Structural Prediction of Impurities in Drugs using MS^n Data

Technical Report vol.3

1. Introduction

Erythromycin is a macrolide antibiotic produced by a strain of bacteria known as *Saccaropolyspora erythraea*. The antibiotic is effective against many gram-positive and some gram-negative bacteria and is often used for people who display allergic reactions to penicillin. Structurally, this compound contains a 14-membered lactone ring (Area C) and two deoxy sugars, D-desoamine (Area A) and L-cladinose (Area B), making it a compound very difficult to produce via synthetic methods.

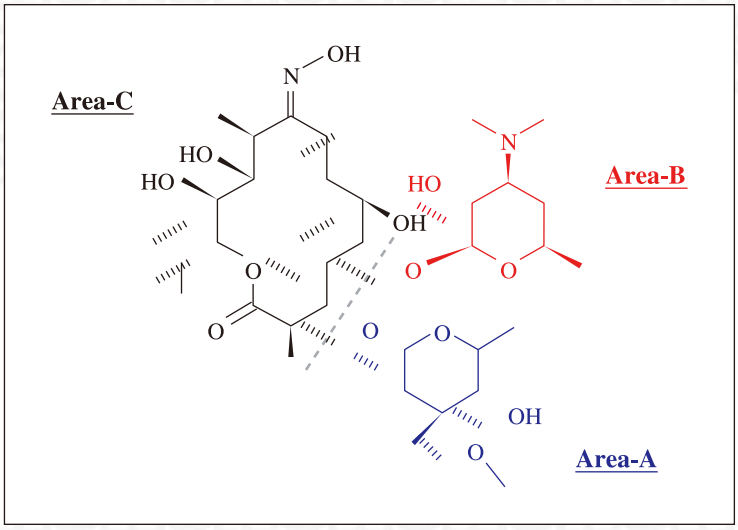


Fig. 1 Structure of Erythromycin A Oxime

This report describes the use of MS^n data with a prediction tool software to identify the formulas and structures of impurities in an erythromycin sample. Discerning the chemical formula or structure of unknowns is a difficult task that can be partially alleviated by acquiring high mass accuracy data; however, data interpretation is tedious and time consuming. By using fragmentation spectra collected from a Shimadzu LCMS-IT-TOF (a hybrid ion-trap time-of-flight mass spectrometer) along with enhanced formula prediction software, samples are rapidly analyzed to identify chemical formulas and structures.

2. Method

1. An erythromycin A oxime sample was dissolved in methanol (1 mg/mL) and then injected (10 μL) onto a heated (40 °C) reversed-phase column (Phenomenex Gemini C18; 150 x 2 mm; 5 μm) using a Shimadzu Prominence Series SIL-20AC autosampler and a CTO-20A column oven.
2. Mobile phase A consisted of 0.1% ammonium hydroxide in water; mobile phase B was acetonitrile. Compounds were eluted from the column at 0.2 mL/min using LC-20AD pumps operated isocratically (60% B) and monitored using an SPD-20A UV detector (200 nm) prior to entering the mass spectrometer.
3. High mass accuracy data was collected on Shimadzu's LCMS-IT-TOF hybrid ion-trap time-of-flight mass spectrometer using negative electrospray operated in full scan MS and MS^2 modes.
4. Data was analyzed using the newly developed Formula Predictor software.

