

Single Ion Monitoring with 0.5 ppm Mass Accuracy Coupled with Data Dependent Decisions in Complex LC-MS/MS Runs

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Key Words

- LTQ FT™
- Data Dependent™
- FT SIM
- Nano-LC-MS/MS
- Proteins

Overview

Purpose: Combination of dynamic range enhancement and improvement of mass accuracy for protein identification in complex mixtures

Methods: Nano-LC-MS and nano-LC-CID-MS/MS using a Hybrid Linear Ion Trap–FTICR Mass Spectrometer (Thermo Scientific LTQ FT)

Results: Demonstration of high dynamic range with high mass accuracy (<0.5ppm) which greatly improves the coverage and identification of proteins using a protein mixture.

Introduction

Recent work in proteomic research demonstrates the need to identify peptides and proteins in complex mixtures from biological sources at extremely low levels with the highest confidence!^[1] This implies the use of a mass spectrometer with high dynamic range and high mass accuracy. Moreover, for MS/MS experiments, fast scan repetition rates at highest sensitivity are needed. As it is well known in mass spectrometry, dynamic range can be gained by sacrificing resolution and mass accuracy. Here we describe an instrument method design that increases the dynamic range without sacrificing mass accuracy. This is demonstrated using a digest of a complex protein mixture derived from a 1 D-SDS band (*Escherichia coli* culture) containing approximately 20 proteins.

Implementation and Experimental Setup

The protein mixture was enzymatically digested and analyzed by nano-LC-MS and nano-LC-CID-MS/MS using an LC equipped with a 75 $\mu\text{m} \times 10$ cm column (5 μm) directly coupled to a hybrid linear ion trap–FTICR mass spectrometer (LTQ FT). Liquid chromatographic separation was performed using a linear gradient containing water, acetonitrile and 0.1% formic acid at a flow rate of 300 nL/min on column. Full scans were acquired using the ICR cell as detector while Data Dependent™ MS/MS experiments were performed in the linear ion trap.

A detailed description of the used workflow is given in Figure 1. An FT master scan at high target values ($5e^6$ ions in the ICR cell) is followed by a series of Data Dependent FT-single ion monitoring (SIM) scans and Data Dependent Ion Trap MS/MS scans. The Data Dependent decision at a given RT time is based on the FT master scan with a mass resolution of 25,000 (scan event 1). In the next step an FT SIM scan (scan event 2) at a mass resolution of 50,000 and an Ion Trap MS/MS scan (scan event 3) are performed in parallel on the most abundant ion. For the FT SIM scan a mass window of ± 5 u is chosen and only $1e^5$ ions are collected in the ICR cell. With this low target value a mass accuracy of better than 0.5 ppm is routinely achieved (Figure 2). In the given setup this pair of experiments (FT SIM and IT MS/MS) is repeated for the 2nd and 3rd most abundant ion. The cycle time of this setup

