Access to **your** success

⊕ **SHIMADZU**

Introduction to MetID Solution Software for Structural Elucidation of Expected and Unexpected Metabolites

Technical Report vol.11

1. Introduction

Identification of metabolites is an important step in the process of drug development. Mass spectrometry (LC/MS) is routinely used for metabolite identification and structural analysis. Typically, the MS^1 measurement of the control sample and actual sample are compared, and the results of metabolite ion masses (m/z) detected in the actual sample are compared to the m/z of expected metabolites. Metabolite identification generally involves sample analysis of urine and blood matrices which are often complex and include many substances. Accordingly, extremely large numbers of peaks are generated. If the m/z detected in the actual sample is very close to the m/z of the expected metabolite, it becomes difficult to distinguish one from the other. Even time-of-flight or Fourier transform mass spectrometers, though capable of accurate mass analysis, can still produce false positives or fail to differentiate among some structurally similar metabolite candidates and the parent compound.

This report introduces the software "MetID Solution", developed to automatically extract and structurally analyze metabolite candidates through comparison of not only MS^1 but MS/MS and MS^n spectral results, such as can be generated by an LCMS-IT-TOF ion trap, time-of-flight mass spectrometer.

2. Metabolite Search from MS^1 Analysis Results

Fig. 1 shows a pattern diagram depicting the metabolite search method (chromatogram comparison) employed using the results of MS^1 analysis.
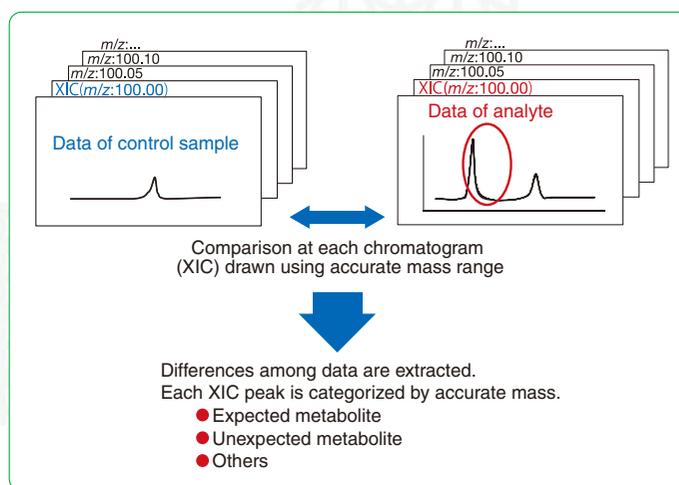
Fig. 1: Metabolite Candidate Extraction by Comparison of MS^1 Chromatograms

As shown in Fig. 1, comparison of chromatograms involves a control sample and an actual sample. The Extracted Ion Chromatograms (XIC) as generated using a high resolution mass spectrometer are compared.

Although chromatogram comparison is a useful means of searching for metabolites, these results obtained from observation of MS^1 alone cannot reliably determine whether or not a substance is actually a metabolite. For this, additional mass spectrometry data is required.

3. Including Structural Information for Comparison

One method for determining whether or not a substance is a metabolite is to compare the MS/MS spectrum of each substance in the actual sample with that of the parent drug. Basically, if a parent compound is metabolized, there is a strong possibility that metabolites will maintain partial structural integrity with respect to the original compound. Thus, commonalities (fragment ions or neutral loss) would be expected in the results of MS/MS measurement of the parent compound and its metabolites.

With the LCMS-IT-TOF, which is capable of high-speed MS/MS analysis, a large number of MS/MS spectra can be obtained from a single analysis of an actual sample.
MetID Solution software automatically finds the precursor ions in the MS/MS spectra having commonalities with the parent compound, and generates a table of common fragment ions and neutral losses. This process is depicted in the pattern diagram below.

Large number of MS/MS spectra obtained

Parent compound MS/MS spectrum

Statistical processing of MS/MS spectra

MS/MS spectra obtained from analyte

Probability of metabolites in case of commonalty in MS/MS spectra

Parent compound MS/MS spectrum and respective MS/MS spectra of analyte are subjected to partial least squares (PLS).

MS/MS spectra having commonalities with parent compound MS/MS spectrum are quickly extracted.

Fig. 2: Metabolite Candidate Extraction by Statistical Processing of MS/MS Spectra

4. MetID Solution Features

MetID Solution software is a tool that utilizes a dual approach to improve the efficiency and reliability of metabolite identification.