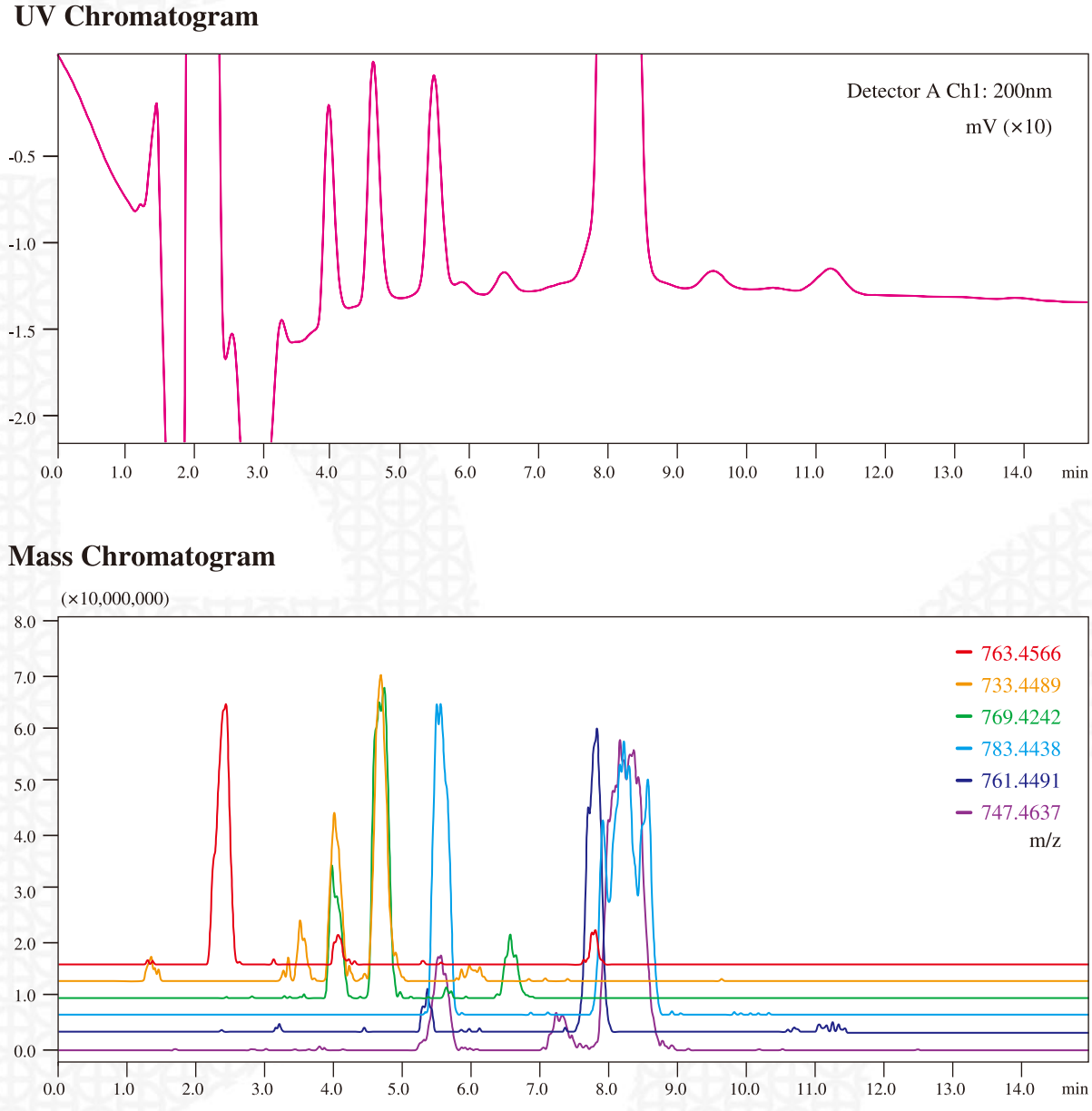


Fig. 2 UV and mass chromatograms of erythromycin A oxime sample

3. Results

Fig.3 shows the mass spectra of erythromycin A oxime. In Fig.3(A) and (B), the precursor ions are marked ▽. The mass difference of 176.1032 is considered to represent the loss of Area A from the erythromycin A oxime structure.

As m/z = 396.238 (highlighted in pink) is Area C, the mass difference of 175.1229 in Fig.3(B) is thought to indicate the loss of Area B from the erythromycin A oxime structure.

