Hybrid Ion Trap–Fourier Transform-Ion Cyclotron Resonance for High-Performance Analysis

by Lester Taylor

The use of mass spectrometry has steadily spread throughout the biological sciences. This single technique enables unprecedented breakthroughs in our understanding of genomics, proteomics, and systems biology. Once used simply as devices to determine molecular weights, the mass spectrometers of today have improved and rapidly evolved to precisely suit the needs of particular scientific disciplines.

A notable example of this evolution is the hybrid ion trap–Fourier transform-ion cyclotron resonance (FT-ICR) instrument (**Finnigan**™ LTQ FT™, **Thermo Electron Corp.**, San Jose, CA) (*Figures 1* and *2*). The ability of an ion trap to capture, fragment, and analyze ions is combined with the extremely high mass accuracy and resolution of an FT-ICR detector to yield an efficient, practical device for the analysis of complex biological samples. The power of such a system can be used to resolve and identify the components of a complex mixture for differential proteomics or metabolomics studies. This power can also be harnessed to unambiguously determine the structure of post-translationally modified proteins. Importantly, the instrument can also be used to fragment intact proteins, opening up vast opportunities for top-down protein analysis.

Figure 1 Photograph of the LTQ FT-MS.

High-resolution, accurate mass determination is key to the analysis of complex samples and mixtures. The higher the mass resolution is, the more complex the mixture that can be analyzed and characterized. Only by resolving individual analyte peaks, which occur at the same nominal mass, can the components in a mixture be determined simultaneously. Knowledge of the accurate mass of target or unknown compounds greatly restricts the number of potential elemental compositions, which can account for specific masses and may even allow for the unequivocal determination of elemental composition. In cases where the target ions are composed of known combinations of atoms (such as amino acids, nucleotides, and carbohydrates), analytical performance becomes even more significant, for example, when used to search protein databases.

Most commercially available high-resolution mass spectrometers achieve mass accuracies of only ~5 ppm with internal calibration. Internal calibration, however, has several associated complications, for example, ionization suppression effects. The LTQ FT routinely provides mass accuracies of better than 2 ppm with external calibration. The instrument can achieve accu-

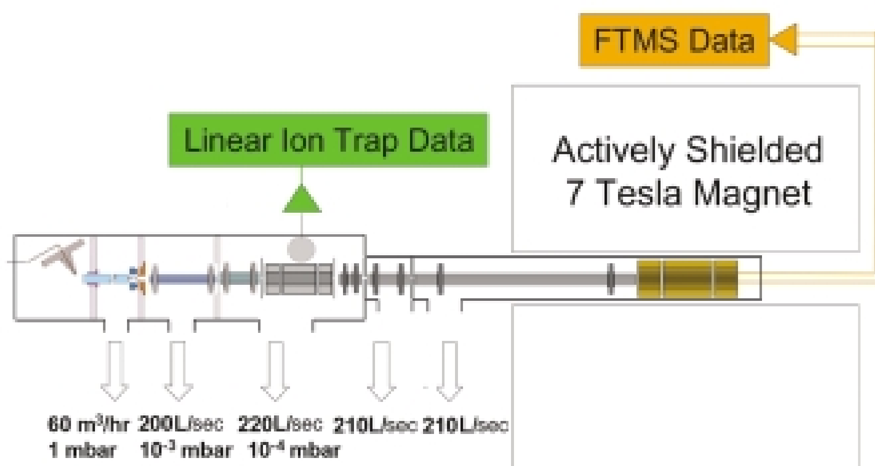

Figure 2 Diagram of the LTQ FT-MS.

rate mass resolution in about 1 sec, allowing FT-MS measurements on a chromatographic time scale. HPLC can be directly coupled to the system and used as an on-line separation technique, accelerating analysis in a variety of areas, such as pharmaceutical research, drug discovery, and metabolic studies, enabling the user to achieve precise results in a shorter time frame.

Routine FT-MS

The Finnigan LTQ linear ion trap is the front end of the hybrid LTQ FT mass spectrometer. The instrument utilizes the ability of the linear trap to isolate, activate, and mass scan ions that can then be detected by the linear ion trap (first mass spectrometer) or passed into the FT-ICR cell (second mass spectrometer) for additional mass analysis. Experiments have shown that a linear trap can function well beyond the intended spectral space charge limit when isolating and activating precursor ions with high efficiency for populations exceeding 5×10^5 ions. This is more than sufficient for the acquisition of high-quality MS-MS data by the FT-MS detector.

