NanoFrontier: NanoLC Trap TOF Mass Spectrometer for Proteomics

Yasushi Terui

OVERVIEW: NanoFrontier is a nanoLC (liquid chromatograph) trap TOF (time of flight) MS (mass spectrometer) for Proteomics. NanoLC is used for sample separation, and it can make gradient-elution at nL/min (nanoliter per minute) flow rates without split. It incorporates splitless gradient flow that provides flow rates down to nL/min. The MS part is a hybrid type that incorporates ion-trap and TOF MS. It can perform MS^n (mass spectrometery) analysis with high mass accuracy measurement. Using NanoFrontier to perform proteome analysis will enable one to clarify life process and discover new knowledge about how to develop new drugs.

INTRODUCTION

NOW that we have the human genome blueprint, post-genome analysis is gaining a lot of attention. In particular, proteome analysis, which is the detailed investigation of all expression proteins in an organism at any one time, is becoming a very important field. Proteomics is useful in many ways from shedding light on organism phenomena to the development of new drugs that target proteins to treat illness. Mass spectrometry has a huge role to play in the analysis of proteomes. In 2003, the global market for mass spectrometers used in proteome analysis was 55 billion yen and the market expanded at a rate of 14% per year[1)].

Proteome analysis measures extremely small samples at minute trace levels, so researchers require instruments with very high sensitivity. NanoFrontier, which went on sale in October 2004, is an LC-MS (liquid chromatography-mass spectrometry) device for protein analysis that responds to researcher demands for high accuracy. The LC part, which is used for sample separation, can make gradient-elution at nL/min (nanoliter per minute) flow rates without split. The MS part is a hybrid trap-TOFMS (time of flight mass spectromter) unit that incorporates an ion-trap spectrometer and a time-of-flight mass spectrometer. The trap-TOFMS can perform MS^n (mass spectrometry) analysis with high mass accuracy measurement.

Fig. 1—NanoFrontier Exterior.
The control computer and the nanoLC are on the left side and the trap-TOFMS is on the right side.

Fig. 1 shows an exterior view of NanoFrontier. The control computer and the nanoLC are on the left side and the trap-TOFMS is on the right side. Below we introduce NanoFrontier's features and provide a measurement example.

NANOLC FEATURES

For the nanoLC to perform sample separation, it requires a stable gradient flow down to flow rates in nL/min. A splitless method is used to achieve nano-level performance and a DEGS (dual exchange gradient system) is used to ensure a high level of reproducibility. Fig. 2 gives an overview of DEGS. Gradient solution formed using the gradient pump is introduced into a 10-port valve then filled into Loop1. After a specified time, the 10-port valve is switched and gradient solution, which was filled into Loop1, is delivered to an analysis column. At the same time, gradient solution formed using the gradient pump is filled into Loop2. After a specified time, the 10-port valve is switched and gradient solution, which was filled into Loop2, is delivered to an analysis column. At the same time, the next gradient solution is filled

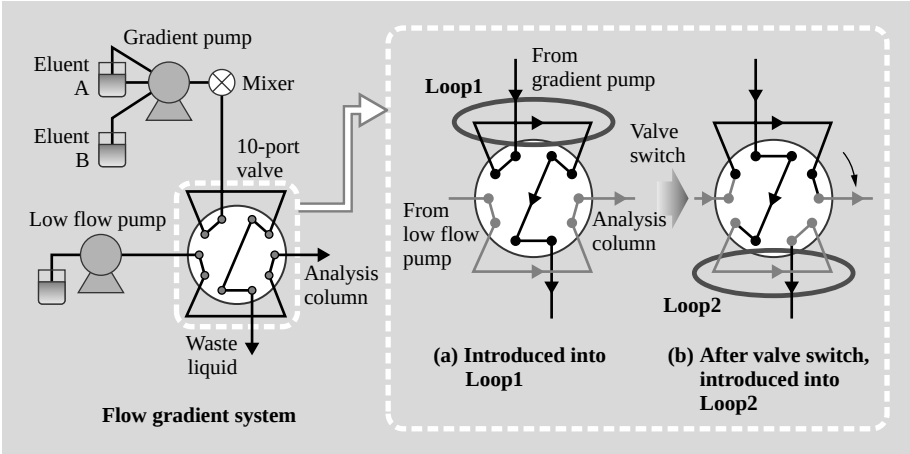
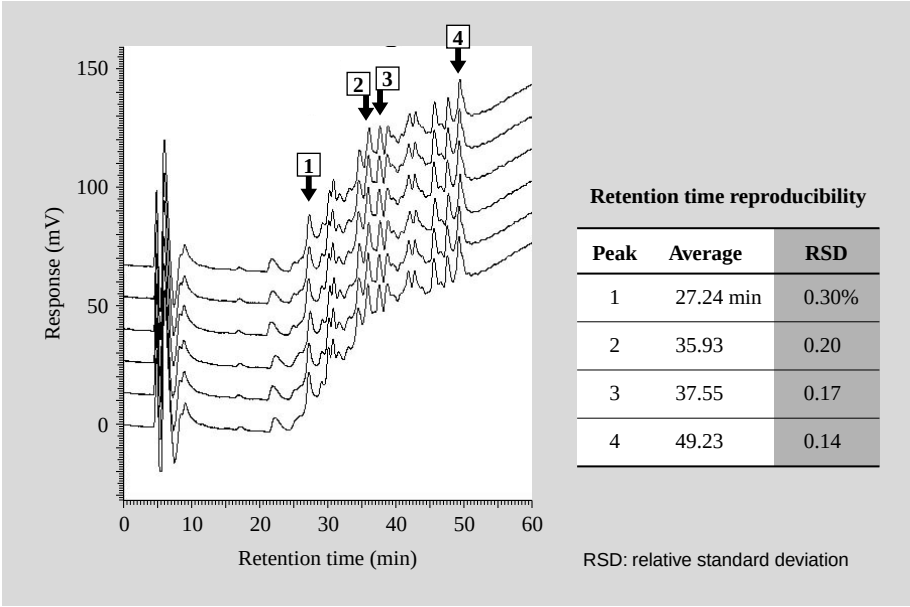


