The resonance excitation process is used for inducing fragmentation in an ion trap. As the amplitude of the applied RF voltage increases, the parent ion dissociates to fragment ions over a narrow range of energies. The collision energy needed to achieve optimum fragmentation efficiency has been shown to follow a linear correlation with \( m/z \) (Figure 1). Increasing the amplitude further does not significantly change the fragmentation pattern.

The collision energy is set using a simple scale of 0-100%. Under the previous regime, setting a value of 30 would apply 30% of the available 5V regardless of the mass of the ion. Figure 2 shows the effect that this would have for three different ions. For an ion at \( m/z \) 200, too much energy would be applied causing the ion and its fragments to be ejected from the trap. For an ion at \( m/z \) 800 the energy would be correct. Not enough energy, however, would be applied for an ion at \( m/z \) 1800 and it would not be fragmented sufficiently. The Normalized Collision Energy principle automatically compensates for this mass dependency. Using a setting of 30% collision energy on an ion at \( m/z \) 1800 applies more energy than to an ion at \( m/z \) 800.

The effect of using Normalized Collision Energy on the same three ions from Figure 2 is shown in Figure 3 below. A collision energy of 30% is now optimized for a wide range of masses.
Data Dependent™ experiments make practical use of Normalized Collision Energy. Using the conventional method, a single value cannot be chosen for the collision energy that will produce good data for compounds with widely different masses. This is particularly relevant when analyzing protein digests where peptides have masses that range from below 200 Da to over 2000 Da. Normalized Collision Energy ensures that good MS\textsuperscript{n} data are collected automatically, whatever the mass of the analyte.

Another aspect of the Normalized Collision Energy is that each Thermo Scientific LTQ™ and LCQ™ series instrument operating under Xcalibur™ software is automatically calibrated for system variations. This means that the MS/MS spectrum obtained on one LCQ using 30\% collision energy will be essentially identical to the MS/MS spectrum obtained on another LCQ using 30\% collision energy.

For the first time MS\textsuperscript{n} spectra are now as reproducible as electron impact spectra. The combination of Normalized Collision Energy with WideBand Activation\textsuperscript{(1)} means that spectral libraries can be built, searched and exchanged. Spectra produced on one instrument will be the same as those from another. Xcalibur software, moreover, comes complete with the industry standard NIST mass spectral search software and a library of pharmaceutical, forensic and environmental compounds.

\textsuperscript{(1)} WideBand Activation, see Thermo Fisher Scientific PSB102.