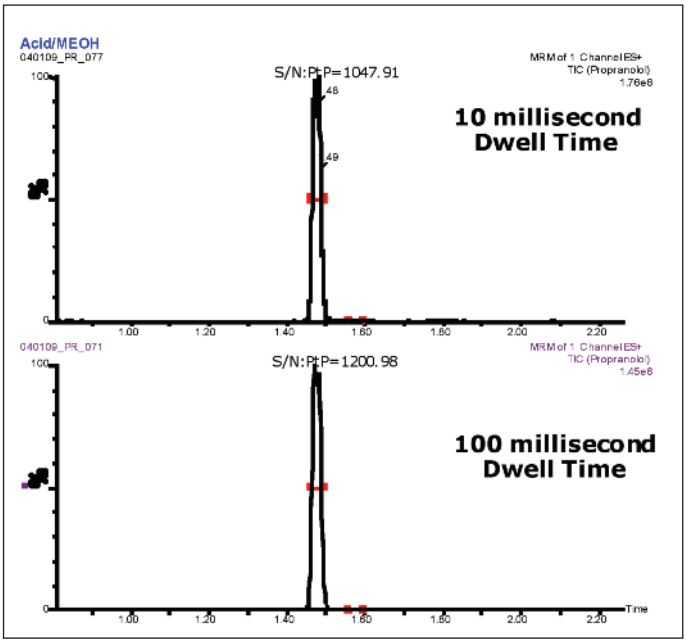

Figure 3: Comparison of signal to noise from a positive ion LC/MS analysis of propranolol in MRM mode using both a 100 ms (top) and 10 ms (bottom) dwell time

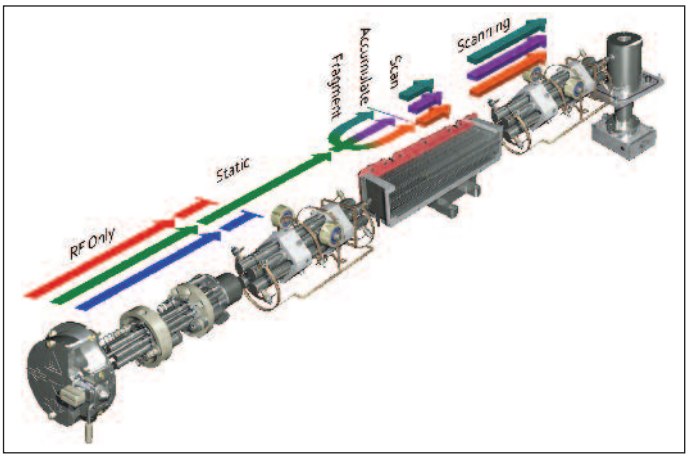

Figure 4: Schematic of ion optics path using TWave collision cell operating in high sensitivity full scan mode

It is not only in quantitative MRM mode that a high MS data capture rate is important for accurate results, this is also the case for qualitative measurements. Sub 2 µm LC has also been quickly accepted in the field of qualitative analysis, such as metabolite ID, metabonomics and impurity profiling. The use of low data acquisition rates in qualitative analysis would result in poor peak definition and the potential contamination of one analyte peak spectra with another. Modern time of flight (TOF) MS instruments have the capability of collecting data at a rate of 50 ms per spectra acquisition or lower, this is achieved without loss of MS resolution. This is not the case for electrostatic ion trap, such as the Orbitrap, MS instruments where the use of high data acquisition rates can reduce the MS resolution to below 10,000 FWHM (Full Width Half Maximum) at speeds greater than 1 spectra per 500 ms.

It is well recognised that the best approach for structural elucidation is via the use of accurate mass measurement [10] for the ability to produce elemental composition information. However, triple quadrupole instruments are still widely used for this application area as they allow both quantification and qualitative analysis. The relatively low duty cycle rate of the tandem quadrupole instruments does not traditionally lend itself to high sensitivity full scan analysis. Again the T-Wave collision cell can be exploited to somewhat address this issue with an approach called ScanWave, Figure 4. Here the collision cell can be operated in such a way that the first 2/3^{rds} are used in a conventional manner to fragment the ions of interest and the final 1/3rd of the collision cell is used to store the ions and eject them into the final resolving quadrupole. The MS/MS system is operated in linked ion guide mode where the ejection of the ions from the collision cell is synchronised with the scanning of the final resolving quadrupole. This is achieved by controlling the release of the ions from the collision cell by the means of an RF barrier. This barrier is gradually reduced during the course of a MS scan causing the heavy ions (greater m/z value) to be ejected first and the lighter ions last. This functionality allows the final quadrupole to be scanned in the same manner (high ⇒ low m/z) and in synchronization with the ejection of the ions significantly improving the duty cycle and hence spectral sensitivity (typically 10 fold - see Figure 5). The main benefit of this approach is that it does not require the accumulation of the ions in the cell prior to ejection like a linear ion trap, meaning that this approach does not affect the data capture rate. This again allows the analyst to take full advantage of speed and resolving power of sub 2 µm LC.

Conclusion

New LC technology such as sub 2 µm particle LC and SFC provide faster analysis and improved resolution. In order to take full advantage of this new LC technology mass

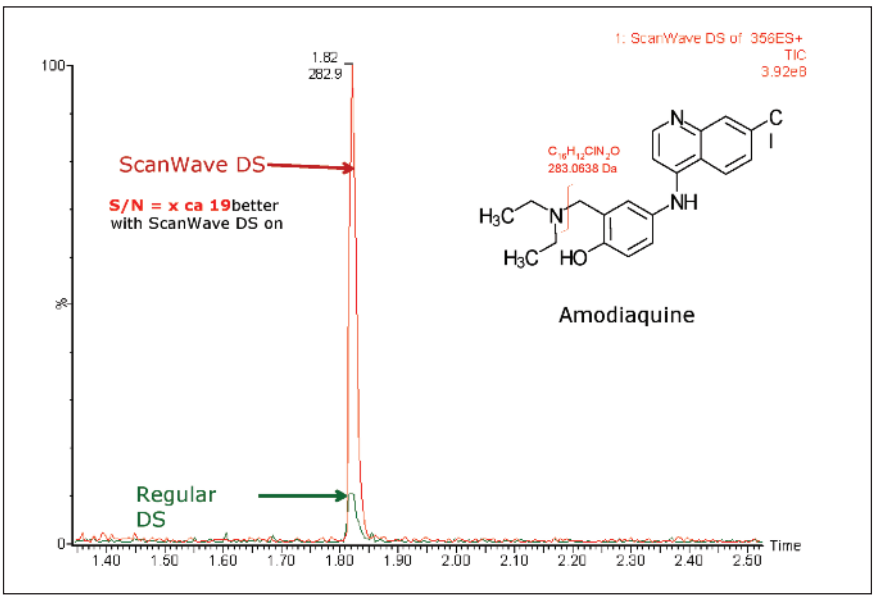

Figure 5: Comparison of full scan MS/MS sensitivity using normal acquisition (green mode and Scan Wave mode (red)

spectrometry must advance with faster data acquisition speeds which do not compromise data quality or sensitivity. Technologies such as the T-Wave collision cell and scan wave allow fast data acquisition, multiple analyte analysis, high sensitivity analysis and collection of spectral data without compromising LC run times. This approach and others will allow scientists to take full advantage of new LC technologies. The author would like to thank Dr. Marian Twohig for the data in Figure 5.

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