3. MODIFICACIONES POSTRADUCCIONALES

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Liquid chromatography—electron transfer dissociation and ion mobility studies on a QTOF mass spectrometer

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ETD (Electron Transfer Dissociation) is a MS/MS technique in which precursor cations are reacted with radical reagent anions to induce fragmentation. Electron transfer from anion to cation, promotes fast and randomised dissociation compared to collision induced dissociation (CID) and hence ETD product ion spectra contain predominantly "c" and "z" type ions and post translational modifications often remain intact providing valuable sequence information.

Here we show how ETD can be implemented and performed on a hybrid Q-IMS-TOF (Waters Synapt) where a supply of reagent from a sealed ampule is delivered to the intermediate pressure region of the nano ESI source and a high voltage discharge pin was added to the ion source to generate the reagent anions. Analyte cations were generated using the standard nanospray source via infusion or nanoACQUITY UPLC system. For ETD, the ion source polarity and the quadrupole set mass were sequentially switched to deliver anions and cations into the TRAP travelling wave (TWAVE) ion guide where they reacted to form ETD product ions. Product ions were optionally separated by ion mobility in the IMS TWAVE ion guide or were accelerated into the TRANSFER TWAVE ion guide to cause 2nd generation collision induced dissociation (CID) ions prior to mass analysis in the TOF.

Bovine serum albumin (BSA) tryptic peptides (250fm and 50fm) were separated by nanoAcquity UPLC and a selection of triply charged precursor masses were subsequently selected to generate LC-ETD spectra. Data were acquired at 1 spectra/second and the fragment ions were database searched with all spectra matching to BSA with high probability. High quality data was observed at the 50fm injection level, with ETD data acquired at 1 spectra/second.

Cleavage was observed at almost every amide bond in the peptide backbone, yielding easy-to-interpret sequence ladders of c and z-ions. This coupled with the inherent mass measurement accuracy and resolution of the oa-TOF mass analyser makes the data easily amenable to de novo sequencing. The signal intensity of the fragment ions seemed to diminish with decreasing m/z, as previously reported. In addition to the ETD experiments the mass spectrometer can acquire alternate scans in CID, and as such data will be compared and contrasted between ETD and CID on a variety of peptides produced and separated by nanoscale chromatography.