Fundamental to the overall performance of the LTQ FT is the automatic gain control (AGC) capability of the linear ion trap. This feature controls the number of ions entering the FT-ICR cell by accurately calculating the optimal injection time for ion collection. Highly reproducible interscan ion populations ensure optimum resolution and mass accuracy of the spectra generated by the instrument.

High-performance MS

FT-ICR MS is fundamentally different from other mass spectrometry techniques. At the heart of the system is a cylindrical cell capable of trapping and storing gas phase ions in a high-field magnet. A stored waveform inverse Fourier transform (SWIFT) waveform excites ions trapped in the cell by applying an RF voltage to electrodes within the cell. These ions incur a radial motion away from the central axis of the cell and adopt a circular motion. Ions are detected by their image currents induced at the detection electrodes.

The resulting frequency of the ion cyclotron motion is dependent on the mass-to-charge ratio of these ions and the magnetic field strength. Mass accuracy is also strongly affected by the total number of ions trapped in the ICR cell, and therefore it is critical to operate the FT-ICR cell with optimum ion populations in order to 1) maximize sensitivity and 2) avoid space charge mass shifts. After the image current of all of the ions circulating in the cell is recorded, a Fourier transform extracts the different frequencies from the signal, and the resulting frequency spectrum is readily converted to a mass spectrum using the relationship between frequency and mass. If required, ions can be stored and analyzed for longer periods of time, which provides higher resolutions, since ion detection is nondestructive (in contrast to other types of MS detection).

No trapping gas is pulsed into the ultrahigh vacuum region to trap ions in the ICR cell of the LTQ FT. Thus, there is no time delay between pumping away gas and detecting the ICR signal, and FT is performed in real time using an ultrahigh-speed signal processor. The amplified signal is digitized by a high-speed A/D converter and is directed through a preprocessing system that performs fast FT, peak detection, and peak centroiding. An FT-ICR MS acquisition duty cycle in excess of 80% is possible at a scan repetition rate of 1 sec.

Improved mass accuracies and stability

The use of AGC on the LTQ FT mass spectrometer ensures that mass accuracies of better than 2 ppm are routinely achieved with full automation, independent of the type of analyte, its concentration, and spraying conditions. A series of experiments using the instrument have confirmed the stability of the mass calibration and measurement for a variety of compound types. In these experiments, a mass range of *m/z* 200–2000 was scanned with a cycle time of less than 1 sec at 100,000 resolving power (FWHM) at *m/z* 400. The same external mass calibration (generated two days earlier) was used for each run. The standard electrospray ionization

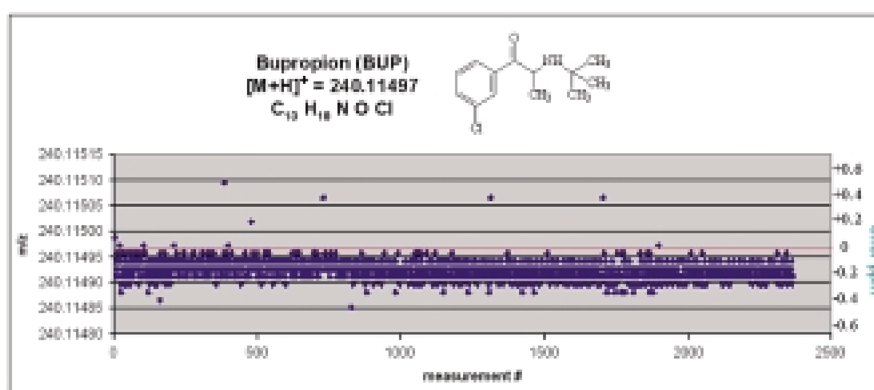

Figure 3 *Mass accuracy for repetitive scans of the MH^+ ion of an antidepressant drug over a period of 45 min. Approximately 2400 spectra were acquired using the NanoMate sample introduction system. The red line indicates the theoretical mass. The average error is –0.2 ppm.*

(ESI) source was replaced by the NanoMate 100 robot (**Advion,** Ithaca, NY).

