Structural Elucidation by Composition Formula Predictor Software Using MS^n Data

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Overview

Erythromycin is a macrolide antibiotic produced from 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 with people that experience an allergic reaction to penicillin. The drug contains two deoxy sugars, **D-desosamine (Area A)** and **L-cladinose (Area B)**, attached to a **14-membered lactone ring (Area C)** ; due to its molecular structure, it is extremely difficult to synthesize.

For commercial purposes, it is essential to completely identify impurities that may be present with the drug of interest.

In this paper, MS^n data was used in conjunction with software prediction tools to identify the formula and structure of unknowns.

Introduction

Discerning the chemical formula or structure of unknowns is a difficult task which 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 novel hybrid ion-trap time-of-flight (IT-TOF) mass spectrometer along with enhanced software, samples are rapidly analyzed to identify chemical formulas and structures.

Methods

An erythromycin sample was dissolved in methanol (1 mg/mL) then injected (10 µL) onto a heated (40 ˚C) reversed-phase column (Phenomenex Gemini C18; 150 × 2 mm; 5 µm) using a Shimadzu Prominence Series SIL-20AC auto sampler and a CTO-20A column oven. 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. High mass accuracy data was collected on a LCMS-IT-TOF hybrid ion-trap time-of-flight mass spectrometer using negative electrospray operated in full scan MS and MS^2 modes. Data was analyzed with novel formula prediction software, Composition Formula Predictor.

Results

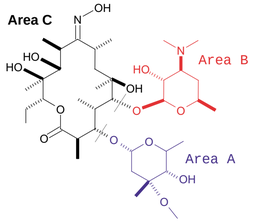


Figure 1. Structure of erythromycin.

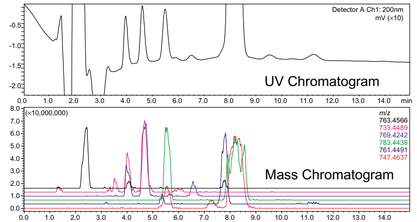


Figure 2. UV and mass chromatograms of erythromycin sample.

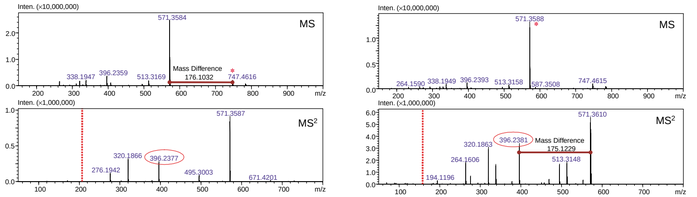


Figure 3. Mass spectra of erythromycin A oxime. The mass difference of 176.1032 is indicative of a loss of Area A from the erythromycin molecule; the mass difference of 175.1229 indicates further loss of Area B resulting in Area C as shown circled in red. Precursor ions are indicated by a ✻.

Results

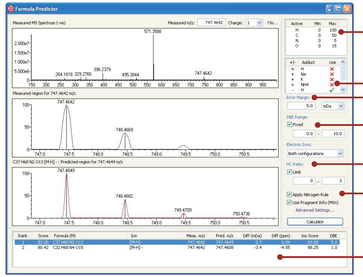


Figure 4. The Formula Predictor software window. Results from a search on the m/z = 571.3588 ion are displayed. The highest score calculated corresponds to the molecular formula $C_{37}H_{68}N_2O_{13}$, a match for erythromycin.

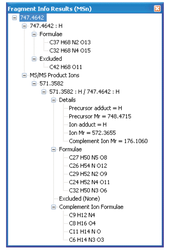


Figure 5. Fragment Info Results window. Right clicking in the blue area of the above Formula Predictor window gives the option to display information generated from fragment data.

Table 1. Mass accuracy data for erythromycin and fragments. Molecular formulas were determined using Composition Formula Predictor software. Mass accuracy was calculated using | (Δmass) | / mass $_{measured}$ × 10^6= ppm.

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
$C_{19}H_{27}NO_4$	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

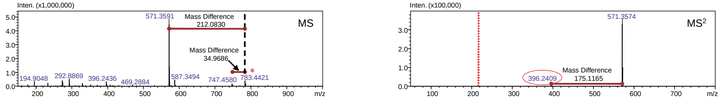


Figure 6. Mass spectra of impurity m/z = 783.4421. Fragmentation data indicates a similar structure to erythromycin A oxime, as ions correspond to Area C (m/z = 396.2409 circled in red) and a loss of Area B (175.1165). The mass difference between the impurity and erythromycin A oxime indicates a 35.9841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

Results

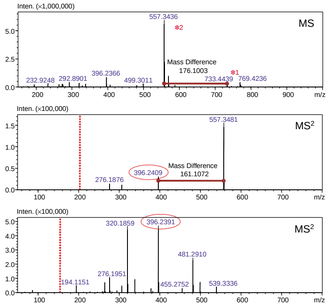


Figure 7. Mass spectra of impurity m/z = 733.4439. The mass difference of 176.1003 indicates a loss of Area A. The ions at m/z = 396.24 circled in red denote Area C. The 14.0164 mass difference between 557.3436 and 571.3600 corresponds to CH_2 (14.0157), resulting in a loss of a methyl group from Area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

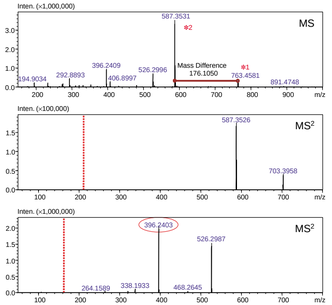


Figure 8. Mass spectra of impurity m/z = 763.4581. The mass difference of 176.1050 indicates a loss of Area A. The ion circled in red at m/z = 396.2403 is Area C. The mass difference of 15.9931 between 571.3600 and 587.3531 corresponds to an O (15.9949) signifying an additional oxygen atom in Area B.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

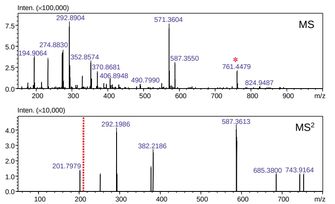
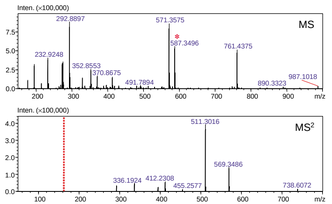


Figure 9. Mass spectra of impurity m/z = 761.4375. Precursor ions are indicated by a ✻. No fragment ion exists at m/z = 396.2392, negating Area C. Also, losses of Areas A (176.1049) or B (175.1208) are not seen indicating a molecule dissimilar to erythromycin A oxime.



Discussion and Conclusions

- Impurities m/z = 733.4439, 763.4581, and 783.4421 are assumed to have similar structures to erythromycin A oxime as their mass patterns are alike and 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 erythromycin A oxime, it is assumed that it was externally mixed into the sample.