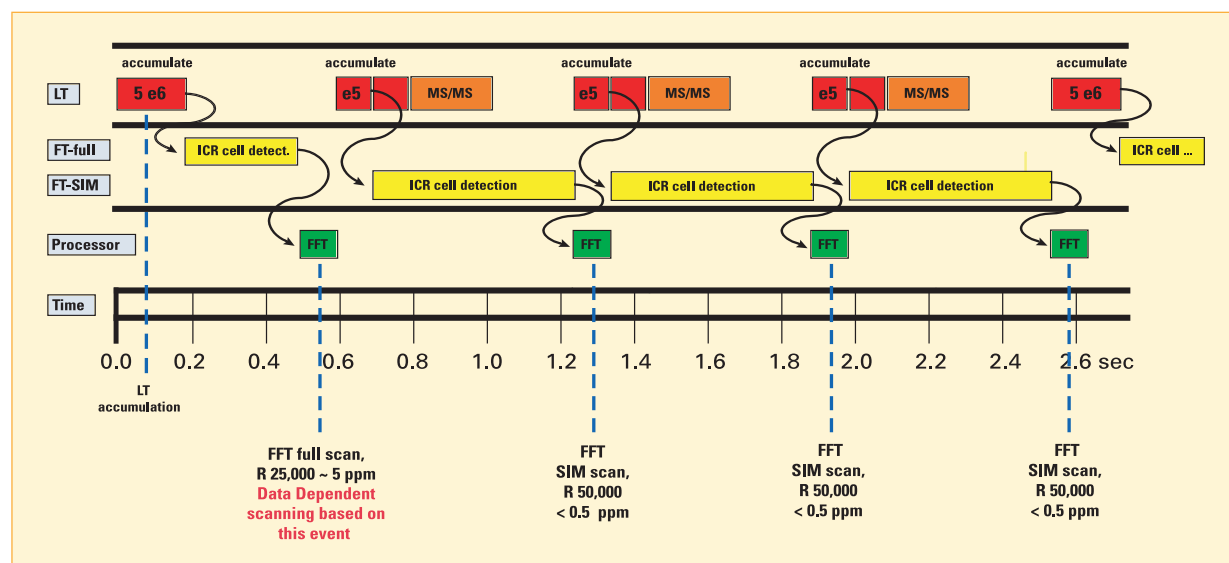


Figure 1: Workflow of FT SIM method

(one FT master scan and three Data Dependent FT SIM and three Data Dependent IT MS/MS) is less than 3 s. For comparison, the same sample was analyzed using a method setup consisting of an FT master scan at R 100,000 with a target value of $5e^5$ ions resulting in mass accuracy of better than 2 ppm followed by six Ion Trap MS/MS scans^[2]

Results and Discussion

Figure 3 shows the base peak chromatogram of the analyzed 1 D-SDS band of an *Escherichia coli* culture used as a reference sample for this study.

A critical parameter influencing mass accuracy and dynamic range on ion trap based hybrid FTICR mass spectrometers is the number of ions trapped in the ICR cell. When a moderate number of ions is introduced into the ICR cell for analysis, the resulting full scan yields ions with mass accuracy of better than 2 ppm and a dynamic range of 1000:1. This setting in combination with Data Dependent ion trap experiments (duty cycle time around 1.5 seconds) lead to the identification of 7 of the 20 proteins in the complex mixture (Table 1).

Deliberately increasing the number of ions to overfill the ICR cell (10-fold increase) results in a higher dynamic range. However, due to space charging effects, the mass accuracy is decreased (outside 2 ppm window), which increases the probability of false positive identifications. To improve the mass accuracy while increasing the dynamic range, the scan cycle is extended by adding a Data Dependent SIM scan^[3]. The analysis of the *E. coli* protein mixture using this method setup allows the identification of 18 of the 20 proteins with greatly improved sequence coverage (Table 1); for example, the sequence coverage for the first hit (Succinyl-CoA synthetase) is increased to 60% (identified by 17 enzymatic peptides) compared to 30% (identified by nine enzymatic peptides) in the first run. This result demonstrates clearly the benefit of the higher dynamic range due to the higher number of ions in the ICR cell for the FT master scan.

The SIM option assures that the determination of the precursor ion is done with highest mass accuracy (which is transferred to the dta-file for data base search). Figure 2

