Access to your success
SHIMADZU

C146-E118

Structural Analysis by In-Depth Impurity Search Using MetID Solution and High Accuracy MS/MS

Technical Report vol.16

1. Introduction

MetID Solution is a software application that was developed to identify drug metabolites (refer to Technical Report No. 11), but the technique for searching out structural analogues using multivariate analysis (PLS: Partial Least Square) can also be applied to the search and structural analysis of impurities, always a critical step in the production of medicinal and chemical products. In the structural prediction of impurities, the comparison of cleavage information with respect to the principal ingredient is important, and MetID Solution identifies only candidate substances detected by LCMS-IT-TOF whose cleavage information is the same as that of the principal ingredient. The software easily confirms whether or not the possible candidates have a fragmentation ion or neutral loss in common with the principal ingredient, powerfully facilitating the process of structural analysis.

Here we present the measurement of an alcoholic beverage sample suspected of containing multiple compounds (sildenafils) similar to sildenafil, a principal ingredient in ED therapeutic drugs. Recently, health foods illegally spiked with sildenafils are appearing on the market, and this is an important social issue wherever it may occur. This article reports on the analysis of the alcoholic beverage in question by automated MS/MS using the LCMS-IT-TOF, the subsequent exhaustive search for sildenafils using MetID Solution, and their structural analysis based on the cleavage information.

2. Method

For the HPLC, the Prominence system was used, and for the LCMS, the LCMS-IT-TOF was used. The LCMS-IT-TOF was used in the Auto MS/MS mode, in which precursor ions are switched automatically.
The details are presented below.

Column	: Phenomenex Gemini 5u C18 2.0 mm I.D. x 150 mmL
Mobile phase A	: 5 mM ammonium acetate - water
Mobile phase B	: acetonitrile
Gradient program	: 5%B (0 min) → 100%B (20 to 35 min) → 5%B (35.01 to 45 min)
Flow rate	: 0.2 mL/min
Injection volume	: 1 µL
Column temp.	: 40 deg. C
Ionization mode	: ESI (+)
Nebulizing gas	: 1.5 L/min
Drying gas pressure	: 100 kPa
Probe voltage	: +4.5 kV
CDL temperature	: 200 deg. C
BH temperature	: 200 deg. C

The work flow up to structural analysis is shown in Fig. 1. In the prediction of impurity structures, it is important to first predict the cleavage positions within the structures of the various fragmentation ions of the principal ingredient.

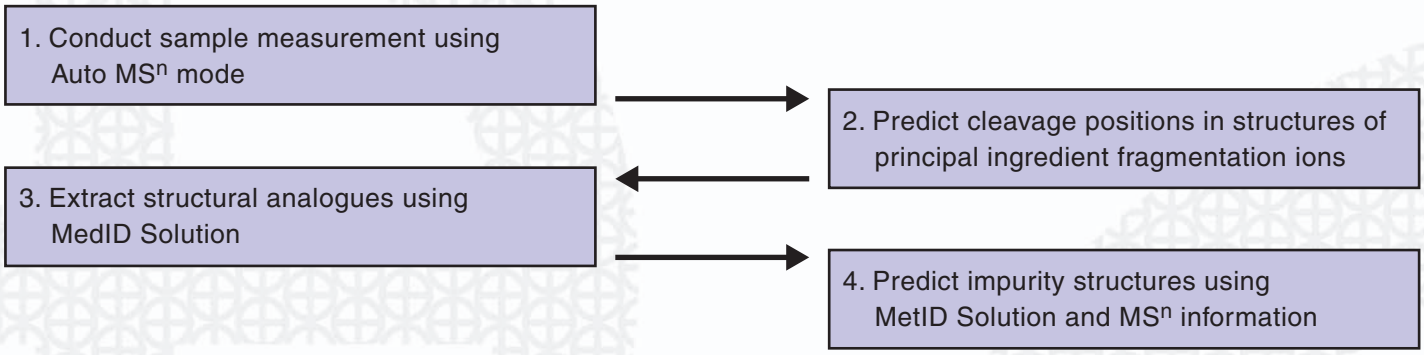

Fig. 1: Work Flow

3. Results

Fig. 2 shows the total ion chromatogram (TIC) and the m/z 475.2122 mass chromatogram corresponding to the sildenafil protonated molecule $[M+H]^+$. Because many peaks are observed in the TIC besides that of the principal ingredient, it is difficult to visually distinguish sildenafils from among all of these.

