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Structural Characterization of Cyclic Peptides using a Quadrupole Time-of-Flight Mass Spectrometer

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Overview

The aim of this study is structural analysis of a cyclic peptide. We developed an analytical method for cyclosporin A using a LC/Q-TOF mass spectrometer and performed LC-MS/MS measurement. As a result, all of the major MS/MS fragments were assigned to predicted fragments from the chemical structure with a 100% structural coverage. In this presentation, we will discuss the workflow of data analysis using an analytical software and the details of qualitative results.

Introduction

Cyclic peptides are expected to be a new class of therapeutic agents because of their higher stability in vivo and higher cell permeability than linear peptides. MS/MS-based peptide sequencing is the most common effective strategy for structural identification of linear peptides. However, in the case of cyclic peptides, sufficient sequence information cannot be obtained with MS/MS measurement as the fragmentation patterns are quite complicated due to the cyclic nature. In order to overcome this issue, we have attempted to confirm the structural characteristics of cyclic peptides only by spectral assignment.

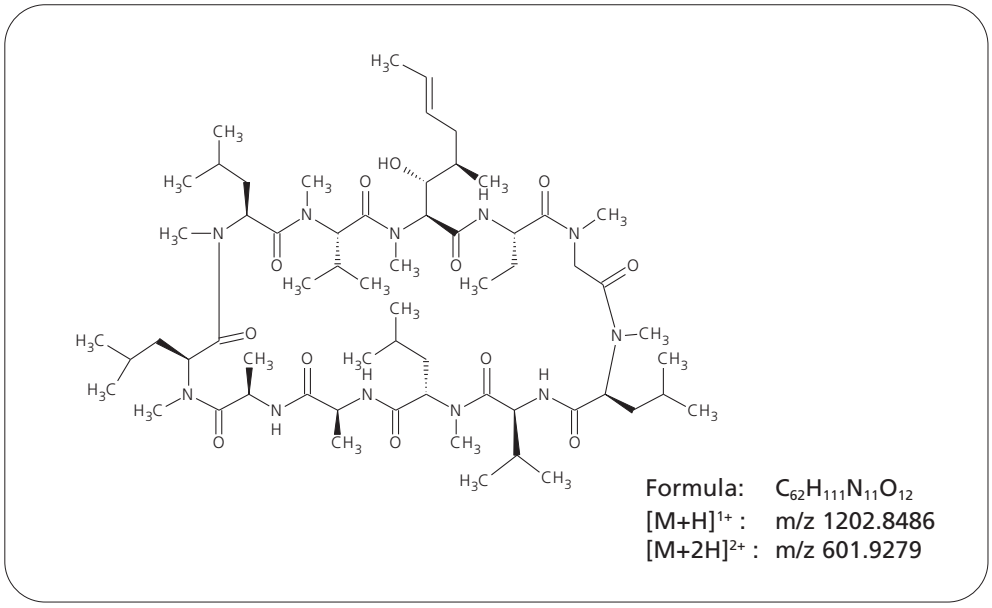

Figure 1 Structure of Cyclosporine A

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Methods

Commercially available compounds were used for this experiment. Standards of surfactants were diluted with Milli-Q water to an appropriate concentration. Cyclosporin A was analyzed using a quadrupole time-of-flight (Q-TOF) mass spectrometer (LCMS-9030; Shimadzu Corporation, Kyoto, Japan) or a quadrupole ion trap time-of-flight (IT-TOF) mass spectrometer (LCMS-IT-TOF; Shimadzu) coupled with conventional flow liquid chromatography (Nexera X2; Shimadzu). LC separation was performed using a Shim-pack GISS column (2.1 x 50 mm, 1.9 µm, Shimadzu) with a binary gradient of 0.1 % formic acid in water and 0.1 % formic acid in acetonitrile. Assignments with MS/MS spectra and fragment predictions from the molecular structure are performed using a third party's software, ACD/MS Workbook Suite (ACD/Labs, Toronto, Canada)

High-Resolution and Accurate Mass Spectrometer

Resolution power: > 30,000 FWHM at m/z 1,972 / 1,626
Mass accuracy: 1 ppm
Maximum acquisition rate: 100 Hz
Minimum polarity switching rate: 1 second

Figure 2 LCMS-9030 quadrupole time-of-flight mass spectrometer

Results

Method development for cyclosporin A

The theoretical m/z was calculated from the formula (Figure 1). Retention time was confirmed from XIC at the theoretical m/z (mass error tolerance = ± 1 ppm) (Figure 3). The mass spectra were created by integrating around the observed peaks on the chromatogram (Figure 4, upper). We further examined the chemical composition of the corresponding peak on the MS spectrum by formula prediction using ACD/MS Workbook Suite software. As a result, the predicted composition with the top score completely agreed with the composition of cyclosporin A with the top score (Figure 5).