In the first experiment, the robotic plate was loaded with 48 different samples at various concentrations, including peptides, proteins, polymers, ligands, and drugs. Each sample was analyzed twice. With a sample cycle time of approx. 1 min, the 96 measurements were carried out in less than 100 min. For each sample, approx. 30 spectra were acquired. The majority of the masses measured displayed mass accuracies of better than 1 ppm, with a total root mean square (RMS) error of less than 2 ppm.

In order to estimate the reproducibility of the mass measurements, an antidepressant drug was analyzed repeatedly over a period of 45 min, resulting in approx. 2400 spectra (*Figure 3*). These were evaluated and found to have an average mass error of –0.2 ppm, with a standard deviation of 0.07 ppm. The evaluation was also carried out for the +1, +2, and +3 isotope peaks of the quasi-molecular ion. Averaged results showed mass measurement accuracies of better than 0.4 ppm.

To demonstrate that mass measurement is independent of the sample concentration, the experiment was repeated with sample concentrations of 10, 5, 1, 0.2, and 0.1 micro g/mL, using a metabolite of the drug. The spectra were analyzed for the accurate masses of the four isotope peaks on the quasi-molecular ion of the compound. All experimental measurements were determined to an accuracy of better than 0.5 ppm.

Identification of post-translational modifications in peptides and proteins

The LTQ FT has been used to develop a highly sensitive two-tiered LC-MS method for the identification of the exact site of post-translational modifications (PTMs) in peptides and proteins. This consists of intact protein analysis, to establish PTM distribution, followed by detailed PTM site mapping by enzyme digestion using high-resolution accurate mass measurement on either full MS, or a combination of MS and MS^n scans to pinpoint site-specific mass changes in a peptide sequence.

Digested protein mixtures containing a variety of PTM (phosphorylation, glycosylation, and disulfide bonds) were separated by on-line HPLC and analyzed using Data Dependent™ mass scans with Dynamic Exclusion™ (both from **Thermo Electron**) (*Figure 4*). Mass spectra were acquired with alternate detection in both the ion trap and FT-ICR mass spectrometers. A BioBasic™-C18 column (0.18 × 10 cm) (**Thermo Electron**) was used for peptide analysis (5–60% acetonitrile [ACN] gradient in 60 min) (*Figure 5*). For intact protein analysis, a purified protein kinase and human growth hormone were analyzed by an LC-MS method using a 0.15 × 100 mm C8 column (Zorbax C8SBW, 20–80% ACN in 30 min, **Micro-Tech,** Vista, CA). Spectra were acquired by Data Dependent scanning with and without neutral loss triggering MS3 scans using Dynamic Exclusion. BioWorks 3.1 (SEQUEST and Biomass™ Deconvolution) software (**Thermo Electron**) determined peptide and protein identification (*Figure 6*). The sites of phosphorylated, disulfide-linked, glycosylated proteins were rapidly determined with a high