Fig. 2—DEGS Overview. DEGS adopts splitless flow method which forms gradient solution with good reproducibility at flow rates down to nL/min.



Retention time reproducibility

Peak	Average	RSD
1	27.24 min	0.30%
2	35.93	0.20
3	37.55	0.17
4	49.23	0.14

RSD: relative standard deviation

Fig. 3—UV Chromatogram and Retention Time Reproducibility for BSA Digest (200 nL/min). To measure DEGS retention time reproducibility, a sample is used in which BSA has undergone an enzyme digestion procedure. A UV detector performs the measurement. The measurement flow is 200 nL/min.

into Loop1. In this way after several repetitions, the dual exchange gradient system forms gradient solution through the external pump that captures a portion of solution each time. We have also developed a new low flow pump with rates from 50 to 200 nL/min.

Fig. 3 shows a UV (ultraviolet) chromatogram and retention time reproducibility for BSA (bovine serum albumin) digest using nanoLC. Four data points are plotted on the chromatogram and checked for retention time reproducibility. After six measurements, reproducibility is 0.3% or below.

MASS SPECTROMETRY FEATURES

The MS part that detects ions and separates mass incorporates a hybrid trap-TOFMS unit that combines both mass spectrometry and high mass accuracy

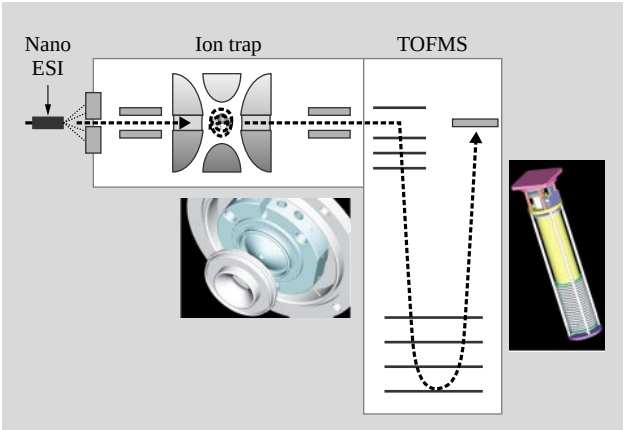


Fig. 4—Trap-TOFMS Overview. NanoFrontier is a hybrid MS that combines an ion trap and orthogonal time of flight.

