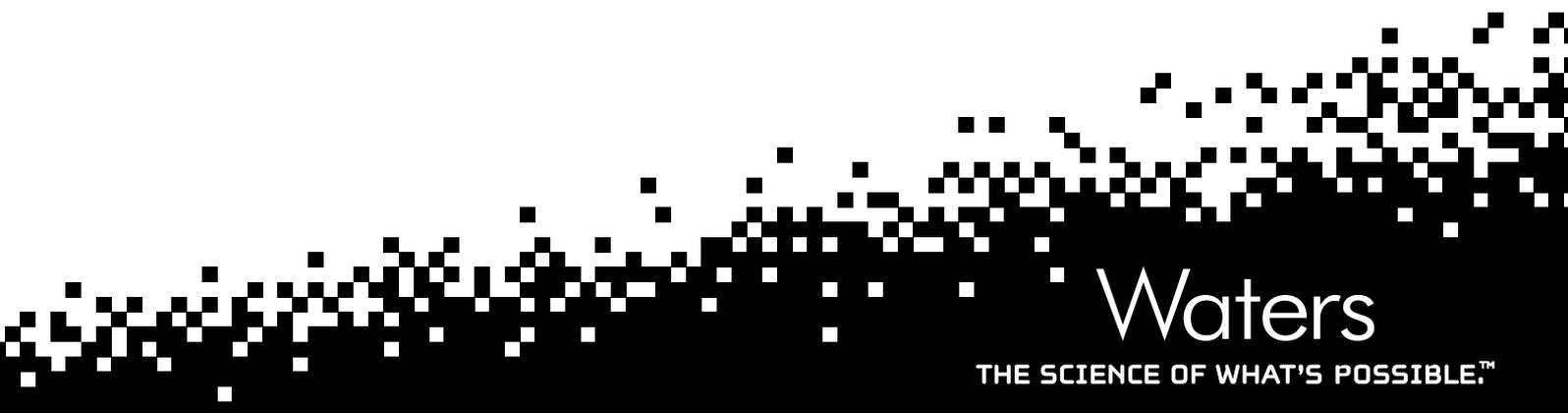


QUANTOF

High-resolution, accurate mass, quantitative
time-of-flight MS technology



Waters

THE SCIENCE OF WHAT'S POSSIBLE.™

Orthogonal-acceleration time-of-flight (oaToF) mass spectrometers are invaluable tools for the detection and identification of chemical compounds in complex mixtures. This is because they are capable of delivering high sensitivity and high resolving power at the high spectral acquisition rates required to fully exploit state-of-the-art separations technologies such as UltraPerformance LC® or UltraPerformance Convergence Chromatography.™ However, traditionally oaToF analyzers have been used primarily for qualitative rather than quantitative applications due, in part, to limited dynamic range compared to triple quadrupole instruments.

This lack of dynamic range presented a significant challenge to scientists who needed to detect, identify and quantify compounds across a wide range of concentrations in complex sample matrices. There was a clear need to improve dynamic range with an ion detection system that gives a proportionate response

across a wide range of signal intensities. It was also essential that any improvements in dynamic range did not compromise the mass resolving power or UltraPerformance LC compatibility. This challenge has been met by Waters' QuanToF™ technology.

QuanToF technology incorporates novel detector electronics and hardware features that enable Waters' modern oaToF mass spectrometers to generate spectra at speeds which enable narrow UPLC® peaks to be accurately profiled without compromising mass resolution. At the same time QuanToF provides a proportionate response across a wide range of signal intensities, regardless of spectral complexity, so that accurate quantitative results can be obtained even in the crude sample extracts that many scientists must deal with. QuanToF technology effectively makes possible the simultaneous acquisition of quantitative and qualitative data in a single LC/MS experiment.

The Challenge of oaToF Ion Detection

In an oaToF mass spectrometer ions are transmitted to the pusher region and accelerated orthogonally by a pusher voltage. The ions then separate according to m/z in the flight tube, are focused in the reflectron region and arrive at the detector where their arrival times are accurately measured (Figure 1).