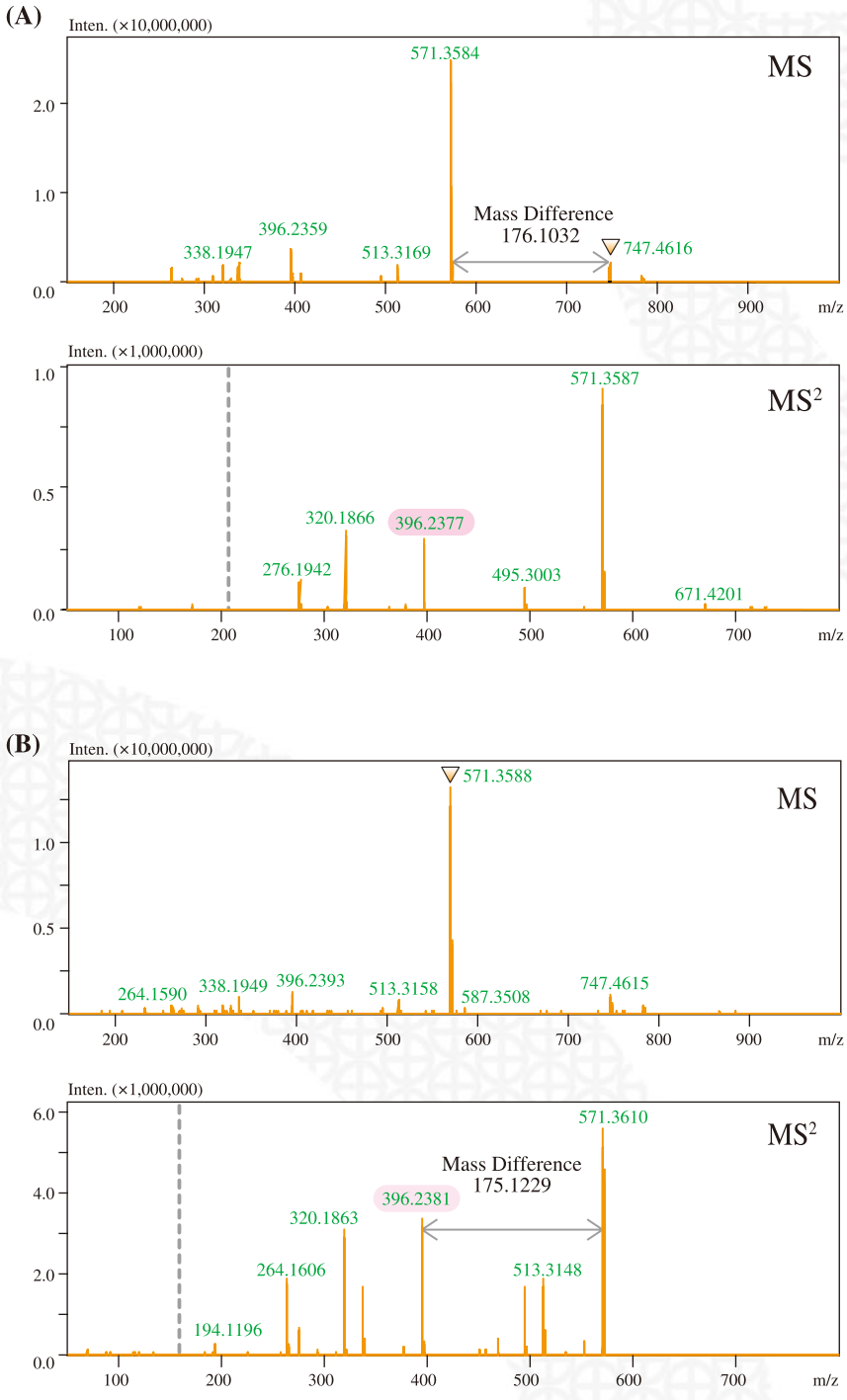
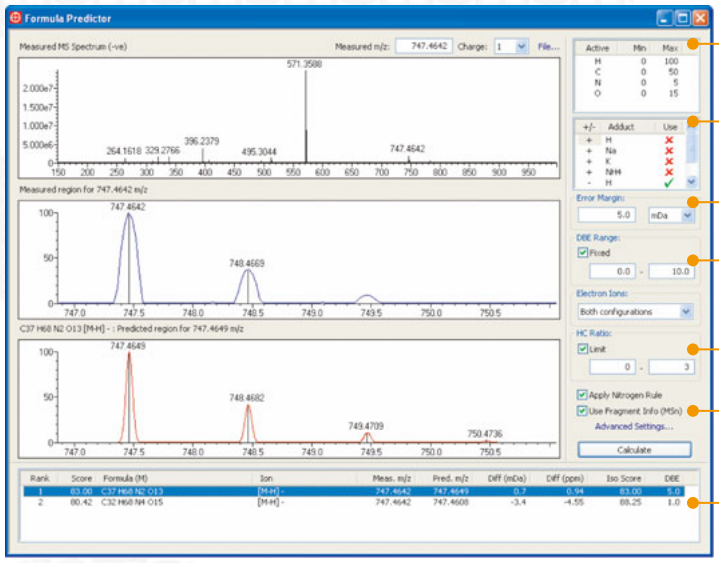


