

Pulsed Q Collision Induced Dissociation (PQD) on Linear Ion Trap Mass Spectrometers

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Among a variety of approaches for precursor ion activation and dissociation in ion trap mass spectrometers, resonance excitation collision induced dissociation (CID) has been the most widely used. Recently, a new dissociation technique called Pulsed Q Collision Induced Dissociation (PQD), has been developed by Thermo Fisher Scientific and implemented exclusively for their linear ion trap mass spectrometers.^{1,2} The patented technique of PQD generates spectra qualitatively similar to CID, however, at the same time, it allows the observation of low m/z fragments that are usually excluded from CID spectra and also helps to access higher energy dissociation channels.^{1,2}

PQD can be regarded as a three-step dissociation process involving the controlled variation of key parameters, such as the resonance excitation amplitude and the main RF amplitude. The first step involves putting the precursor ion at a high Q value (0.6 ~ 0.8), and using a short (~100 μ s), high amplitude resonance excitation pulse, as shown in the schematic in Figure 1. In this step, the ions with m/z resonant to this excitation pulse absorb energy and become kinetically excited. Next, ions are held at the high Q value for a short time period (delay time ~ 100 μ s), which is long enough for the kinetic energy of the ions to be converted into internal energy through collisions, but not long enough for significant dissociation to occur. Subsequently, the precursor ions' Q value is pulsed to a low value by rapidly dropping the RF amplitude and then allowing the precursor ions to undergo fragmentation at this low Q value. The combination of activating at high Q values (high energies) and collecting fragments at low Q values (to trap low m/z fragments) results in an information-rich mass spectrum.

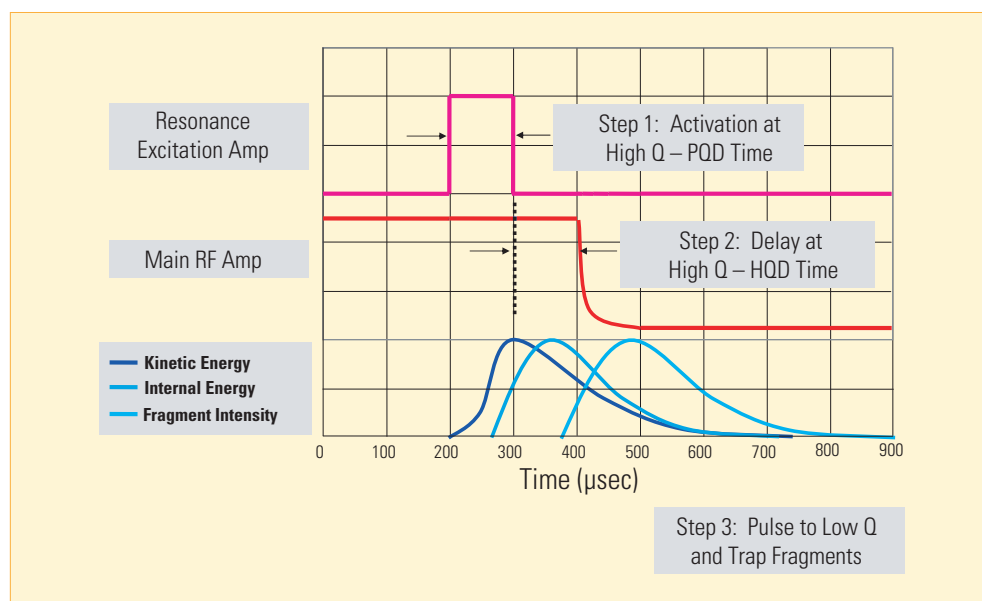


Figure 1. Schematic of Pulsed Q Collision Induced Dissociation Process

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PQD provides comprehensive structural information and enables new applications. For example, a comparison of PQD and CID product ion spectra of taurocholic acid, a conjugate form of bile acids, is depicted in Figure 2. The low mass cut-off typically observed with resonance excitation CID excludes detection of low m/z fragments of taurocholic acid including product ions that cannot be generated via MS^n . On the other hand, PQD provides access to these low m/z product ions, particularly, m/z 80, which may then be used for quantification or screening purposes.