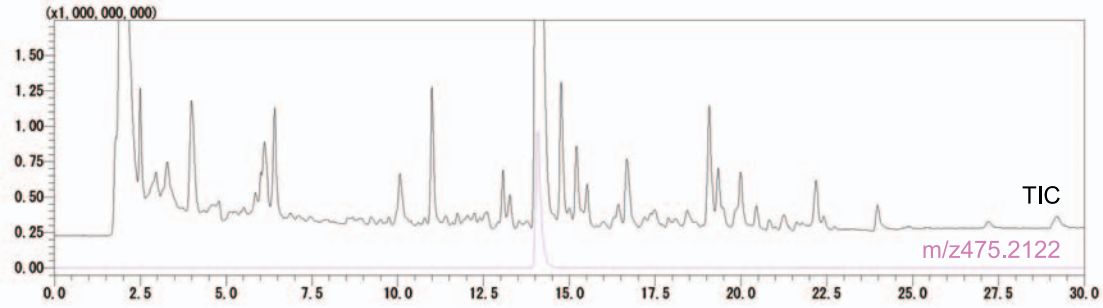

Fig. 2: Total Ion Chromatogram (TIC) of Alcoholic Beverage and Sildenafil Mass Chromatogram (m/z 475.2122)

Fig. 3 shows the mass spectra. The respective cleavage positions were predicted from the measurement values of the main fragmentation ions obtained in MS^2 analysis, as indicated by the arrow markings in the structural formula (Fig. 4). As stated above, it is important to first predict the cleavage positions shown for the various fragmentation ions with respect to the compound used for comparison (sildenafil).

