SHIMADZU

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High-throughput and high resolving power analyses of monoclonal antibody subunits using multi-turn TOF-MS coupled to HPLC system

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1. Overview

A new multi-turn time-of-flight mass spectrometer has been combined with HPLC for high-resolution and high-throughput analyses. High resolving power of over 100k and high-throughput, fast LC gradient of 8 min has been confirmed for reduced monoclonal antibodies analysis.

2. Introduction

The analysis of antibody drugs requires high mass resolving power to detect modifications of these high mass molecules through small mass differences. It is applied to quality control (QC) such as impurity analyses due to changes in the structure. While high-throughput analyses are an important factor for QC, many of the proposed methods are performed coupled to nanoflow liquid chromatography (nanoLC) to improve sensitivity, at the expense of throughput. Therefore, we have been exploring the possibility of analyzing antibody drugs with both high throughput and high resolution using a multi-turn TOF-MS [1] coupled to a high-performance liquid chromatography (HPLC).

3. Materials and Methods

3-1. Multi-turn TOF-MS system

Our new multi-turn TOF-MS consists of rotationally symmetric sector electrodes. Ions fly along a long 3D open-loop multi-turn orbit (nearly 50 m), thereby high mass resolving power can be obtained with compact size. The diameter and height of the outer sector are 500 mm and 240 mm, respectively (Figure 1).

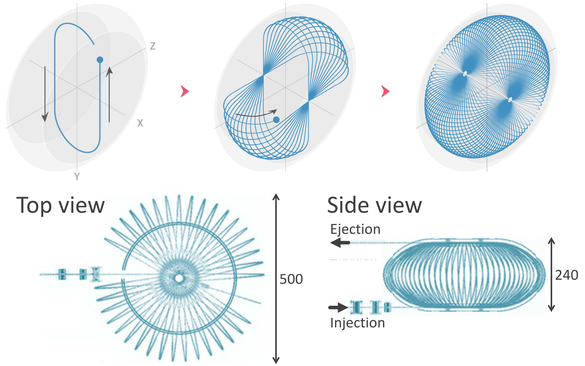

Figure 1 Ion trajectory of the multi-turn TOF-MS.

Figure 2 shows overview of the multi-turn TOF-MS system. The system consists of electrospray Ionization (ESI) source, quadrupole (QP), collision cell (CC), linear ion trap (LIT), and multi-turn TOF. Ions passed through QP and CC are accumulated and compressed in LIT. Then these ions are ejected and accelerated toward the multi-turn TOF.

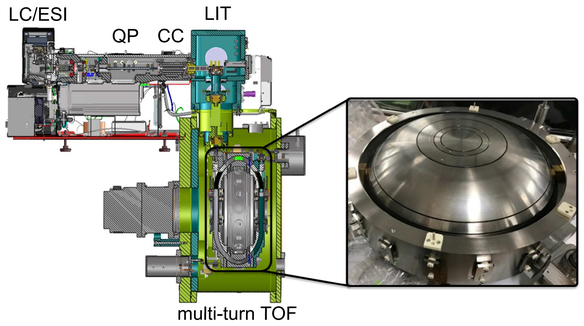

Figure 2 Overview of multi-turn TOF MS system.

In our previous study, mass spectra of myoglobin (~17,600 Da) were observed with high resolving power of over 100k (FWHM). A clearly isotope-separated spectrum was obtained (Figure 3). In this study, subunits of monoclonal antibodies were measured with the multi-turn TOF-MS coupled to an HPLC system.

Myoglobin (~17,600 Da, 15+)

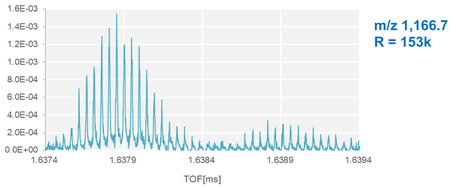

Figure 3 TOF spectrum of Myoglobin.