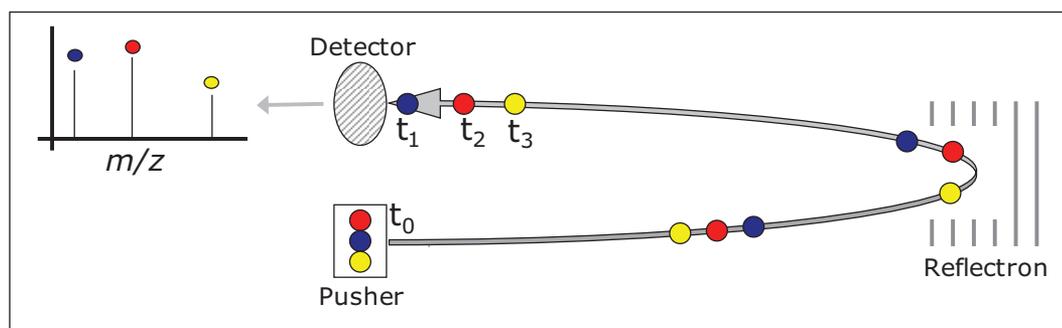


Figure 1. Ions of different m/z travel at different velocities in the ToF MS analyzer and arrive separately at the ion detector. Arrival times are then converted to a mass spectrum.

A spectrum from a single ion push can take between 30 and 100 μs to acquire. This means that data from an oaToF MS system is acquired at speeds of up to 30,000 oaToF spectra per second. Individual oaToF Spectra are summed and a composite spectrum is recorded as raw data (Figure 2).

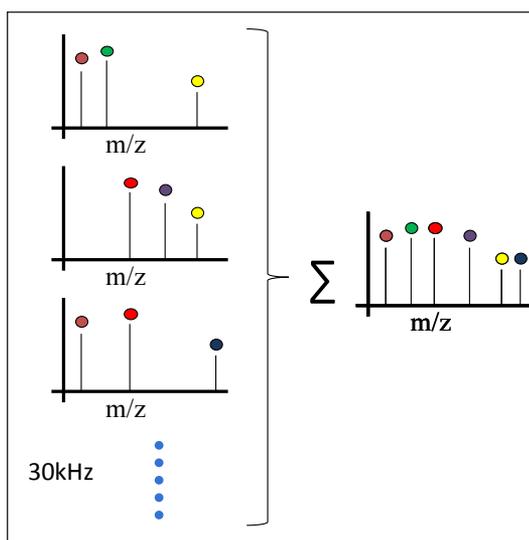


Figure 2. Signals from a large number of individual oaToF MS spectra are summed to produce a composite spectrum, which is recorded as raw data.

The signals produced by the detector from the arrival of a single ion and from the near-simultaneous arrival of two ions are shown (Figure 3). The detector produces an output signal from an ion arrival with a width of only 600ps, allowing ion arrival times to be measured with a high level of accuracy. The detector output is also proportional to the total charge on the particles striking it at any given point in time and displays an inherently wide dynamic range of response.

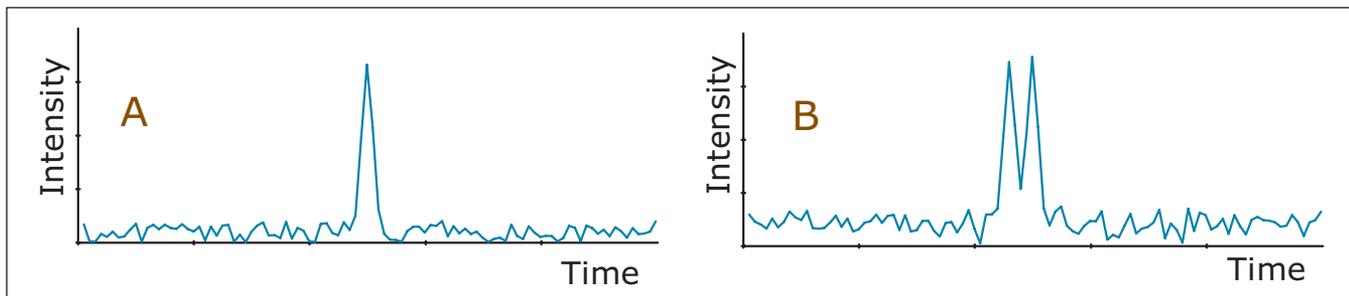


Figure 3A. Detector output from a single ion arrival event.