	Chromatogram Comparison	MS/MS Spectral Comparison
Data used	MS^1	MS/MS (up to MS^n)
Method	Differences are extracted by comparing control sample and actual sample chromatograms (XIC).	MS/MS spectra having commonalities with referenced parent compound MS/MS spectrum are extracted.
Features	Conceptually easy. Possible with any mass spectrometer.	Fast, highly reliable.
Advantages	Possible with any mass spectrometer. Applicable for non-targeted search (no automated reference).	Control sample is not required. Uses PLS statistics to help find metabolites quickly. High robustness with respect to analytical conditions.
Shortcoming	Depends greatly on data repeatability. It takes time to accurately compare the XIC for all mass regions. Requires control samples	

5-1. Measurement Example

A metabolite sample of verapamil ($C_{27}H_{38}N_2O_4$: MW 454.283), a compound in which the principle metabolites are known, was measured by LCMS-IT-TOF, and data processing was conducting using MetID Solution.
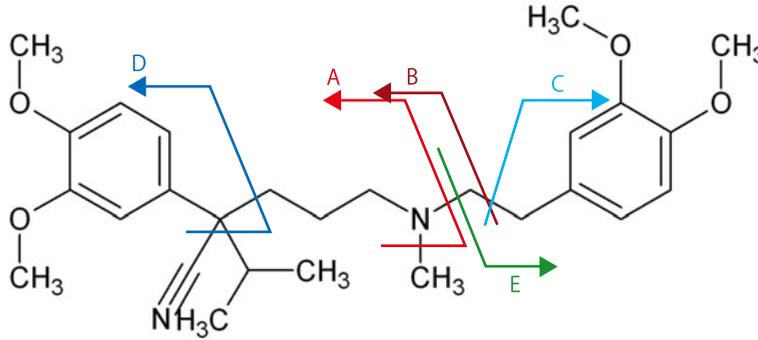
Fig. 3: Verapamil Structural Formula — A to E indicate cleavage sites.
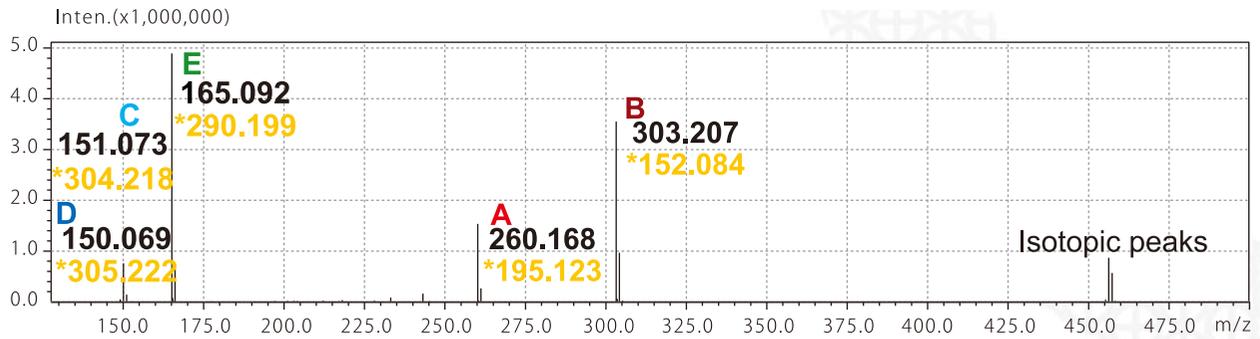
Fig. 4: Verapamil MS/MS Spectrum — * indicates neutral loss.

5-2. Determination of Metabolites by Chromatogram Comparison

Peak absent in control sample XIC is present in actual sample XIC.

Possibility of metabolite

XIC (control sample)

XIC (actual sample)

Transformation list

MS spectrum

Peak table of expected metabolites

Peak table of unexpected metabolites

Peak table of others
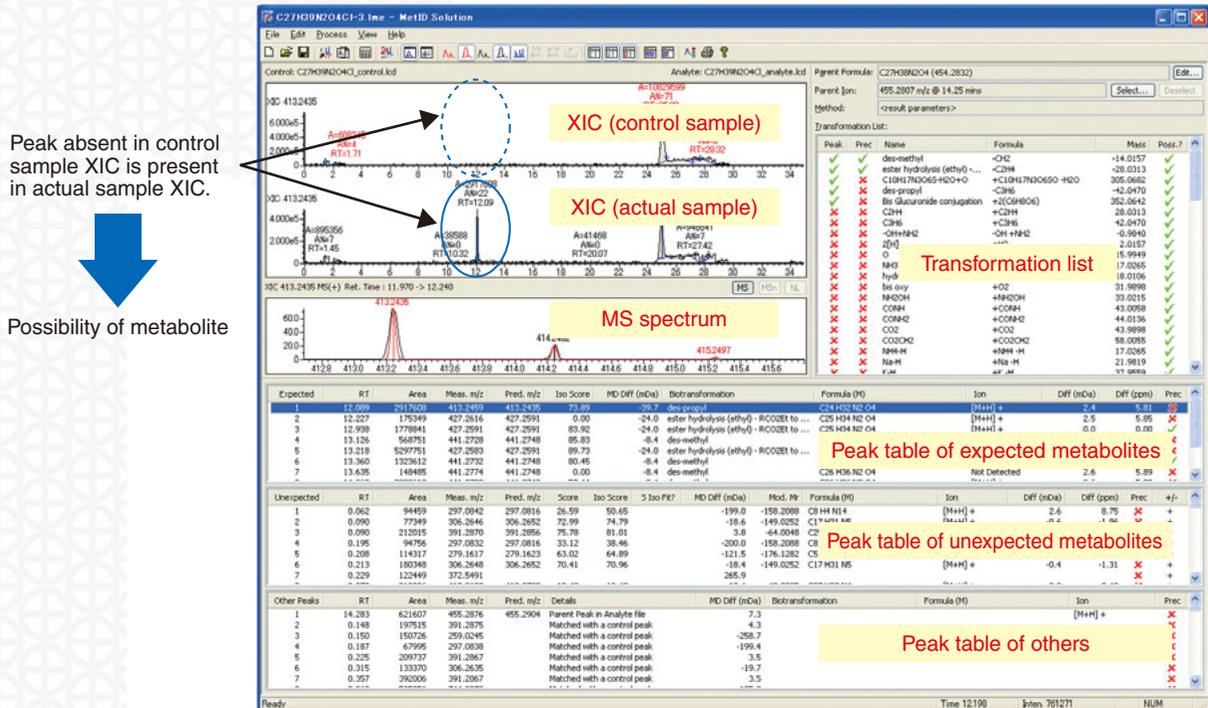
Fig. 5: Chromatogram Comparison Results Window

