# Electron Transfer Dissociation within a RF Travelling Wave Ion Guide Collision Cell of a QTOF

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## OVERVIEW

**A QTOF mass spectrometer has been modified to allow Electron Transfer Dissociation (ETD).**

**ETD reactions occur within the RF travelling wave (TWAVE) collision cell.**

**The effect of TWAVE parameters such as wave speed and amplitude are shown.**

## INTRODUCTION

ETD (Electron Transfer Dissociation) is a technique for MS/MS in which precursor cations are mixed with radical reagent anions. Electrons are transferred from anion to cation, promoting fast and randomised dissociation compared with collision induced dissociation (CID). ETD product ions are predominantly of the "c" and "z" type and post translational modifications often remain attached to the fragment ions providing valuable sequence information.

## METHODS

A Waters QTOF-Premier ion source "lockmass" probe (normally used for calibration) was modified to provide a negatively charged APCI beam of reagent anions (***Figure 1***). Analyte cations were generated using the standard electrospray probe (***Figure 3***). Both ion sources, the quadrupole set mass and polarity of all associated ion lenses between the source and the collision cell were sequentially switched every 2 seconds to deliver ions into the TWAVE collision cell (***Figures 2 and 4***) containing helium buffer gas at $10^{-2}$ mB. The TOF mass analyser recorded positive ion products.

Analyte samples were dissolved at 1pm/µl in 50:50 acetonitrile/water and infused at 2.5 µl/min into the +ESI source. 1mg/ml of Azobenzene was infused via the –APCI source. Typically, 10 seconds of analyte data were summed for each spectrum. Custom Waters research enabled software (WRenS) was used to control the ion optics and source switching whilst Masslynx acquired TOF MS/MS fragment data at 10000 FWHM resolution.

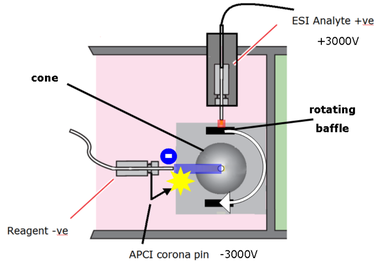


***Figure 1*** *Reagent anions generated via "lockspray" calibration probe modified for negative APCI*

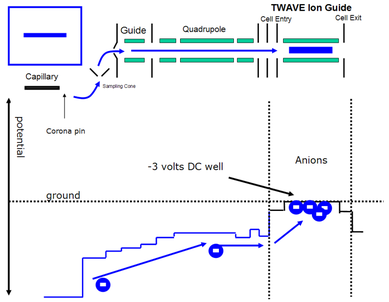


***Figure 2*** *Potential Energy when selecting and retaining anions*

## RESULTS

***Figures 5, 6 and 7*** illustrate MS/MS spectra of the triply charged precursors of Renin Substrate (m/z 587), Substance-P (m/z 450) and Bovine Casein Monophosphopeptide (m/z 688) respectively, reacted with Azobenzene (m/z –182) at a TWave speed of 200m/sec and amplitude of 0.3V. Fragment ions are predominantly "c" and "z" type indicative of the ETD ion-ion reaction. Approximately 1 million anions were present in the TWAVE ion guide.