Figure 3B. Detector output from two ions arriving in close proximity.

The challenge is to take full advantage of these beneficial properties of the ion detector and the critical next step is the conversion of the detector signal into recorded digital information in a way that allows high dynamic range, high mass resolution and high mass accuracy to be obtained simultaneously in the final mass spectrum.

Analogue to Digital Conversion

At the heart of QuanToF is an analogue-to-digital converter (ADC) that records the intensity of detector response over time. This enables the very fast signals produced by the detector to be correctly represented and arrival time and intensity to be calculated accurately (Figure 4). This allows oaToF spectra to be recorded with high mass resolution, high mass accuracy and high dynamic range at very fast data acquisition rates.

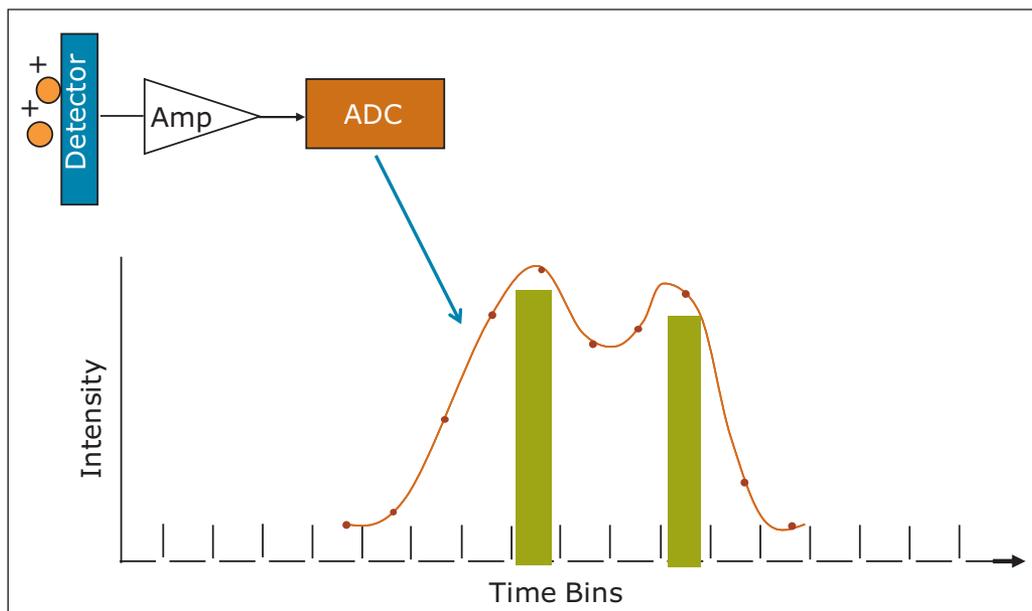


Figure 4. The near-simultaneous arrival of two ions at the detector is shown. The signal is amplified and produces two partially overlapping peaks. The ADC is able to accurately record both the arrival time and intensity of both ions.

The ADC is an 8 bit device that is able to record a maximum of 25 ions arriving simultaneously during a single time-of-flight spectrum from a single orthogonal sampling of the ion beam (Figure 5).