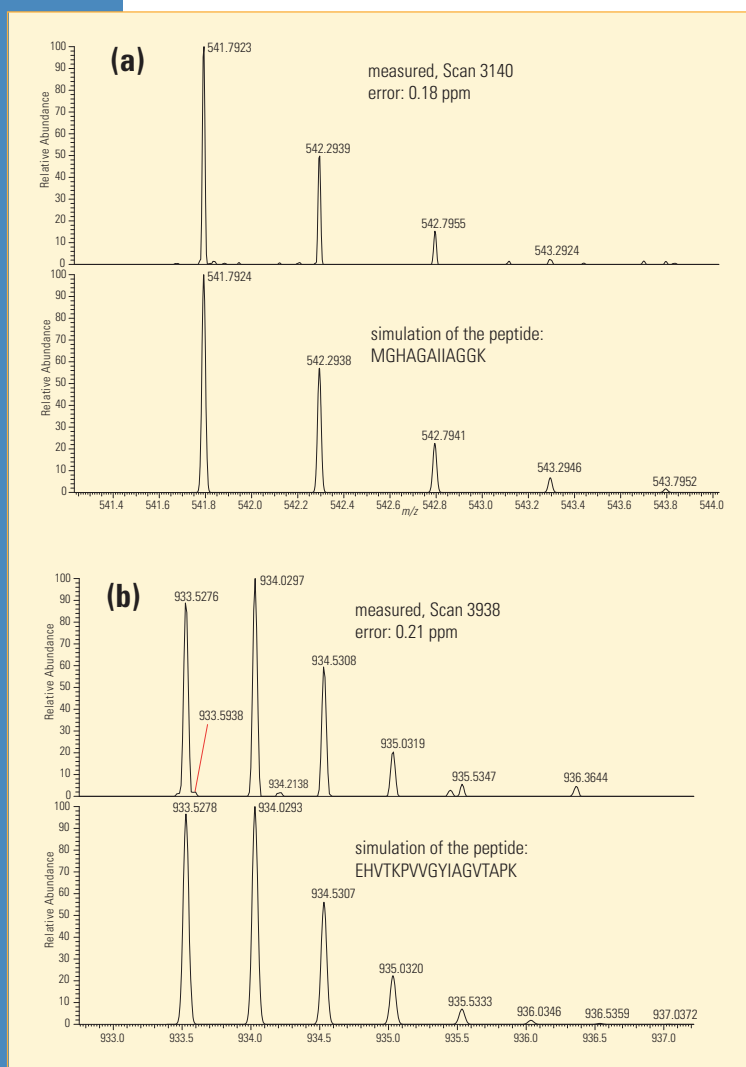


Figure 2: Examples for mass accuracy of the precursor ion determination using SIM scan. Shown are the measured doubly charged ions and the corresponding simulation of two enzymatic fragments of Succinyl-CoA synthetase: **(a)** fragment 245-256, **(b)** fragment 229-241

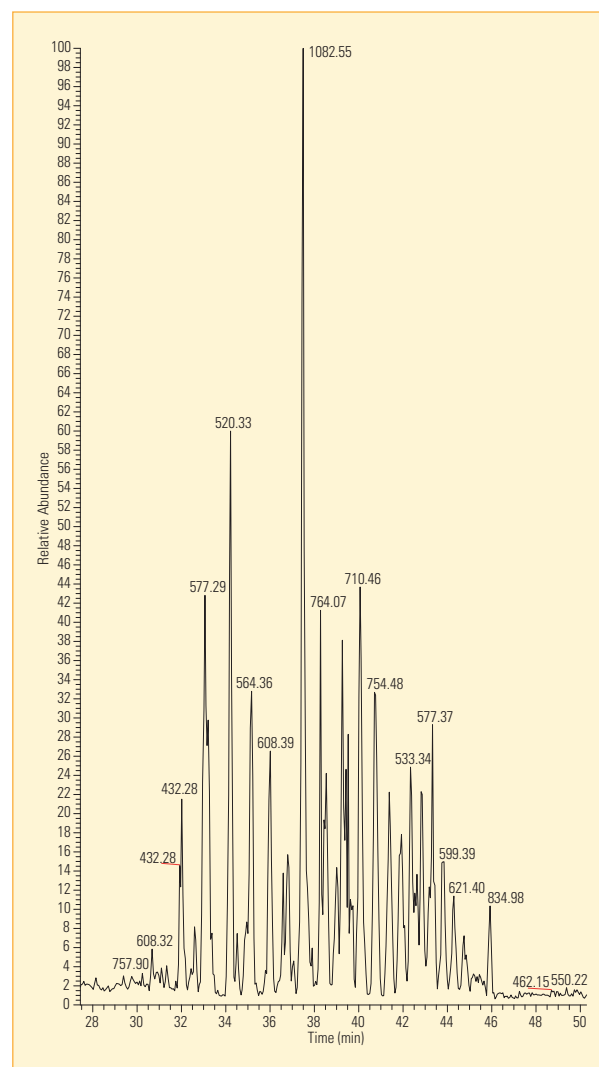


Figure 3: Nano-LC-MS base peak chromatogram of an enzymatic digest of D-SDS band of an *E. coli* culture