5-3. Determination of Metabolites by MS/MS Spectral Comparison

Points of commonality between the parent compound MS/MS spectrum and the precursor ion in the analyte are indicated with positive values in the horizontal axis direction.
In this example, four types of metabolites were detected.
In addition, fragment ions in common and neutral loss information are automatically summarized in the table.
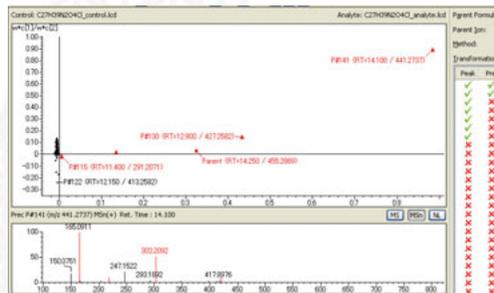
Fig. 6: MS/MS Spectral Comparison Results Window (partial)

5-4. Metabolite Structure Prediction by MS/MS Spectral Comparison

Looking at P#141 (m/z 441.2737, RT 14.100) as the most relevant ion indicated in the horizontal axis, the mass difference (-14.0151 Da) with respect to the parent compound suggests that it corresponds to the neutral loss of CH_2 (14.0157), or in other words, that demethylation occurred. In addition, signals associated with the

B neutral loss and the fragment ions corresponding to D and E shown in Fig. 3 were also found in the MS/MS spectrum of P#141. This result suggests that the structures of the B neutral loss part and those of D and E are consistent with the overall structure of the original compound and P#141.

Demethylation probably occurred to one of these methyl groups.
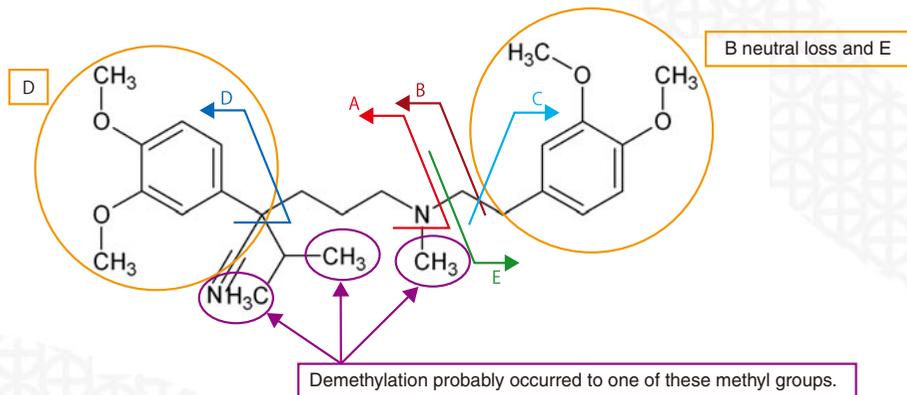
Fig. 7: Predicted Structure for P#141

One of the principle metabolites of verapamil is norverapamil ($C_{26}H_{36}N_2O_4$: MW 440.267). This structure does not contradict the information obtained from the MS/MS spectrum of P#141.

Reference: S. Yamaguchi et al., J.Mass Spectrom. Soc. Jpn., 55, 83(2007) et al
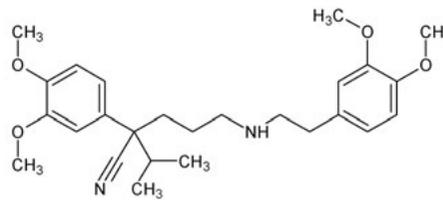
Fig. 8: Structural Formula of Norverapamil