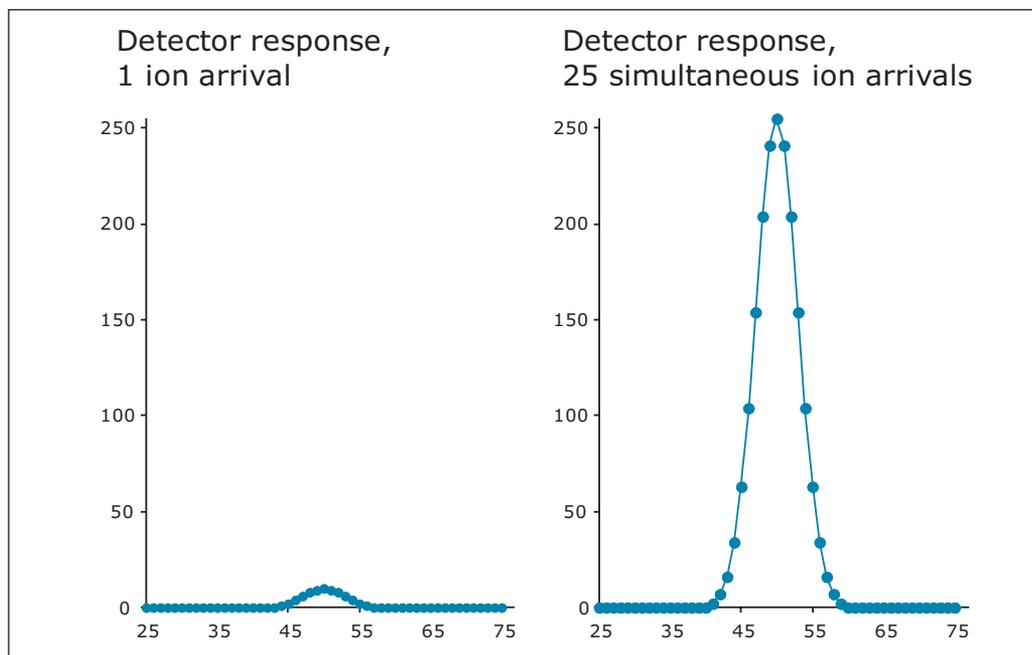


Figure 5. The area under the detector signal peak is directly proportional to the number of charges arriving at the detector simultaneously, up to a maximum of 25.

The theoretical dynamic range of the detector may be calculated as follows:

- Assume an analyte compound separated by UPLC chromatography elutes with a chromatographic peak width of 1.5 seconds at half height.
- The number of individual orthogonal sampling events which can occur in 1.5 seconds, at a rate of 30,000 oaToF spectra per second, is 45,000.
- Assume 10 ions is the minimum number needed to generate a mass spectral measurement of an analyte peak. This may be equated with a limit of detection for the analyte.

Average number of ions observed per orthogonal sampling event at the limit of detection is therefore:

$$\frac{10}{45,000} = 0.0002 \text{ ions per push}$$

The upper limit of linear dynamic range has already been defined as 25 ions per orthogonal sampling event. The theoretical maximum dynamic range is therefore:

$$\frac{25}{0.0002} = 125,000$$

This equates to a potential maximum of over 5 orders of magnitude dynamic range.

Matrix Tolerant Dynamic Range

Complex samples can give rise to intense background ions from the many co-extracted components in the sample matrix. Some types of mass analyser require gain-control or signal-attenuation techniques to cope with the high total ion current (TIC) that may be generated. A Waters oaToF mass analyser does not suffer from charge-capacity limitations such as those experienced in 3D, linear or electrostatic ion trap systems, so the high dynamic range of the QuanToF detector can be used to maximum effect, generating mass spectra where both very intense and very weak signals can be recorded simultaneously, regardless of overall TIC (Figure 6). The QuanToF detector has the added advantage of maintaining instrument resolving power across the full dynamic range.

The result is high-resolution, accurate mass data across the widest dynamic range of signal intensities, so that no detectable analytes or isotope peaks are missed and low limits of detection are achieved, even in the most challenging types of sample.