No.	Identified protein	SIM method*	Standard method*
1.	Succinyl-Co synthetase	59.9	30.7
2.	FKBP-type peptidyl-prolyl cis trans isomerase	47.5	30.7
3.	2,3,4,5-tetrahydropyridine-2-carboxylate N-succinyltransferase	40.5	35.0
4.	50S ribosomal subunit protein L2	38.5	28.9
5.	Pantothenate synthetase	27.2	3.9
6.	2-dehydro-3-deoxyphosphooctulonatealdolase	23.3	2.8
7.	Hypothetical protein (<i>e. coli</i> K12)	5.2	8.5
8.	Serine acetyltransferase	16.2	ni
9.	Orf, hypothetical protein	21.9	ni
10.	Cysteine synthase B	12.2	ni
11.	glucokinase	7.8	ni
12.	Thymidylate synthetase	5.0	ni
13.	Affects formate dehydrogenase	3.9	ni
14.	Taurine-binding periplasmic protein precursor	3.2	ni
15.	Dihydroxynaphthoic acid synthetase	3.5	ni
16.	Enoyl-[acyl carrier-protein] reductase (NADH)	3.8	ni
17.	Membrane-bound ATP synthase, F1 sector	2.8	ni
18.	agmatinase	3.3	ni

Table 1: List of identified proteins using Thermo Scientific BioWorks™ software showing the sequence coverage of each protein. *: peptide tolerance for database search 0.5 ppm; †: peptide tolerance for database search 2 ppm. ni: not identified by standard method

gives examples of doubly charged ions of two different enzymatic fragments of Succinyl-CoA synthetase. The measured signals (top) are compared to simulations (bottom) and reveal mass accuracies which are well below 0.5 ppm. In addition, this comparison shows very good accordance of the relative abundance of the measured isotopes of each cluster with the calculated distribution of the doubly charged ions.

The identification of the sequence of the proteolytic fragments was done on the basis of MS/MS data acquired in the linear ion trap. As an example the MS/MS-spectrum of the enzymatic fragment EHVTKPVVGYIAGVTAPK is displayed in Figure 4b, additionally a table of possible theoretical a, b, and y-type fragment ions is given (Figure 4a). The highlighted fragment ions represent matched signals in the MS/MS spectrum showing a very good coverage for the b- and y-type fragment ions of the peptide and demonstrate the reliability of the identification by Ion Trap MS/MS data.