Fig. 3 Mass spectra of erythromycin A oxime. The mass difference of 176.1032 is indicative of a loss of Area A from the erythromycin molecule; the mass difference of 175.1229 indicates further loss of Area B resulting in Area C as shown highlighted in pink. Precursor ions are indicated by a ▽.

Right-clicking a compound highlighted in blue at the bottom of the search window (Fig.4) will show the detailed results of fragment data (MS^n) (Fig.5).



Specifies the number and types of elements to be used for the calculation.

Adducts can be specified.

Specifies the allowable margin of error for the calculation.

Specifies whether or not double bond equivalency (DBE) is used, and the allowable limits.

Specifies whether or not the hydrogen-to-carbon ratio is used, and the allowable limits.

Specifies whether or not the nitrogen rule will be applied, and whether or not fragment data (MS^n) will be used to help eliminate unwanted matches.

Search results are displayed in order of score. The isotopic distribution (ISO score) as well as mass accuracy contribute to the final score. Predicted spectra are displayed by clicking the result.

Fig. 4 The Formula Predictor software window. Results from a search on the m/z = 747.4642 ion are displayed. The highest score calculated corresponds to the molecular formula $C_{37}H_{68}N_2O_{13}$, a match for erythromycin A oxime.

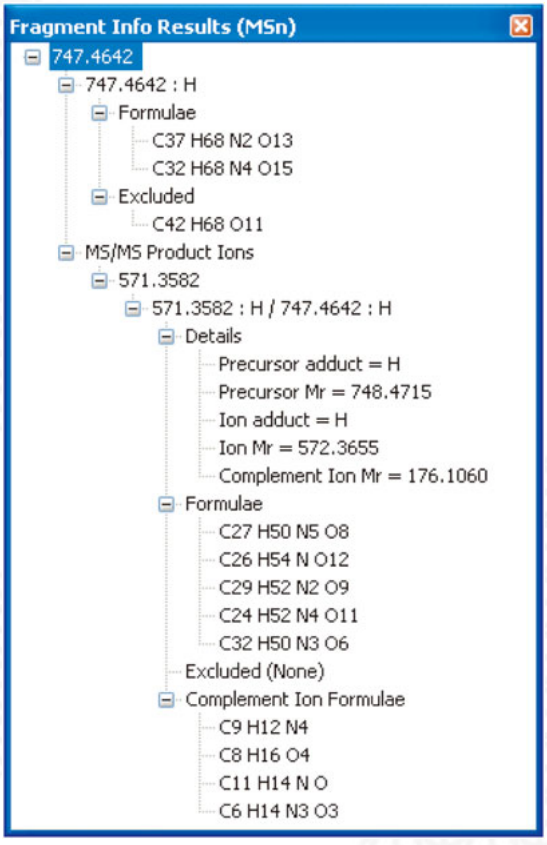


Fig. 5 Fragment Info Results window. Right-clicking in the blue area of the Formula Predictor window gives the option to display information generated from fragment data.

Fig. 6 shows the mass spectra of impurity m/z = 783.4421. As the m/z = 396.2409 ions corresponding to Area C and the mass difference of 175.1165 indicating the loss of Area B in the MS^2 spectrum are the same as for erythromycin A oxime in Fig.3, this impurity is thought to be a 35.9841Da change in Area A of erythromycin A oxime.