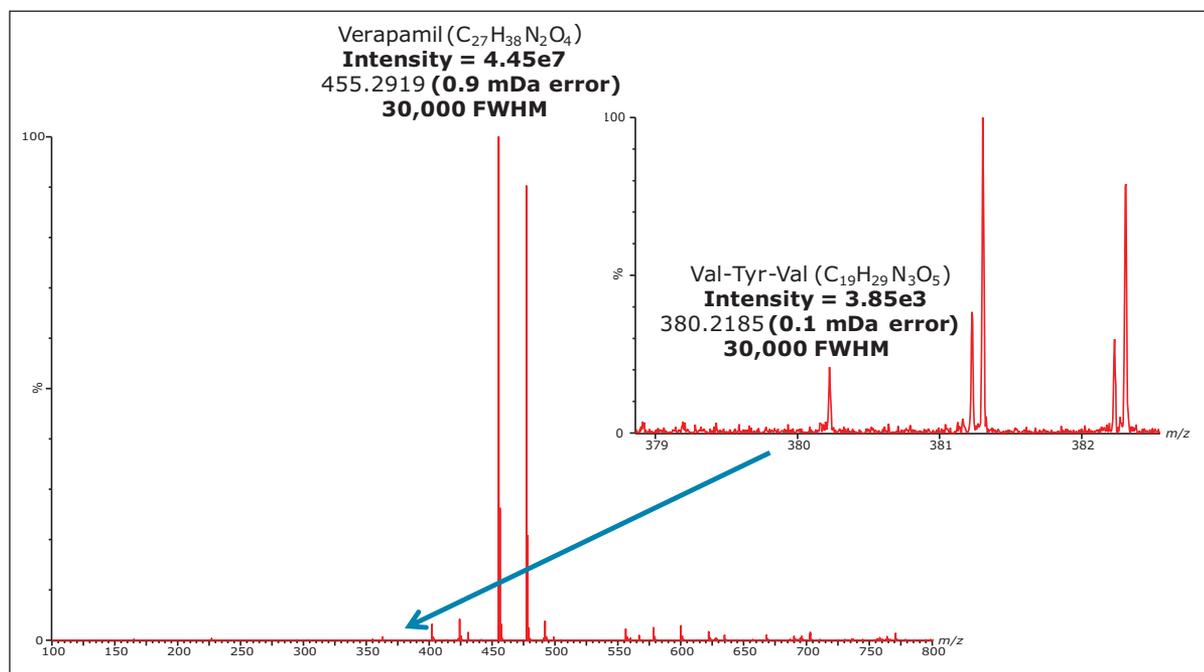


Figure 6. The peptide Val-Tyr-Val was spiked at low level into a solution containing Verapamil. Despite the presence of intense background ions, this Waters oaToF mass spectrometer shows excellent mass accuracy over greater than 4 orders of signal intensity, while maintaining a spectral resolution of 30,000 FWHM.

Quantitative Capability

The high dynamic range displayed by the QuanTof ADC detector electronics gives Waters' oaToF mass spectrometers inherently excellent quantitative capabilities. The quantitative data shown (Figure 7) was obtained without the need for gain-control or signal-attenuation techniques.