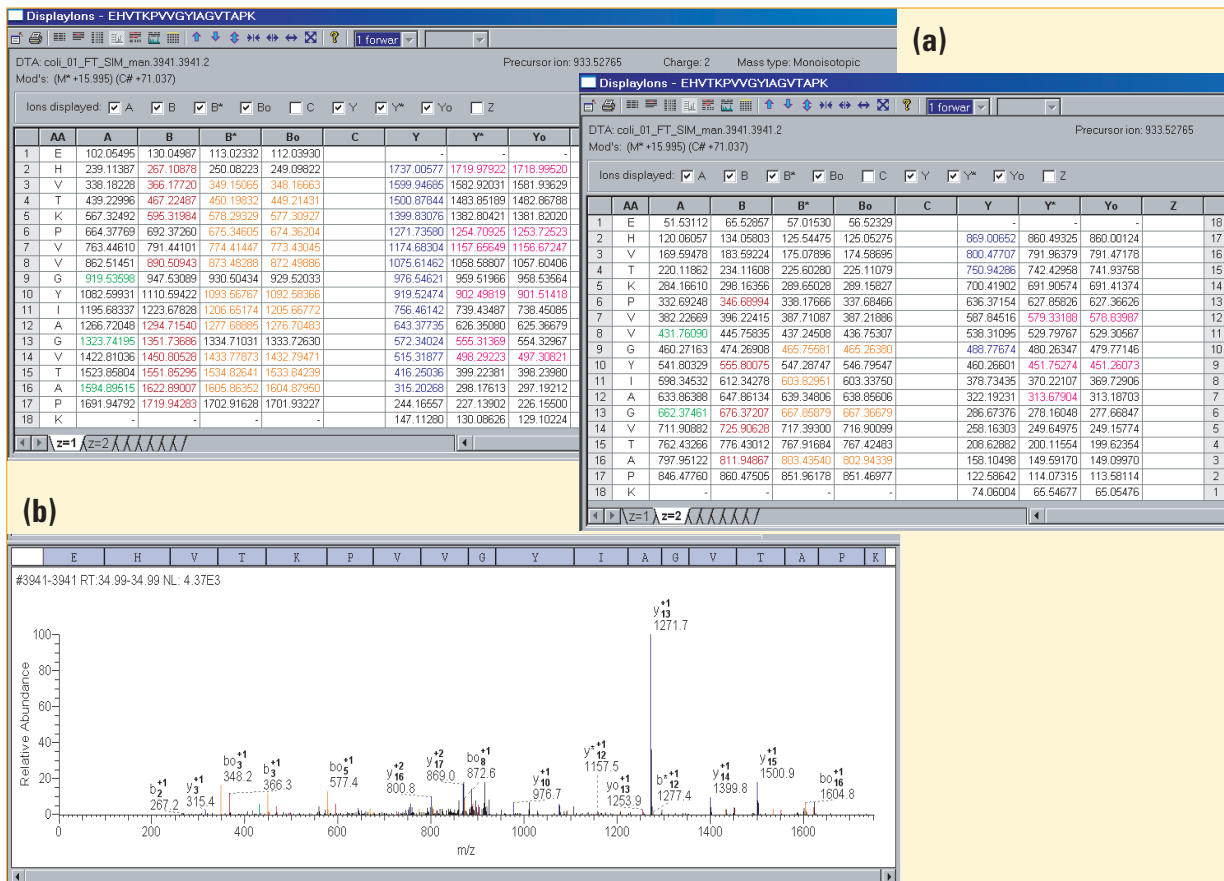


Figure 4: Interpretation of the identified enzymatic fragment EHVTKPVVGYIAGVTAPK (a) and a table of theoretical a, b and y-type fragment ions for singly (left) and doubly (right) charged fragments, fragments found in the raw file are color-coded. (b) Corresponding ion trap MS/MS-spectrum

Conclusions

- The described method setup offers mass accuracy of better than 0.5 ppm for the precursor ion;
- Improved dynamic range by accumulating more ions (10-fold increase) for the FT master scan;
- Improved dynamic range results in more identification with higher sequence coverage;
- SIM scan in the ICR cell and the MS/MS experiment in the linear trap are performed in parallel;
- Shorter database search times due to excellent mass accuracy;
- The probability of false positive hits is greatly reduced.

References

- ^[1] Zolg, J.W., Langen, H. (2004) How industry is approaching the search for new diagnostic markers and biomarkers. *Molecular & Cellular Proteomics* 3, 345-354.
- ^[2] Strupat, K., Metelmann-Strupat, W., Peterman, S. High-Throughput Analysis in Proteomics: Parallel mass detection using the LTQ FT. Application Note 30046, Thermo Fisher Scientific.
- ^[3] Andersen, J.S., Lam, Y. W., Leung, A.K.L., Ong, S.-E., Lyon, C.E., Lamond, A.I., Mann, M. (2005) Nuclear proteome dynamics. *Nature* 433, 77-83.

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