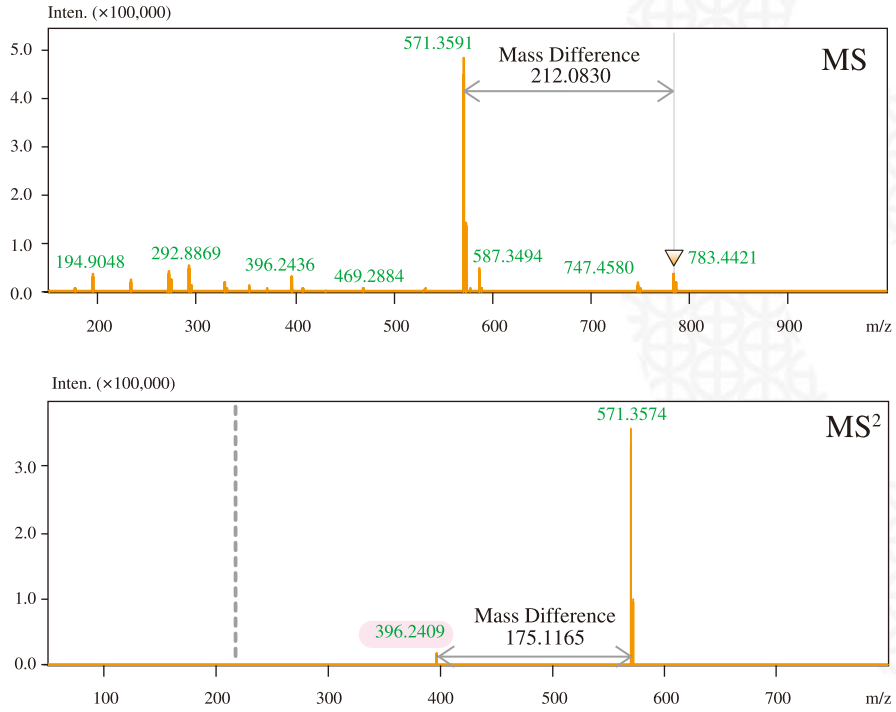


Fig. 6 Mass spectra of impurity m/z = 783.4421. Fragmentation data indicates a similar structure to erythromycin A oxime, as ions correspond to Area C (m/z = 396.2409 highlighted in pink) and a loss of Area B (175.1165). The mass difference between the impurity and erythromycin A oxime indicates a 35.9841Da change in Area A. The precursor ion is indicated by ▽ .

Formula [M]	$[M-H]^-$ (calculated)	$[M-H]^-$ (peak avg.)	Mass Accuracy (ppm)
$C_{37}H_{68}N_2O_{13}$	747.4649	747.4627	-2.9
$C_{29}H_{52}N_2O_9$	571.3600	571.3617	+3.0
$C_{26}H_{46}N_2O_8$	513.3181	513.3161	-3.9
$C_{26}H_{44}N_2O_7$	495.3076	495.3057	-3.8
$C_{21}H_{35}NO_6$	396.2392	396.2383	-2.3
$C_{18}H_{29}NO_5$	338.1973	338.1948	-7.4
$C_{18}H_{27}NO_4$	320.1867	320.1845	-6.9
$C_{17}H_{27}NO_2$	276.1969	276.1967	-0.7
$C_{15}H_{23}NO_3$	264.1605	264.1605	±0.0
$C_{11}H_{17}NO_2$	194.1187	194.1181	-3.1

Table 1 Mass accuracy data for erythromycin A oxime and fragments. Molecular formulas were determined using Formula Predictor software. Mass accuracy was calculated using $|(\Delta \text{ mass})|/\text{mass}_{measured} \times 10^6 = \text{ppm}$.

Fig.7 shows the mass spectra of impurity m/z = 733.4439. The MS spectrum confirms the loss of the mass difference of 176.1049, which represents Area A. In the MS^2 spectra, the ions at m/z = 396.2409 represent Area C. The mass difference of 14.0164 557.3436 and m/z = 571.3600 for erythromycin A oxime in Fig. 3 corresponds to CH_2 (14.0157) and is assumed to result from the loss of a methyl group from Area B. Formula $C_{36}H_{66}N_2O_{13}$ was the top scoring hit in the Formula Predictor software.