3-2. Sample Preparation

NIST mAb purchased from Sigma-Aldrich (St Louis, USA) was diluted to 1 mg/mL in 50 mM ammonium bicarbonate. 100 µg of intact protein was reduced to its heavy chain and light chain by adding 8 M Urea, 50 mM Tris-HCl, and 50 mM DTT [2].

3-3. Analytical Conditions

nanoLC conditions (LC-20ADnano)

Column	EX-Nano Inertsil WP300 C4 5 µm, 150 mm x 0.1 mm
Mobile Phase A	0.1 % formic acid in H_2O
Mobile Phase B	Acetonitrile
Mode	Gradient elution (Total 30 min)
Flow Rate	400 nL/min
Injection volume	10 µL

HPLC conditions (LC-30AD)

Column	Restek C4 5 µm, 150 mm x 2.1 mm
Mobile Phase A	0.1 % formic acid in H_2O
Mobile Phase B	Acetonitrile
Mode	Gradient elution (Total 8 min)
Flow Rate	0.4 mL/min
Injection volume	10 µL

MS conditions (multi-turn TOF-MS)

Ionization	ESI	Interface voltage	+4 kV
Nebulizing gas flow	3.0 L/min	Drying gas flow	10.0 L/min
Heating gas flow	10.0 L/min	DL temp.	200 ºC
HB temp.	400 ºC	Interface temp.	300 ºC

4. Results

4-1. Analyses of mAb subunits using multi-turn TOF-MS system

At first, a nanoLC was combined with the multi-turn TOF-MS system. The total analysis time took more than 30 minutes. Isotopically resolved spectra were obtained for the light chain (Figure 4). For the heavy chain, modified glycans (galactose: 162 Da, etc.) could be identified (Figure 5).

Light chain (~23,000 Da, 13+)

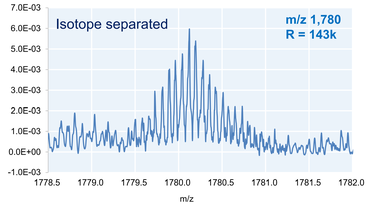

Figure 4 Mass spectrum of LC subunit of NIST mAb.

Heavy chain (~51,000 Da, 30+)

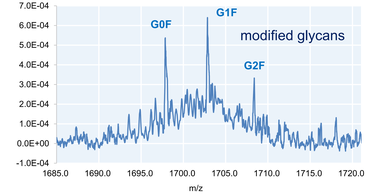

Figure 5 Mass spectrum of heavy chain of NIST mAb.

4-2. High-throughput analyses of mAb subunits

To perform high-throughput analyses, a HPLC has been combined with the multi-turn TOF-MS system. The analysis column used was a Restek C4 (2.1 mm I.D. x 150 mm L. , 5 µm). Figure 6 shows the total ion chromatogram (TIC) of the HPLC and the nanoLC analysis. The HPLC system separated the light and heavy chains in high-throughput fast gradient time of less than 8 min, which is several times faster than the nanoLC analysis.

(a) HPLC (b) nanoLC

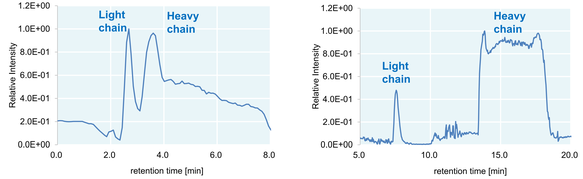

Figure 6 Comparison of TIC for (a) HPLC and (b) nanoLC analysis.

5. Conclusions

- Clearly separated isotope peaks of mAb light chain have been observed with over 100k mass resolution by using the new multi-turn TOF-MS.
- High-throughput fast gradient time less than 8 min with an HPLC system have been demonstrated for light and heavy chain subunits analysis.

References

[1] 69th ASMS Conference on Mass Spectrometry (Philadelphia, 2021), FP-410.
[2] “Monoclonal antibody workflows on the Shimadzu Q-TOF LCMS-9030 using the Protein Metrics Software Suite” (Shimadzu application news, LCMS-103)