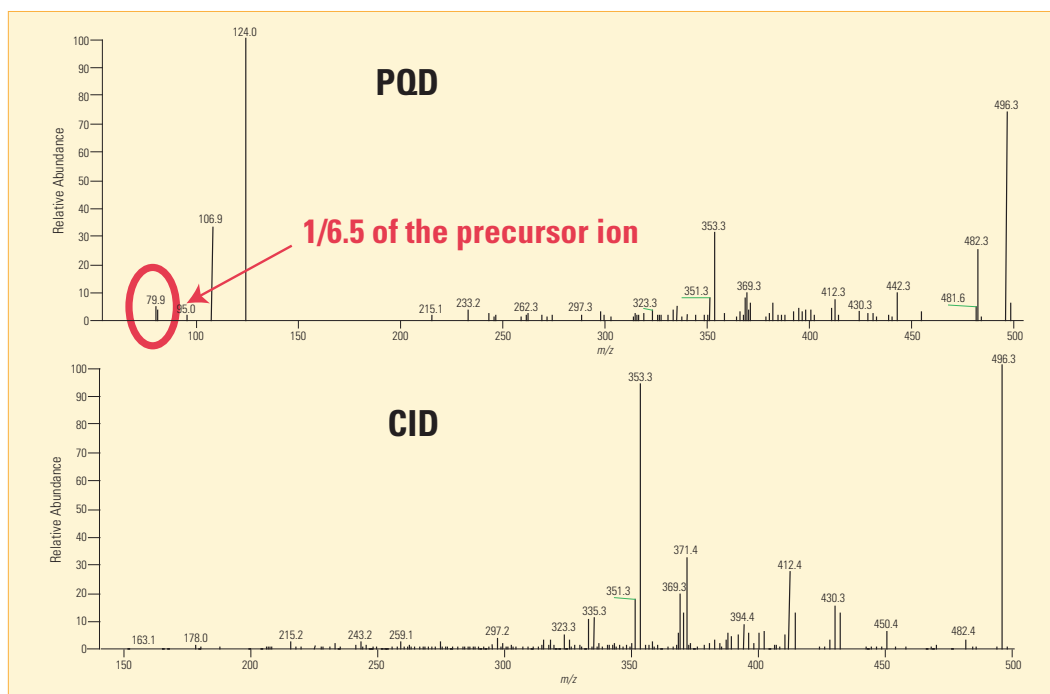


Figure 2. Comparison of PQD and CID spectra for taurocholic acid

With the ability to trap and detect lower m/z product ions, PQD has been applied successfully to peptide quantification utilizing iTRAQ™ labels. The iTRAQ label attaches to the N-terminal amino group of peptides and the epsilon amino group of lysine. It fragments, in MS/MS, from the peptide to produce the iTRAQ reporter ions (114, 115, 116, or 117 m/z) which may be used to perform peptide quantification for up to four different samples by comparing the intensities of the four iTRAQ reporter ions plus one iTRAQ signature ion (m/z 145). With resonance excitation CID in an ion trap mass spectrometer these ions are typically not observed due to the low mass cut-off mentioned earlier. Thus, usually, an additional MS^3 scan is required in order to observe these low m/z ions. With PQD, MS/MS spectra alone are sufficient, as demonstrated in Figure 3.

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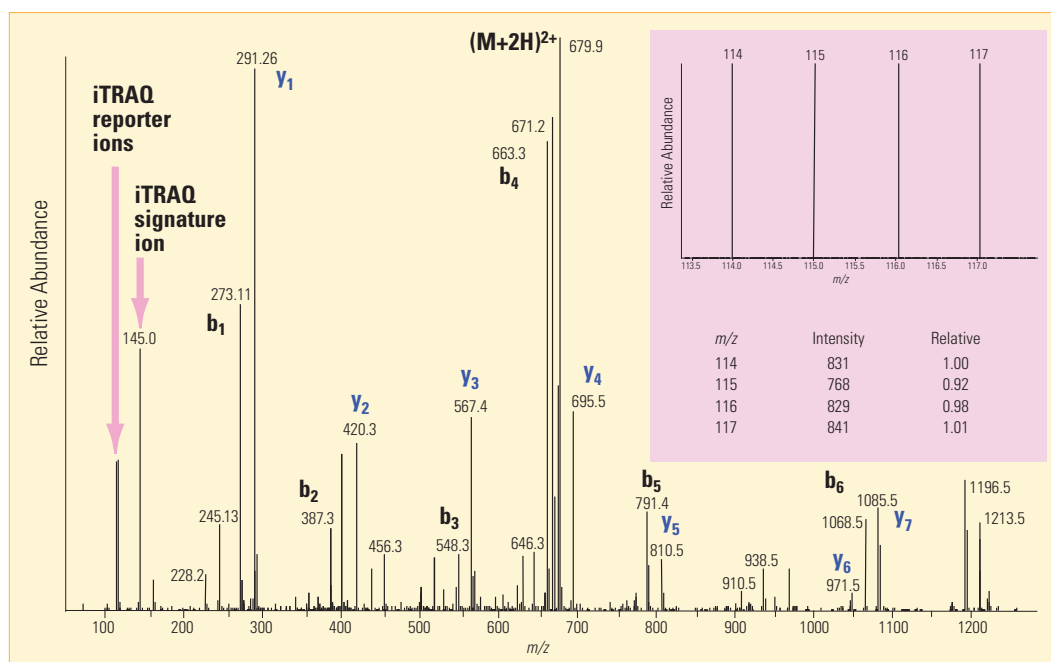


Figure 3. PQD spectrum for QNCDQFEK in BSA digest derivatized with the four iTRAQ reagents mixed together at a ratio of 1:1:1:1.

The PQD spectrum of a +2 ion of a bovine serum albumin (BSA) digest peptide, QNCDQFEK (m/z 679.9), derivatized with the four iTRAQ reagents shows the extended mass range observed in PQD relative to CID.³ In this spectrum, the four iTRAQ reporter ions, as well as the b1 and y1 fragments are clearly observed. The relative ratios of the four iTRAQ reporter ions are in excellent agreement with the theoretical values (see inset in Figure 3).

In summary, PQD is a powerful technique that offers new analytical strategies for both small molecule and proteomics applications. The ability to observe more fragments both at the lower mass range and from higher energy fragmentation pathways provides important structural information complementary to CID. PQD is available exclusively with the Thermo Scientific LXQ,[™] LTQ XL,[™] and hybrid LTQ[™] mass spectrometers.

¹ Schwartz, J.C. U. S. Patent 6,949,743 B1

² Schwartz, J. C.; Syka, J.E.P.; Quarmby, S.T. The 53rd ASMS Conference on Mass Spectrometry and Allied Topics, San Antonio, TX, June 5-9, 2005.

³ Schlabach, T.; Zhang, T.; Miller, K.; Kiyonami, R. The 2006 ABRF Conference, Long Beach, CA, Feb. 11-14, 2006

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