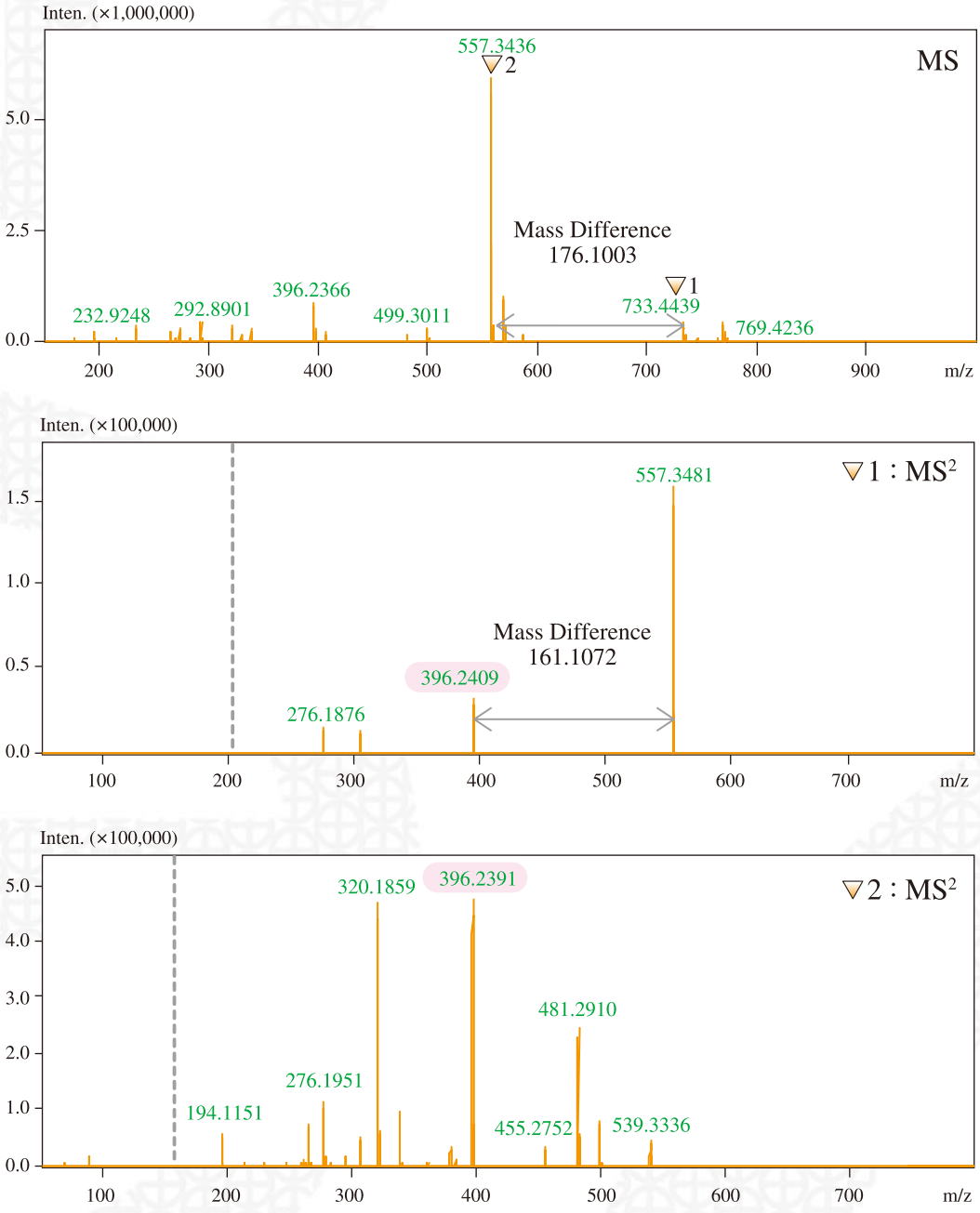


Fig. 7 Mass spectra of impurity m/z = 733.4439. In the MS spectrum, the mass difference of 176.1003 indicates a loss of Area A. In the MS^2 spectra, the ions at m/z = 396.24 highlighted in pink denote Area C. The 14.0164 mass difference between 557.3436 and 571.3600 corresponds to CH_2 (14.0157), resulting in a loss of a methyl group from Area B. Formula $C_{36}H_{66}N_2O_{13}$ was further supported as it was the top scoring hit from Formula Predictor. Precursor ions are indicated by ▽.