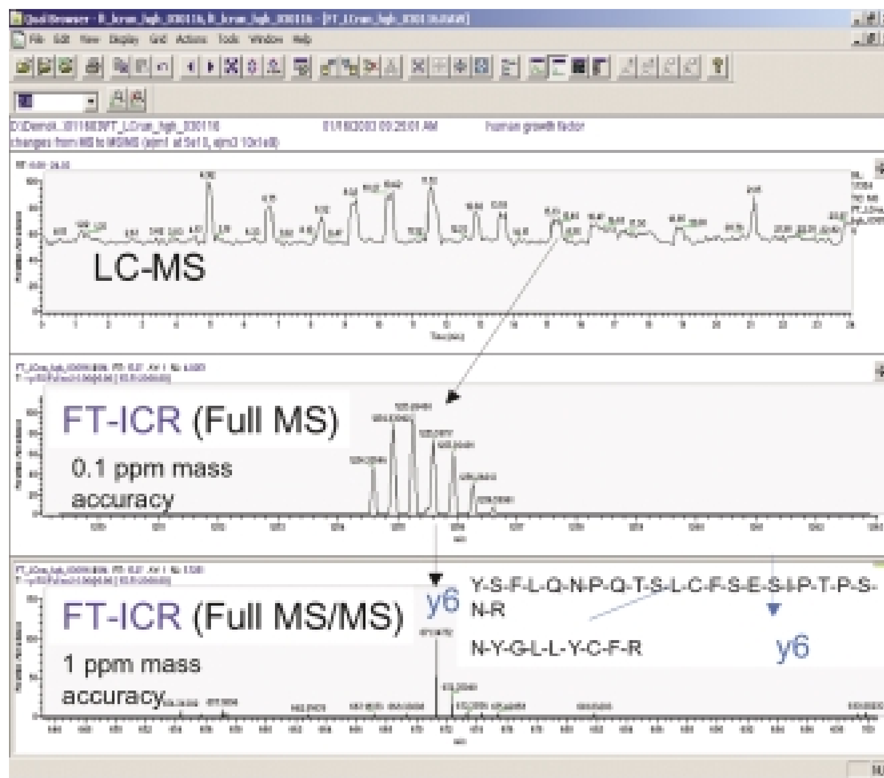

Figure 4 Precise PTM (disulfide–peptides) assignment. LC-MS analysis of an enzyme digest from a mixture of nonreduced proteins containing human growth hormone (top panel). The mass spectrum at 15.27 min shows the portion of the full MS (center panel) along with the full MS-MS spectra (lower panel) obtained using FT-ICR as the detector. The high-resolution accurate mass measurement on full MS spectra (0.1 ppm mass accuracy and 3+ charge) confirms that the two cysteine-containing peptides are linked, and on full MS-MS spectra (1 ppm mass accuracy and 1+ charge) further increases the confidence of the assignment of the two disulfide-linked peptides.

degree of confidence. A large number of peptides can therefore be identified in complex mixtures from a single, automated LC-MS^n experiment, including the identification of PTMs using a combination of the linear ion trap and FT-ICR MS detectors.

The higher-sensitivity and rapid mass scanning provided by the linear ion trap allows the detection of low-abundance ions, which can be fragmented by collisionally induced decomposition (CID) and detected in MS-MS experiments. The FT-ICR instrument is then used for high-resolution accurate mass measurement. The overall hybrid design is optimized to perform these experiments on the chromatographic time-scale.

Versatility and flexibility

Multiple ionization modes are available with the LTQ FT, including ESI, atmospheric pressure chemical ionization (APCI), atmospheric pressure photoionization (APPI), and matrix-assisted laser desorption ionization (MALDI). Ions are transferred by octapole and quadrupole lenses into the ion trap. The Finnigan NanoSpray ion source (**Thermo Electron**) supports a number of micro- and nanoflow operational modes, which provide flexibility in the selection of the type of experiments required for a given analysis. Nanoflow operates both in static and dynamic flow modes. The dynamic/flow nanospray probe allows connectivity to micro- and nano-LC columns. The static nanospray probe enables the analysis of low-volume solutions over extended periods of time.

The LTQ FT is controlled by a PC, incorporating an expanded version of the Xcalibur® software suite (**Thermo Electron**). The powerful and flexible MS data handling system for Microsoft® Windows™ (**Microsoft Corp.**, Redmond, WA) provides fully automated control of