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UHPLC (Nexera X2)	
Analytical Column	: Shim-pack GISS 2.1x50mm, 1.9µm (P/N 227-30048-01)
Mobile Phase A	: 0.1 % formic acid / water
Mobile Phase B	: 0.1 % formic acid / ACN
Gradient Program	: 60%B (0-0.25min) -95%B (2.5-3.0min) -60%B (3.01-4.00 min)
Flow Rate	: 0.3 mL/min
Column Temperature	: 50 °C
Injection Volume	: 1.0 µL
MS (LCMS-9030)	
Ionization	: ESI positive / Negative
Acquisition mode	: MS, MS/MS
Nebulizing Gas Flow	: 3 L/min
Drying Gas Flow	: 10 L/min
Heating Gas Flow	: 10 L/min
Interface Temperature	: 350 °C
DL Temperature	: 150 °C
HB Temperature	: 500 °C
TOF range	: m/z 120 - 1000
CID	: 230 kPa, (MS), 270 kPa (MS/MS)
CE (MS/MS)	: 55 ±15 (Spread)

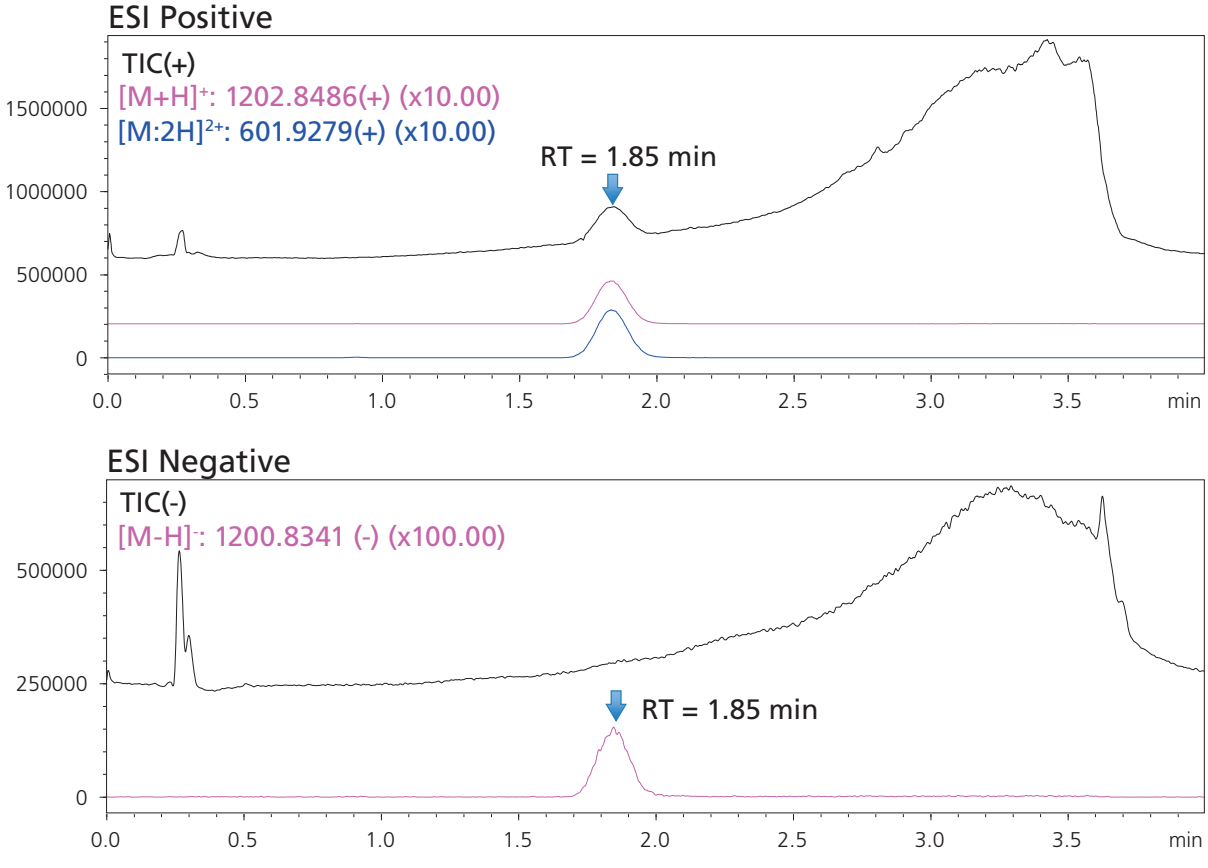

Figure 3 Mass chromatograms of Cyclosporin A (concentration : 1 ppm)

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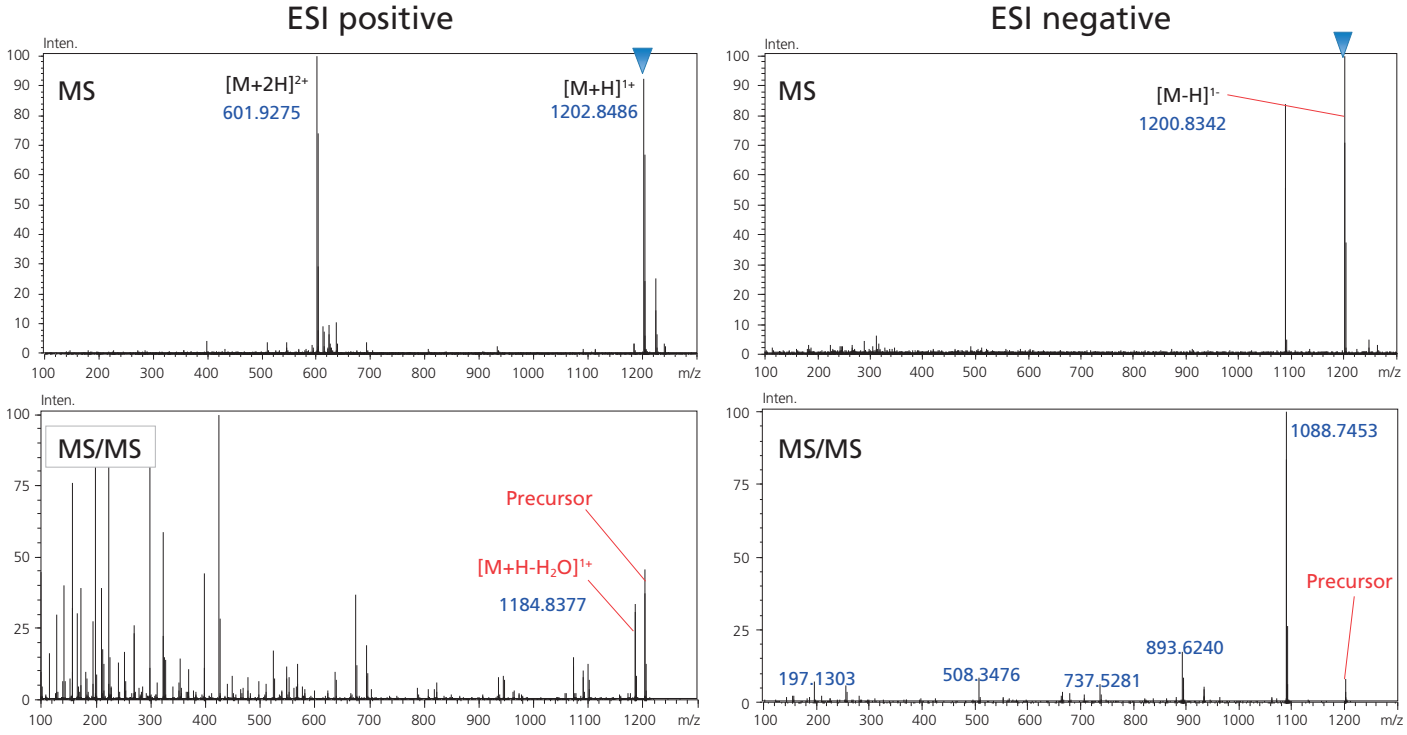
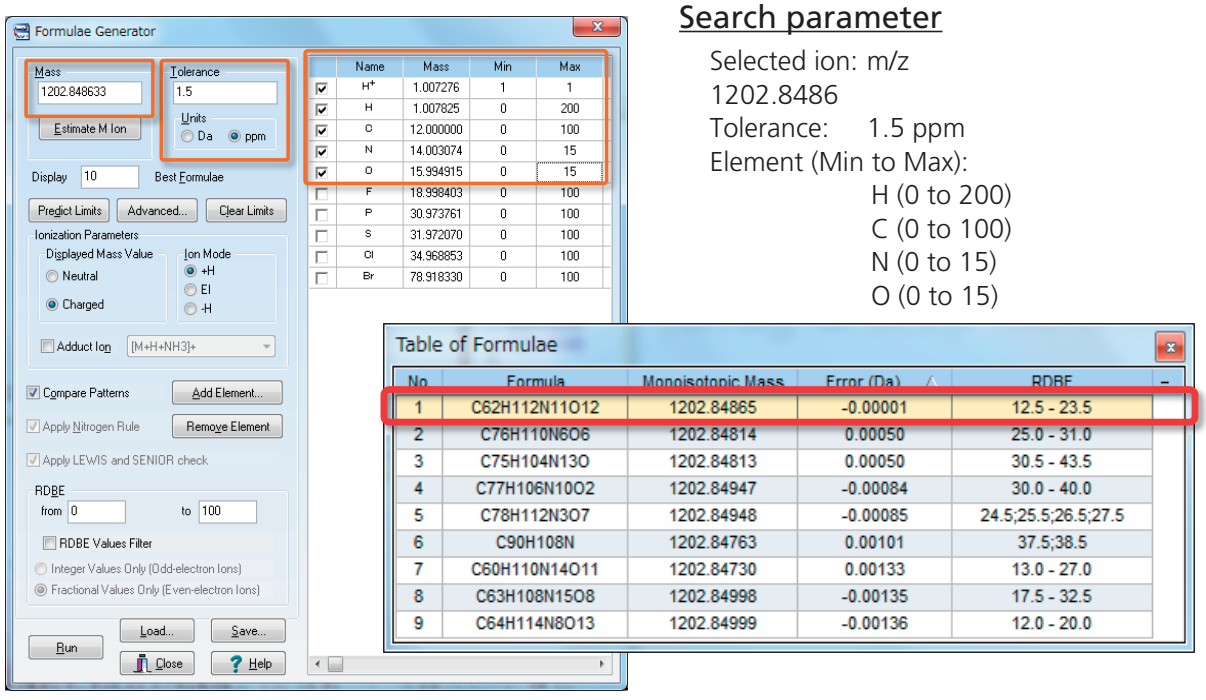