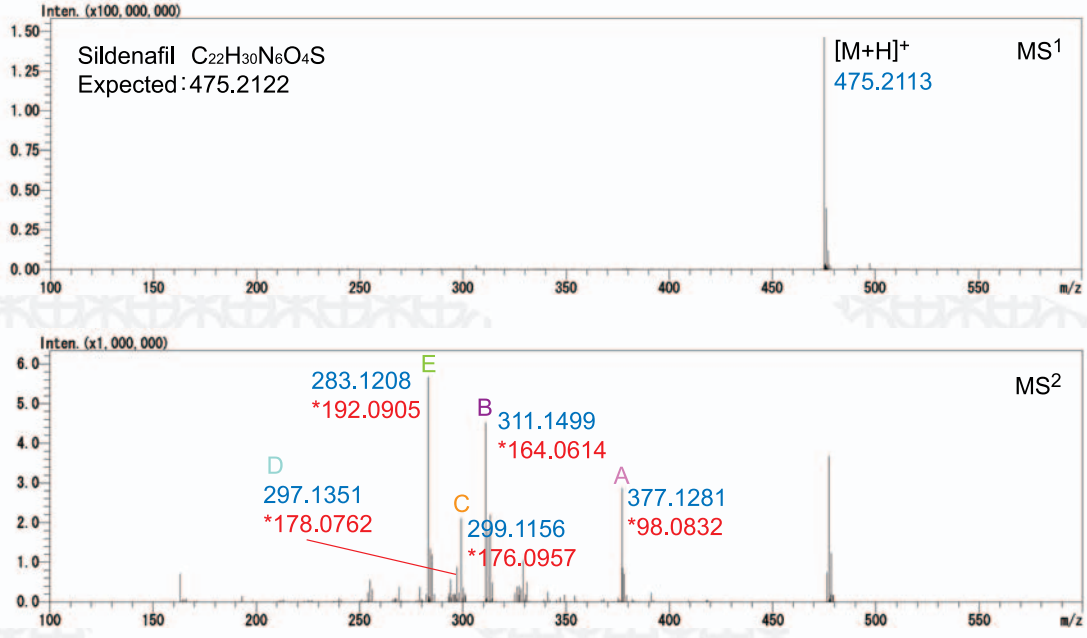

Fig. 3: Sildenafil Mass Spectra (* indicates neutral loss)

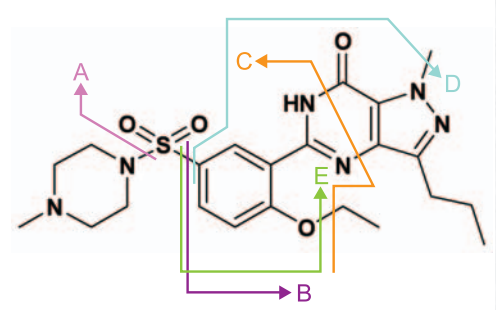

Fig. 4: Sildenafil Structural Formula and Predicted Cleavage Positions

Next, the data file is processed using MetID solution. Fig. 5 shows the MetID Solution results window of the processed alcoholic beverage data file. In the PLS analysis, the substances with fragmentation ions or neutral loss in common with the principal ingredient take positive values along the X axis. Among the substances picked out as possible candidates, those in the Transformation List that have undergone change are listed as Expected, and substances other than those are listed as Unexpected. Substances that can be predicted to be impurities in the sample can be manually added to the Transformation List beforehand, as the list can be freely edited. This makes it possible to accurately search for the existence of relevant substances. (Here, the list was not edited, and consists of metabolites that have been placed there according to the default settings.) In addition, right-clicking on the main window displays the type of table shown at the lower right in Fig. 5. The items displayed in the columns in the table indicted as [I:xxx] are fragmentation ions observed in the principal ingredient (sildenafil), and the columns indicted as [C:xxx] indicate neutral loss. Substances assumed to have commonality with the principle ingredient can be quickly confirmed based on common elements of the cleavage information.