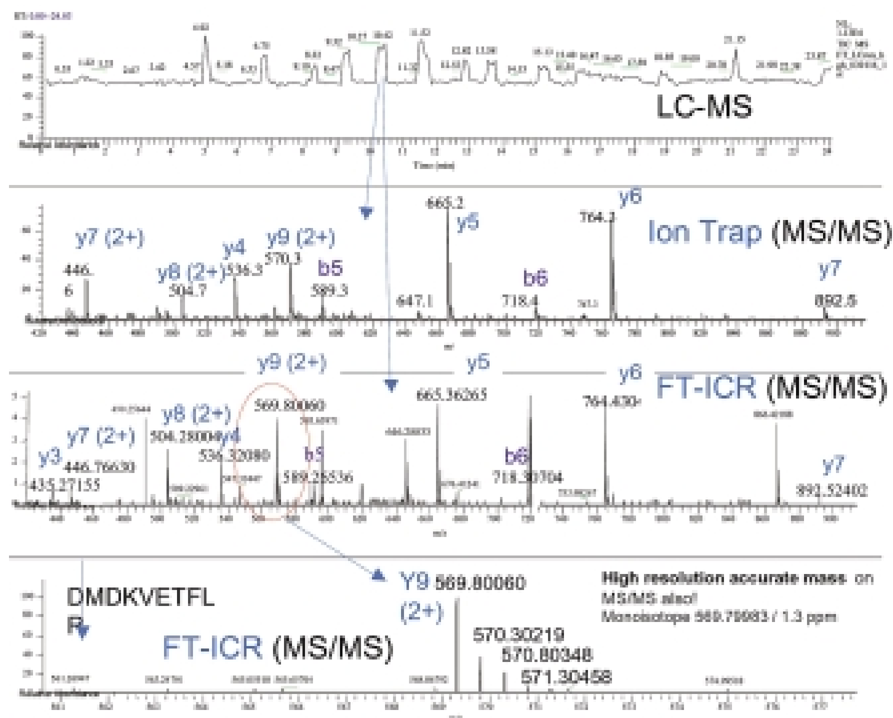

Figure 5 Precise MS-MS measurement for peptide analysis. LC-MS analysis of an enzyme digest from a mixture of reduced proteins that contained human growth hormone (hGH) at the level of approx. 5 pg/mL (upper panel). The mass spectrum at 10.48 min shows the full MS-MS scan using the linear ion trap as the detector (center panel) along with the MS-MS scan obtained using FT-ICR as the detector (lower panel). The zoom-in window from the full MS-MS of the lower panel shows the high-resolution accurate mass of that MS-MS scan (1.3 ppm mass accuracy). The results of applying BioWorks 3.1 (SEQUEST®) search for the above MS-MS data are shown in Figure 6. (Note: Similar fragmentation patterns (both y and b ions) are observed in both ion trap and FT-MS.)

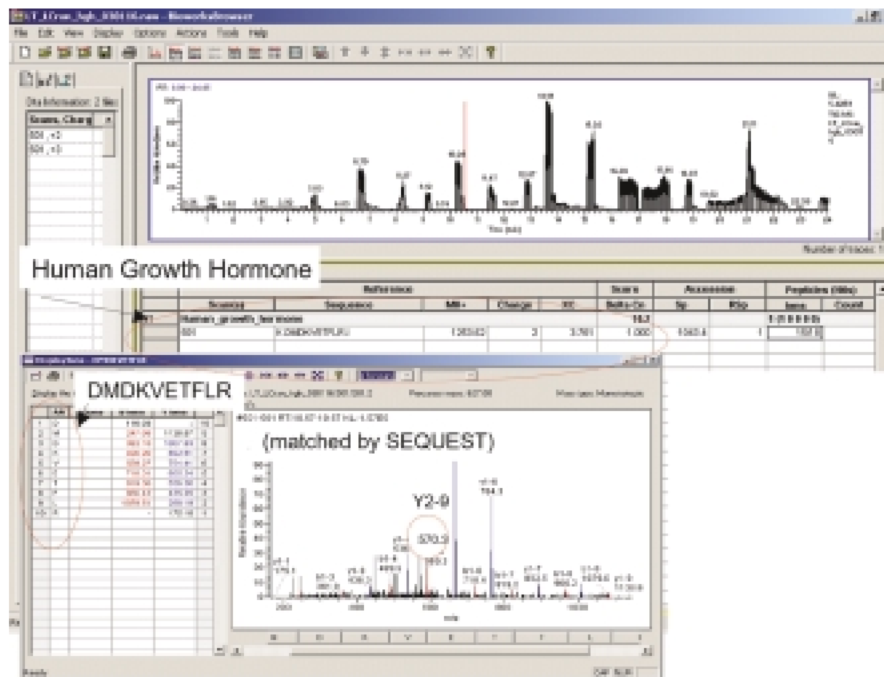

Figure 6 Identification of the peptide by BioWorks 3.1.

the spectrometer, the Surveyor LC (**Thermo Electron**), and other related LC devices. BioWorks, DenovoX™, Mass Frontier, and Metabolite ID (all from **Thermo Electron**) are all integrated to permit the acquisition of raw data for protein/peptide sequencing, small-molecule analysis, and the review of drug metabolism LC-MS-MS samples. High-resolution MS, MS-MS, and MS^n are fully supported by advanced data-dependent features such as Dynamic Exclusion.

Conclusion

Commercial FT-ICR mass spectrometers manufactured to date have been complex, research-grade instruments that required operation by highly skilled mass spectrometry specialists to achieve the most reliable results. This has limited their usefulness and acceptance for routine applications in discovery research and proteomics environments. The LTQ FT hybrid high-performance mass spectrometer combines advanced linear ion trap and FT-ICR technologies in a single instrument. Metabolic studies, proteomics analysis, pharmaceutical discovery, and any application demanding the most rigorous structural characterization can be significantly improved using the analytical power of the LTQ FT.

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