Figure 4 Mass spectra of Cyclosporin A (concentration : 1 ppm)



Search parameter

Selected ion: m/z
1202.8486
Tolerance: 1.5 ppm
Element (Min to Max):
H (0 to 200)
C (0 to 100)
N (0 to 15)
O (0 to 15)

Figure 5 Formula prediction of a precursor ion (Software: ACD/MS Workbook Suite)

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Structural analysis by MS/MS assignment

We performed qualitative analysis of cyclosporin A using a LC/Q-TOF MS system. As a result of MS/MS measurement, all of the major MS/MS fragments were assigned to predicted fragments with a mass error far below 1 mDa. In addition, the coverage of MS/MS fragments to the overall structure of cyclosporine was 100%. The cleavages occurred randomly but a certain regularity in fragmentation pattern were observed since most of the major fragmentations occurred as either a-, b-, c-, x-, y-, or z-series at amide bonds of peptides. Several MS/MS fragments were also analyzed with MS3 using a LC/IT-TOF MS system (data not shown). The fragment ions observed in MS3 measurement were almost the same as the MS/MS fragments. Thus, MS/MS was suggested to be enough to characterize the sequence information of cyclosporine A.

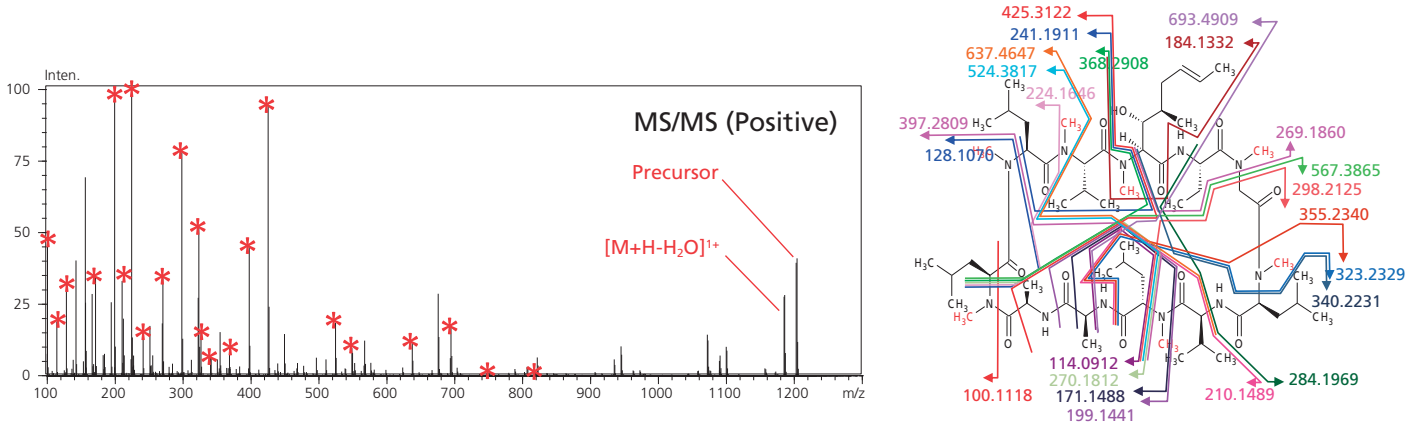

Figure 6. Fragment ion assignment (left) on a MS/MS spectrum and fragmentation pattern (right) of cyclosporin A by a LC/Q-TOF MS system

Figure 7 Theoretical m/z and mass error (Da) of the fragment ions.

m/z Exp.	Formula	m/z Calc.	Error (Da)
100.1118	C6H14N	100.1121	-0.0003
114.0912	C6H12NO	114.0913	-0.0001
128.1067	C7H14NO	128.1070	-0.0003
171.1488	C9H19N2O	171.1492	-0.0004
184.1328	C10H18NO2	184.1332	-0.0004
199.1441	C10H19N2O2	199.1441	0.0000
210.1484	C12H20NO2	210.1489	-0.0004
224.1645	C13H22NO2	224.1645	0.0000
241.1904	C13H25N2O2	241.1911	-0.0007
270.1815	C13H24N3O3	270.1812	0.0003
298.2125	C15H28N3O3	298.2125	0.0000
323.2327	C18H31N2O3	323.2329	-0.0002
326.2448	C17H32N3O3	326.2438	0.0010
340.2223	C17H30N3O4	340.2231	-0.0008
368.2913	C20H38N3O3	368.2908	0.0005
397.281	C20H37N4O4	397.2809	0.0000
425.3125	C22H41N4O4	425.3122	0.0003
524.3811	C27H50N5O5	524.3806	0.0004
567.386	C28H51N6O6	567.3865	-0.0004
637.4655	C33H61N6O6	637.4647	-0.0007
693.4914	C36H65N6O7	693.4909	0.0005
750.5492	C39H72N7O7	750.5488	0.0004
818.5756	C43H76N7O8	818.5750	0.0006

Conclusions

- Most of the major MS / MS peaks could be assigned to the predicted fragment with a mass error of 1 mDa or less.
- The coverage of the MS / MS fragment to the entire structure of cyclosporin was 100%.
- These results indicate that MS/MS measurement with high mass accuracy by LCMS-9030 is useful not only for sequence analysis of linear peptides but also for accurate structural characterization ofcyclic peptides.

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