

# Relative Quantitation of iTRAQ Labeled Proteins Using IRMPD on an LTQ FT Ultra

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## Abstract

**Purpose:** Simultaneous protein identification and quantitation are requirements for many current proteomics experiments. Performing both the precursor and fragment ion measurements in the FTICR cell delivers excellent mass accuracy which is important in order to minimize false positive identifications.

**Methods:** The data were acquired in a Data Dependent™ LC-MS/MS mode using an LTQ FT Ultra™ instrument. The fragmentation of peptides was induced by an IR laser in the ICR cell (IRMPD).

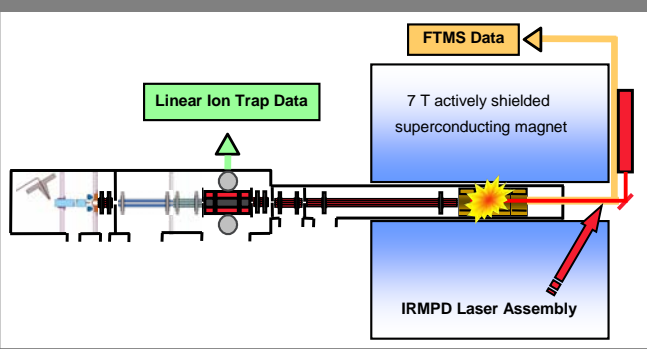
**Results:** The IRMPD technique produces MS/MS spectra which contain a wealth of structural information for peptide identification. Moreover, the iTRAQ™ reporter ions are easily detected in the low mass region of the spectra allowing for reliable quantitation using the same data set.

## Introduction

An important aspect of proteomics is not only to identify all proteins from complex biological samples but also to accurately measure their relative abundances. To address this challenge, many analytical approaches have been developed, including isotope labeling techniques such as iTRAQ. The method relies on the measurement of specific reporter ions in MS/MS spectra of analyzed peptides. The type of instrumentation and fragmentation technique employed influences the outcome of the quantitation. The reporter ions lie in a low *m/z* region of the spectra, and they need to be detected with significant signal/noise ratios.

The LTQ FT Ultra is renowned for its superb mass accuracy, crucial for minimising false positive identifications (1,2). This system is highly flexible and offers several methods for peptide fragmentation, i.e. CID in the linear ion trap, and ECD and IRMPD in the ICR cell. The IRMPD fragmentation is of particular interest, because it delivers abundant b- and y-fragment ions, with high mass accuracy, and it captures the low mass region of the spectrum with iTRAQ reporter ions.

FIGURE 1. The LTQ FT Ultra is a hybrid mass spectrometer combining a linear ion trap and an ICR cell. It enables accurate mass measurement of both precursor and fragment ions. The fragmentation by IR laser irradiation produces rich peptide backbone fragmentation and intense iTRAQ reporter ions in the MS/MS spectrum.



## Materials & Methods

The sample (digest of six standard proteins) was labeled either with 114 or 117 iTRAQ reagent according to the manufacturer's instructions. The labeled samples were mixed in specific ratios (1:2, 1:5, and 1:10) and the mixtures (2 µL injected) were separated via Surveyor™ LC equipped with MicroAS™ auto-sampler (Thermo Fisher Scientific) using a peptide trap (NS-MP-10 C18 100 mm, 2 cm, NanoSeparations) and a C18 column at a flow rate of 200 nL/min. A gradient of 10 - 30% acetonitrile in 65 minutes was used.

The LTQ FT Ultra (Thermo Fisher Scientific) performed a full MS scan (RP 50,000) followed by 3 Data Dependent MS/MS scans (RP 25,000). The IRMPD fragmentation used 80% laser power, 120 ms duration, with laser tuned on substance P and optimised on its phenylalanine immonium ion (*m/z* 120).

FIGURE 2. Automated Data Dependent acquisition enables minor peptides to be selected for fragmentation. A peptide (*m/z* 587.8) eluting at 11.60 min is very weak; nevertheless it is measured with excellent mass accuracy.