Fig. 8 shows the mass spectra of impurity m/z = 763.4581. In the MS spectrum, the mass difference of 176.1050 confirms a loss of Area A. In the MS^2 spectra, the ion at m/z = 396.2403 indicates Area C. The mass difference of 15.9931 between 587.3531 and m/z = 571.3600 for erythromycin A oxime in Fig. 3 corresponds to O (15.9949) and indicates an additional oxygen atom in Area B. Formula $C_{37}H_{68}N_2O_{14}$ was the top scoring hit in the Formula Predictor software.

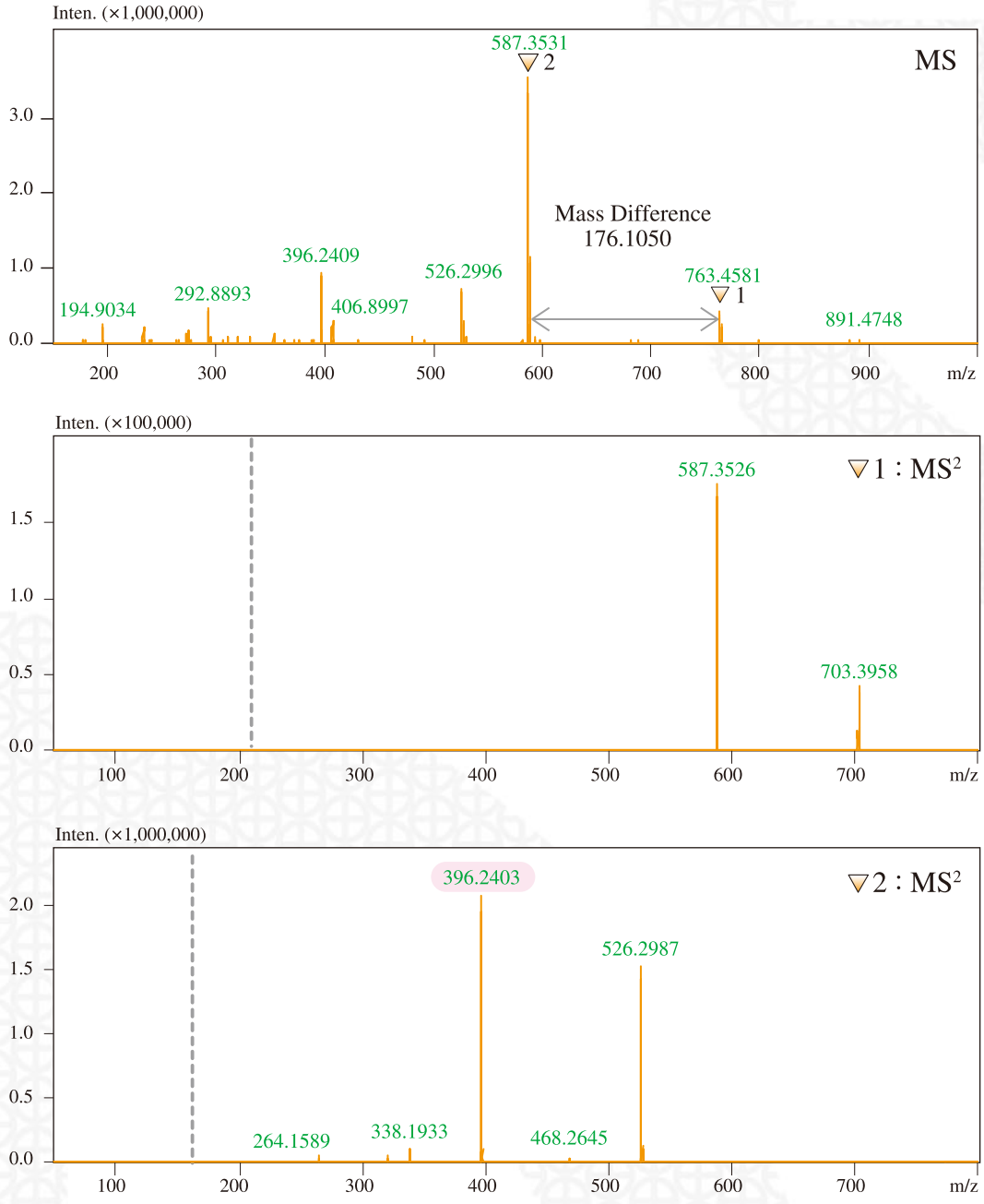


Fig. 8 Mass spectra of impurity m/z = 763.4581. In the MS spectrum, the mass difference of 176.1050 indicates a loss of Area A. In the MS^2 spectra, the ion highlighted in pink at m/z = 396.2403 is Area C. The mass difference of 15.9931 between 571.3600 and 587.3531 corresponds to an O (15.9949), signifying an additional oxygen atom in Area B. Formula $C_{37}H_{68}N_2O_{14}$ was further supported as it was the top scoring hit from Formula Predictor. Precursor ions are indicated by ▽.