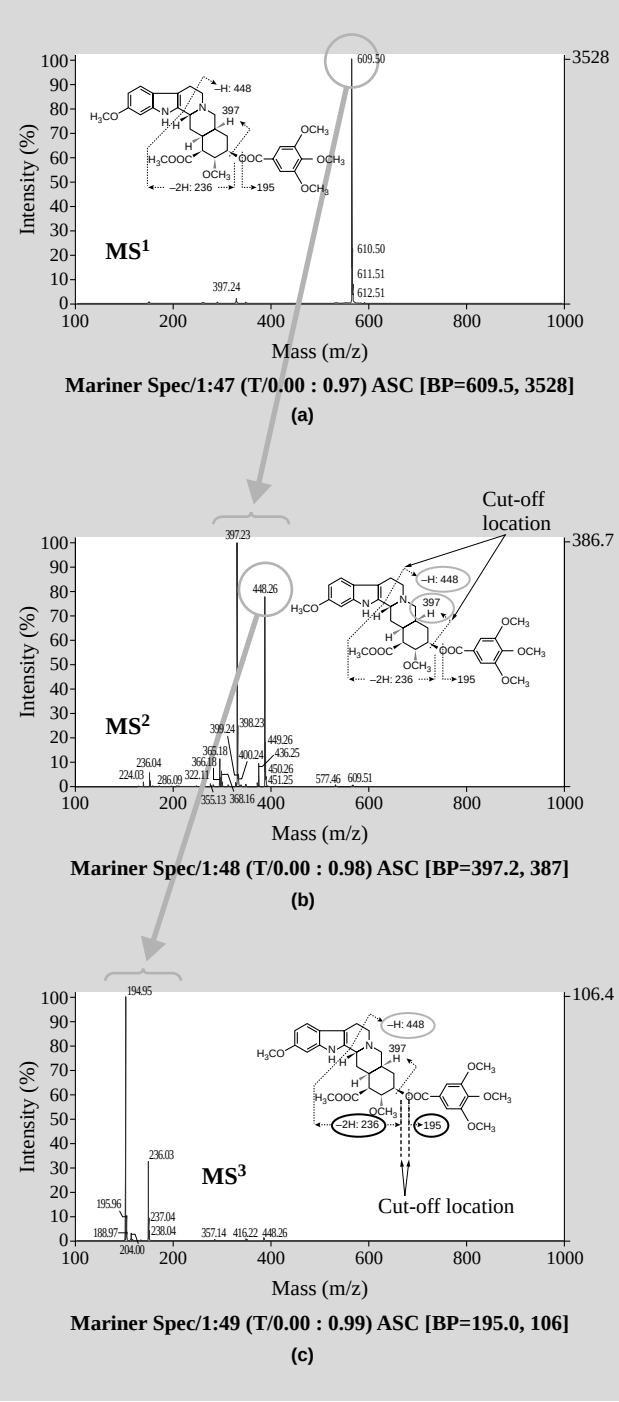


Fig. 5—Reserpine MS^3 Analysis.
An outstanding feature of NanoFrontier is shown in MS^n analysis for the MS spectrum of reserpine.

measurement.

Fig. 4 gives an overview of trap-TOFMS. Measurement sample separated in nanoLC is ionized through nano ESI (electrospray ionization) and introduced into an ion trap. The ion trap repeatedly accumulates, isolates, and divides ions and thereby achieves mass spectrometry analysis. Ions emitted by the trap are introduced into TOFMS for mass separation. One outstanding feature of TOFMS is its ability to provide high mass accuracy measurement.

Fig. 5 (a) shows analysis results for reserpine at MS^2 and MS^3 using a concentration of 100 μg/L with MeOH solvent. The atomic mass number for MS^1 analysis peaks at 609 (m/z). This peak indicates the ions formed by proton addition in reserpine. Taking the peak at 609 (m/z) as the parent ion, fragment ions are observed at 397 and 448 (m/z) during MS^2 analysis. Taking the peak at 448 (m/z) as the parent ion, fragment ions are observed at 195 and 236 (m/z) during MS^3 analysis. Each mass spectrum resolution is 8,000 or above.

Fig. 5 (b) shows reserpine structure. Repeated MS analysis increases the cut-off locations and enables a more detailed measurement of the molecular structure.

TABLE 1. Sample Concentration (10 fmol), Mixed Sample Cover Rate, and Detected Peptides

	Hgα	Hgβ	Lactα	Lyso	OvalA
Number of detected peptides	4	9	2	3	7
Cover rate	36%	62%	6%	22%	24%

MEASUREMENT EXAMPLE

In this example, we used NanoFrontier to measure a mixed protein sample then we search in the Mascot* database. The results appear in Table 1. The sample is a mixture containing hemoglobin α, hemoglobin β, lactalbumin α, lysozyme, and ovalbumin A. Each item has undergone trypsin digestion, and each item has a concentration of 10 fmol. The sample flow is 50 nL/min. Eluent A is 0.1% HCOOH and eluent B is 0.1% AcCN. The gradient condition is 5 to 60% B in 80 minutes.

For hemoglobin α, 4 peptides are detected at a cover rate of 36%. For hemoglobin β, 9 peptides were detected at a cover rate of 62%. For the rest, the average cover rate for five types of protein samples was 30%.

CONCLUSIONS

NanoFrontier is an LC-MS device for protein analysis that provides sample flow rates down to nano liters per minute. To achieve this level of performance,

* Mascot is a trademark of Matrix Science Ltd.

NanoFrontier combines nanoLC, which enables a stable gradient flow, and trap-TOFMS, which enables tandem mass spectrometry (MS^n) with high mass accuracy measurement. With NanoFrontier, researchers can analyze proteomes, shed light on the phenomena of living organisms, and discover new insights into drug design.

REFERENCE

(1) SDI Report 2002.

ABOUT THE AUTHOR

Yasushi Terui

Joined Hitachi, Ltd. in 1994, and now works at the Biosystems Design Department, Naka Division of Hitachi High-Technologies Corporation. He is currently engaged in the development of mass spectrometer. Mr. Terui can be reached by e-mail at terui-yasushi@naka.hitachi-hitec.com.