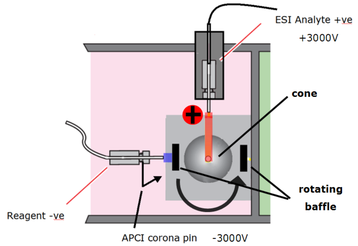


***Figure 3*** *Analyte cations generated via standard ES probe*

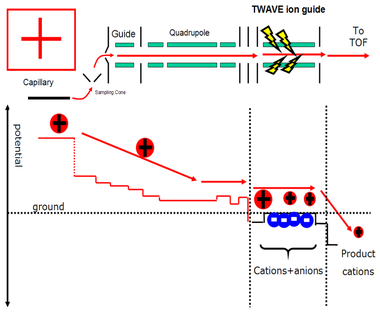


***Figure 4*** *Potential Energy when selecting and reacting cations*

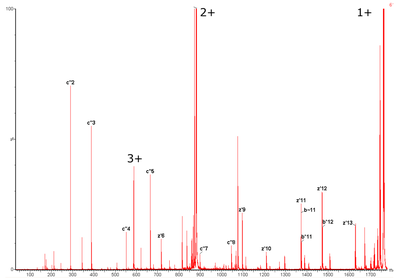


***Figure 5*** *ETD MS/MS spectrum of Renin Substrate 3+*

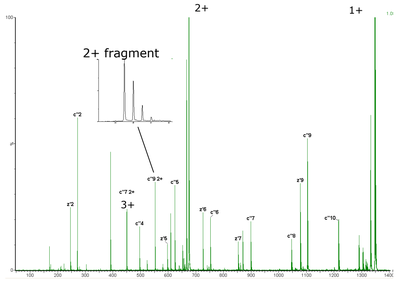


***Figure 6*** *ETD MS/MS spectrum of Substance-P 3+*

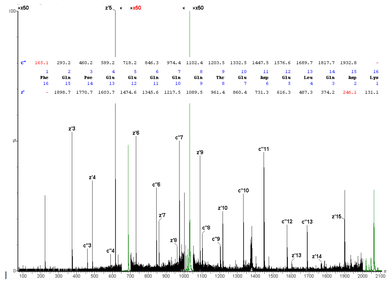

***Figure 7*** *ETD MS/MS spectrum of Monophosphopeptide 3+*

***Figure 8*** illustrates the relative abundances of the 3+ precursor ion of Substance-P as a function of TWAVE amplitude (at a TWAVE speed of 200m/sec) Also the 2+ and 1+ charge reduced product ions and the sum of the "c" and "z" ETD fragment ions are plotted.

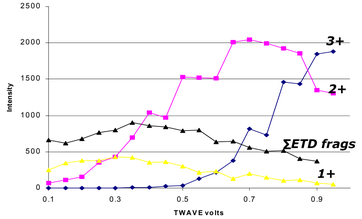


***Figure 8*** *Intensities of 3+ precursor, 2+ product, 1+ product and sum of ETD fragments as a function of TWAVE amplitude*

TWAVE speed is also a parameter that affects relative abundance of different product ions. ETD spectra for 3+ Substance–P at TWAVE velocities of 100m/sec, 10m/sec and 1m/sec equivalent to ion-ion reaction times of 1.8,18,180 msecs. (TWAVE amplitude of 1Volt) are shown in ***Figure 9***. For the longer reaction time, higher charge state ions and many singly charge state product ions are neutralised.

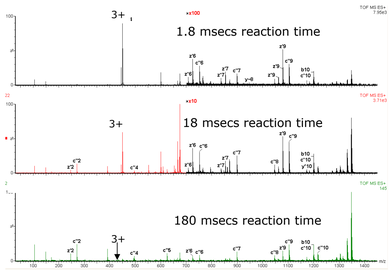


***Figure 9*** *Subtance-P ETD data for TWAVE speeds of... a)100 m/sec, b) 10m/sec and c) 1m/sec*

***Figure 10*** illustrates the complementary information available from CID and ETD, the top spectrum is CID of the doubly charged Renin Substrate using Argon collision gas. The lower spectrum is ETD of the triply charged Renin Substrate. Similarly, ***Figure 11*** shows Bovine Insulin 6+ by CID and ETD.

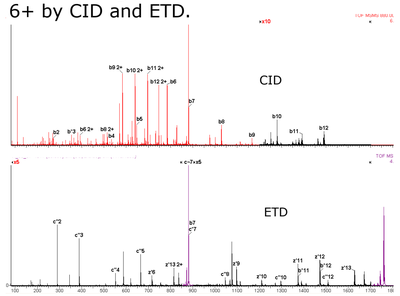


***Figure 10*** CID and ETD comparison of Renin Substrate

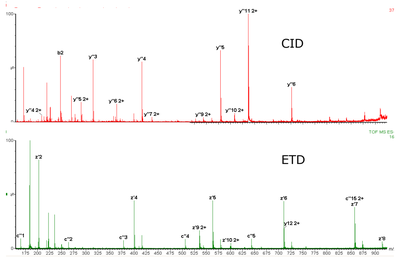


***Figure 11*** CID and ETD comparison of Bovine Insulin

## CONCLUSION

- ETD within the QTOF-P TWAVE gas cell has been demonstrated and is a complementary fragmentation technique to CID.
- The TWAVE parameters such as speed and amplitude provide control over the reaction dynamics.

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