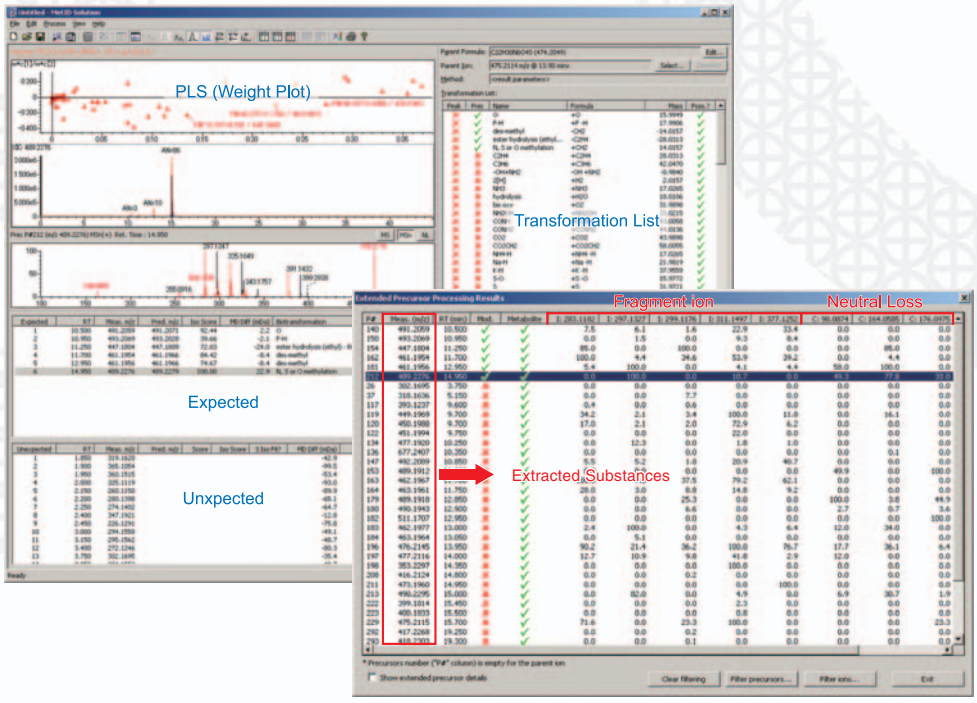

Fig. 5: PLS Analysis Results Using MetID Solution

As an example of structural analysis of sildenafils, m/z 489 (P#212), shown as highlighted in Fig. 5, was selected. Fig. 6 shows the mass spectra of P#212.

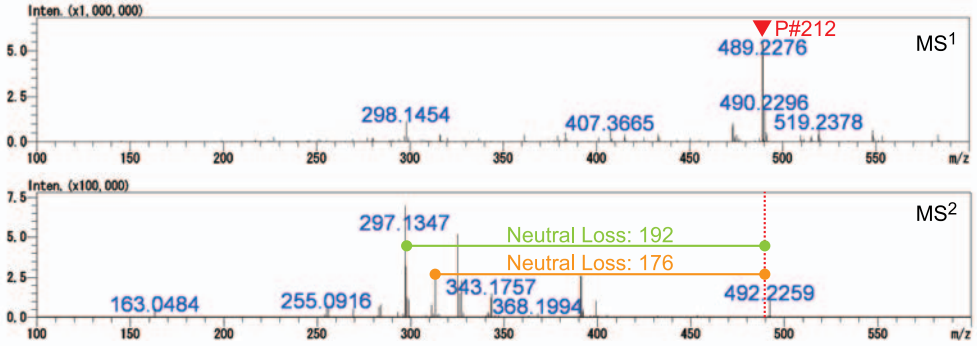

Fig. 6: Mass Spectra of P#212

The measured value of the P#212 precursor ion is m/z 489.2276, and this value corresponds well with the values of the vardenafil and homosildenafil protonated molecules, known to be sildenafils (theoretical value of $C_{23}H_{32}N_6O_4S$: $[M+H]^+$ is 489.2279). Checking P#212 in the table of Fig. 5, the fragmentation ions (indicated as F) have common elements with the sildenafils m/z 297, 311, and neutral loss (indicated as NL) have common elements with the sildenafils m/z 98, 164, 176, and 192, but among these, the existence of NL176 and NL192 becomes a critical factor in predicting the structure of P#212.