Fig. 9 shows the mass spectra of impurity m/z = 761.4375. The lack of a fragment ion at m/z = 396.2392 in the MS^2 spectrum precludes the existence of Area C. Also, as no losses of Area A (176.1049) or Area B (175.1208) are seen, the molecule is not thought to be an erythromycin A oxime-related compound.

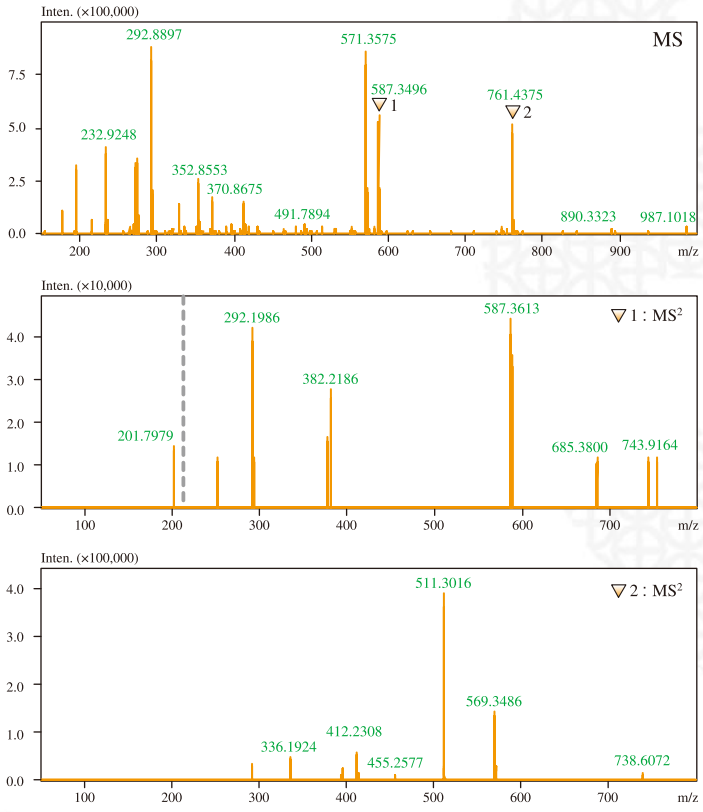


Fig. 9 Mass spectra of impurity m/z = 761.4375. Precursor ions are indicated by ▽. No fragment ion exists at m/z = 396.2392, precluding the existence of Area C. Also, losses of Area A (176.1049) or Area B (175.1208) are not seen, indicating a molecule dissimilar to erythromycin A oxime.

4. Conclusions

- Impurities m/z = 733.4439, 763.4581 and 783.4421 are assumed to have structures similar to that of erythromycin A oxime since their mass patterns are alike. They are therefore believed to be derived from erythromycin A oxime.

- Since the MS^2 spectrum patterns of the impurity at m/z = 761.4375 are different from that of erythromycin A oxime, it is assumed that it was externally mixed into the sample.

Holly M. Shackman; Joy M. Ginter; Joseph P. Fox; Masayuki Nishimura, Shimadzu Scientific Instruments, Columbia, MD

Printed in Japan 3295-09719-30ANS