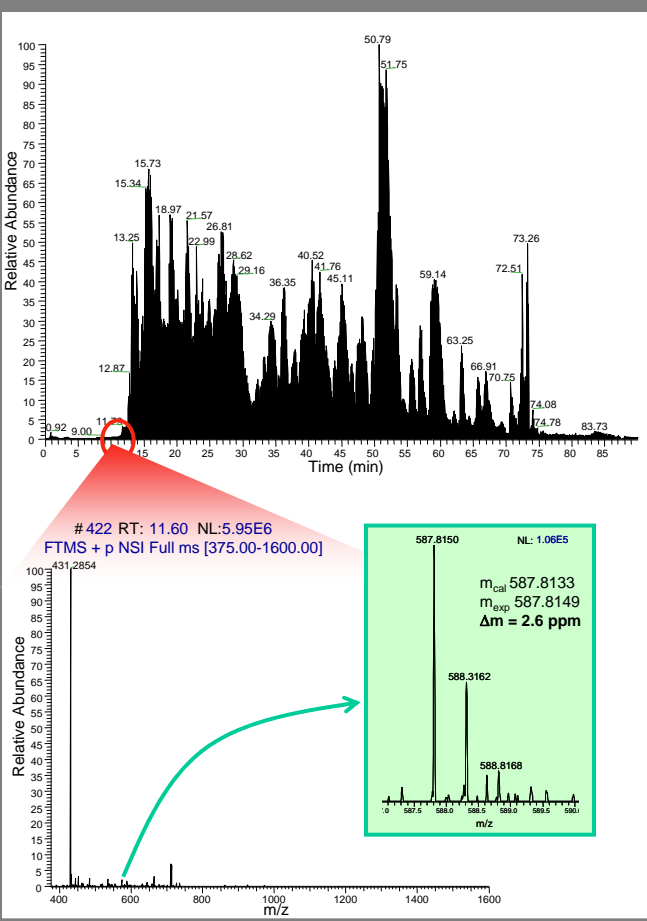
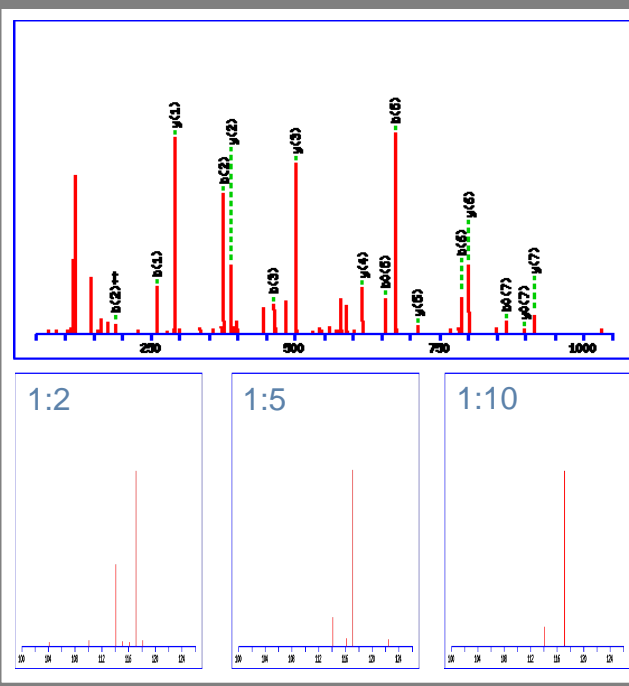


FIGURE 3. MS/MS of peptide DDSPDLPK obtained by IRMPD technique allows for confident identification based on accurate mass measurement of peptide backbone fragments (top panel). The inserts below show the detailed iTRAQ reporter ion region.



## Materials & Methods continued

The data were searched with Mascot™ version 2.1.04 (Matrix Science) using NCBI nr database (060613, 3682060 entries) considering the following modifications: Met +15.9949 Da; Lys, Tyr, N-terminus +144.1021 Da. The intensities of 114 and 117 ions were extracted from the search results using an in-house software tool. The data were used to calculate the average ratio for labelled samples and corresponding RSD values (Table 1).

TABLE 1. Summary of the quantitation results (over 2000 peptide spectra) for each sample prepared by mixing 114 and 117 labeled peptide samples.

Expected ratio 114/117	Measured ratio 114/117	RSD (%)
1:2	1:2.0	20.1
1:5	1:6.3	21.1
1:10	1:12.6	23.1

## Results

The LTQ FT Ultra (Figure 1) is capable of using multiple fragmentation techniques. This enables the user to optimise analysis for various proteomics applications. Here, we have focused on optimizing the IRMPD technique for peptide identification and quantitation.

At the average data acquisition speed of 1 scan in 0.7 sec, we determined that IRMPD is compatible with high throughput nano-LC separation of complex peptide mixtures.

The ability to detect peptides at very low levels is illustrated in Figure 2. The peptide shown is approximately 6000-fold less abundant than the base peak peptide, but its mass is measured with excellent accuracy. Moreover, its MS/MS spectrum provides ample information for its sequence identification and quantitation (Figure 3).

The quality of MS/MS spectra was very good even though no data averaging was used during the acquisition.

The confidence of identification with Mascot was greatly enhanced by the use of accurate mass for both parent and fragment ions.

IRMPD captures fragments at low *m/z* of MS/MS spectra thus enabling the detection of iTRAQ reporter ions. A simple software tool created in-house was used to extract the ion intensities of the reporter ions and calculate their ratio.

The ratios were determined with a relative standard deviation of approximately 20%. This was obtained without considering iTRAQ label correction factors or any normalisation for sample amount.

## Conclusions

- IRMPD delivers rich fragmentation spectra for peptide identification.
- MS/MS spectra are acquired with high resolution accurate mass which adds additional confidence to the database search results.
- IRMPD spectra allow for the quantitation to be performed for iTRAQ-labelled peptides with good accuracy.

As the next step, we would like to evaluate the method for a greater range of analyte concentrations. Also, it would be useful to monitor the effect of increased number of microscans on the accuracy of quantitation.

## Acknowledgement

We would like to acknowledge Torsten Ueckert for writing the software tool.

## References

1. Yates, J.R. et al., *Anal. Chem.*, 2006, 78, 493-500.
2. Zubarev, R., Mann, M., *Mol. Cell. Proteomics*, 2007, 377-381.

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