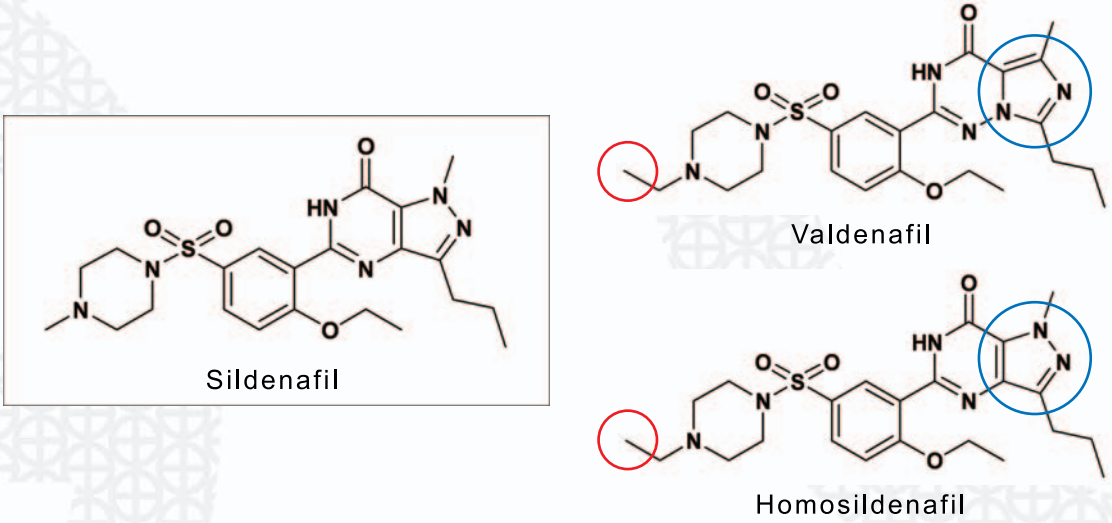

Fig. 7: Structural Formulas of Sildenafil, Vardenafil and Homosildenafil

As shown in Fig. 7, the three compounds resemble each other. The major difference between vardenafil and homosildenafil with respect to sildenafil is that CH_2 has been added to positions encircled in (red) ◯. The differences between vardenafil and homosildenafil are indicated by (blue) ◯.

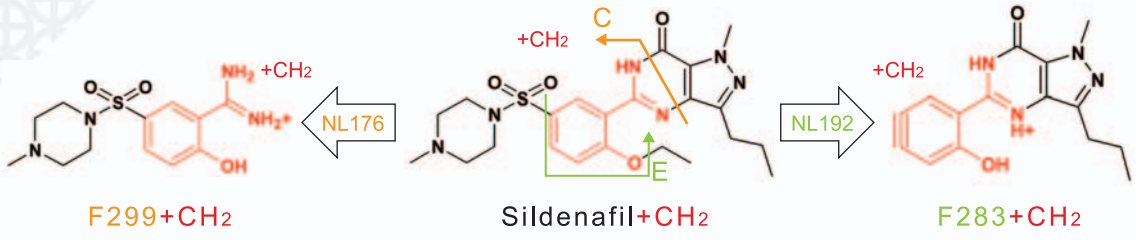

Fig. 8: Structure Prediction of P#212

NL 176 and NL 192 correlate with the neutral losses of F299 and F283, respectively (corresponding to Fig. 4 C and E). That is, it can be judged that the positions where CH_2 was added in P#212 are parts (displayed in red in structural formulas of Fig.8) where F299 overlaps with F283. In other words, P#212 is seen to differ from vardenafil and homosildenafil in the positions of their methyl groups. Neither of the structures of the parts enclosed in a (blue) ◯ in Fig.7 can be determined from this result, but P#212 is clearly neither vardenafil nor homosildenafil, and it is possible to narrow the positions where CH_2 was added.

4. Conclusion

Using the PLS analysis function with the MS^n information provided by MetID Solution enabled efficient extraction of substances with structures in common with the principal ingredient. Moreover, as it is possible to quickly call up all cleavage information indicating commonalities with the principal ingredient, the considerable work associated with the subsequent structural analysis was greatly reduced. MetID Solution, as demonstrated with this example, is a software application that is applicable not only to drug metabolite searches, but can also be effectively applied in a wide range of applications, including impurity analysis.

First Edition: January, 2008

Founded in 1875, Shimadzu Corporation, a leader in the development of advanced technologies, has a distinguished history of innovation built on the foundation of contributing to society through science and technology. We maintain a global network of sales, service, technical support and applications centers on six continents, and have established long-term relationships with a host of highly trained distributors located in over 100 countries. For information about Shimadzu, and to contact your local office, please visit our Web site at **www.shimadzu.com**

SHIMADZU

SHIMADZU CORPORATION. International Marketing Division
3. Kanda-Nishikicho 1-chome, Chiyoda-ku, Tokyo 101-8448, Japan
Phone: 81(3)3219-5641 Fax. 81(3)3219-5710
URL http://www.shimadzu.com

Printed in Japan 3295-02809-30ANS