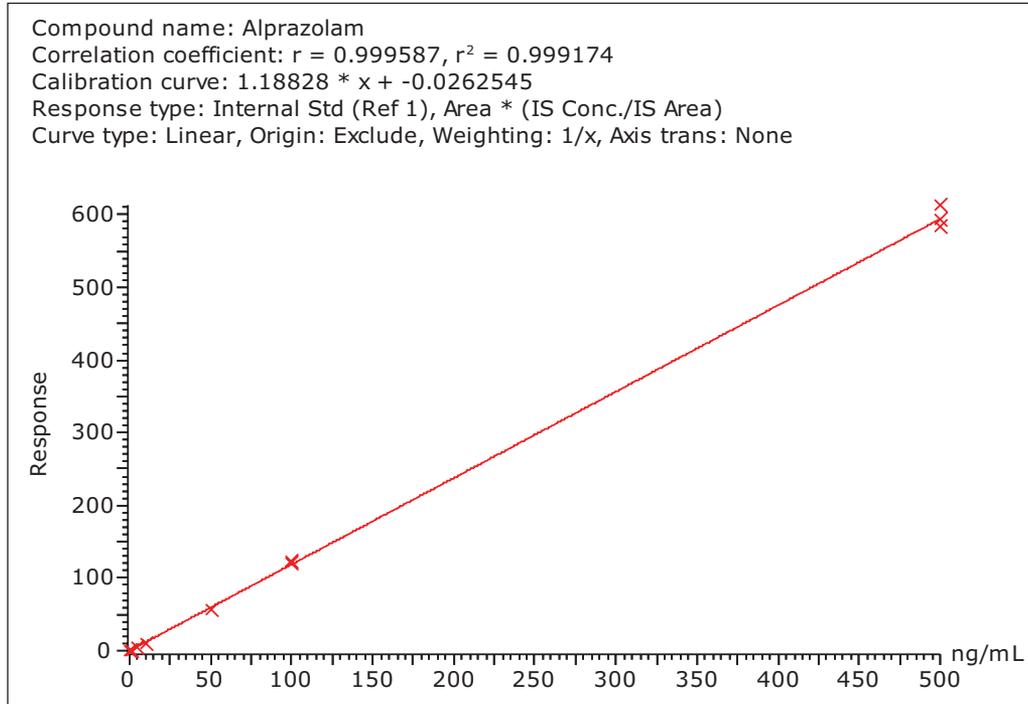


Figure 7. Calibration curve for Alprazolam spiked into plasma at concentrations from 0.05 ng to 500 ng/mL, equivalent to 4 orders of linear dynamic range.

UPLC Compatible Mass Resolution

Since Waters' oaToF mass spectrometers are able to generate individual oaToF spectra at speeds of up to 30,000 per second and record composite mass spectra at speeds up to 30 per second, they make ideal partners for fast, high-resolution separation techniques such as UltraPerformance Convergence Chromatography, UltraPerformance LC, capillary GC or ion mobility separations. Data can be recorded quickly enough to define narrow chromatographic or mobility peaks without any compromise to resolving power (Figure 8). This enables combined, overall selectivity from both the chromatographic system and the mass spectrometer to be maximized.

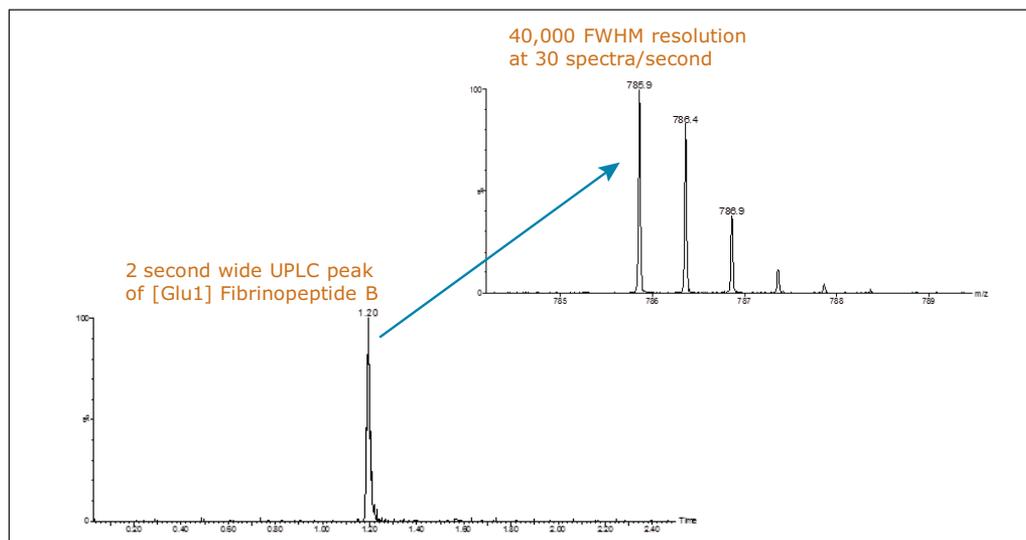


Figure 8. A UPLC/MS acquisition of [Glu1]-Fibrinopeptide B showing mass resolution of 40,000 FWHM at a data acquisition rate of 30 spectra per second. The chromatographic peak is approximately 2 seconds wide at base.

CONCLUSIONS

Waters' QuanTof technology enables oaTof mass spectrometers to deliver fast, high-resolution, accurate mass, quantitative results simultaneously. When coupled to high-efficiency separation techniques such as UltraPerformance Convergence Chromatography, UltraPerformance LC, capillary GC or ion mobility, they deliver superior quantitative and qualitative information, even from the most complex and challenging types of sample.

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Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
www.waters.com

