PSB 114

Isotope Pattern Recognition Using Metabolite ID 2.0 Software

LC/MS has become a widely utilized technique in the drug discovery process, especially for metabolite identification. Many drug compounds contain chlorine or bromine, which produce a distinctive mass spectral isotope pattern corresponding to their natural isotopic abundance. Metabolite ID 2.0 software takes advantage of this diagnostic isotope pattern to identify potential metabolites. By searching for compounds that show the same isotope pattern as the parent compound, metabolites can be quickly associated with the parent drug. To demonstrate the capabilities of isotope pattern searching, in-vitro metabolism of diazepam was studied using LC/MS in conjunction with Metabolite ID 2.0 software.

In vitro metabolites of diazepam were generated by incubation of the drug with rat liver microsomes. The structures of diazepam and its major metabolites, temazepam and *n*-desmethyldiazepam, are shown in Figure 1. The mixture was analyzed using a Thermo Scientific Surveyor™ MS Pump Plus, Autosampler Plus and LCQ™ Deca XP Plus ion trap mass spectrometer. A Thermo Scientific AQUASIL C18 column was used with the LC conditions listed in Table 1. Extracted ion chromatograms and full-scan MS spectra of diazepam and its two major metabolites are shown in Figure 2. The molecular ion of each compound shows a +2 amu peak with about 30% intensity, which corresponds to the ^{37}Cl isotope contribution for each compound. Using the isotope pattern search feature in Metabolite ID 2.0, potential metabolism products are readily identified based on their isotope pattern. The Metabolite ID 2.0 isotope search results for diazepam are shown in Figure 3.

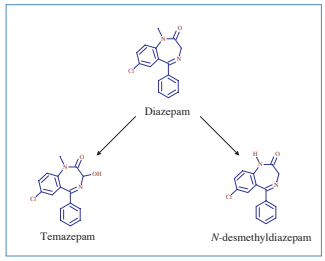

Figure 1

Table 1:
LC Experimental Conditions

Mobile Phase A: H_2O with 0.1% formic acid
Mobile Phase B: ACN with 0.1% formic acid
Flow rate: 400 µL/min

Time (min)	%A	%B
0.00	80	20
1.00	80	20
8.0	20	80
10.0	20	80
10.5	80	20
15	80	20

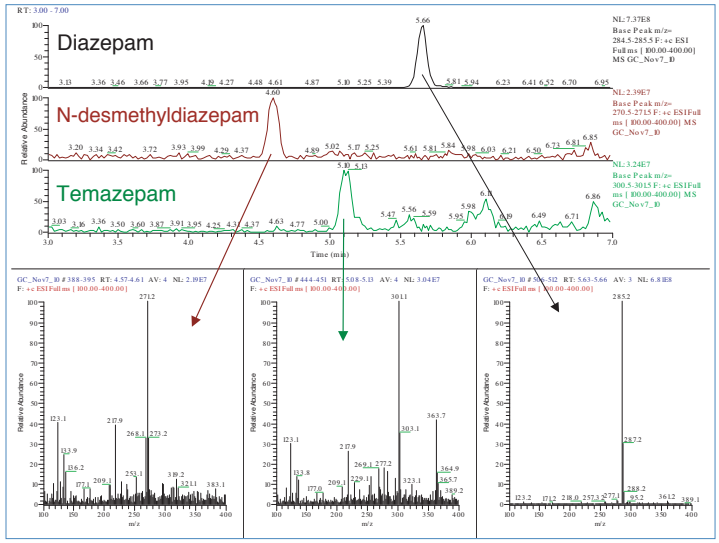

Figure 2: Extracted ion chromatograms and full-scan MS spectra of diazepam and its two major metabolites.

Isotope Pattern Recognition Using Metabolite ID 2.0 Software

The isotopic pattern of diazepam and its metabolites are presented in Figure 4. As can be seen from Figures 3 and 4, Metabolite ID used the ^{35}Cl and ^{37}Cl isotope pattern to detect diazepam and each of its metabolites. The results demonstrate that Metabolite ID 2.0 software can utilize characteristic isotope patterns to identify potential metabolites from an LC/MS analysis.

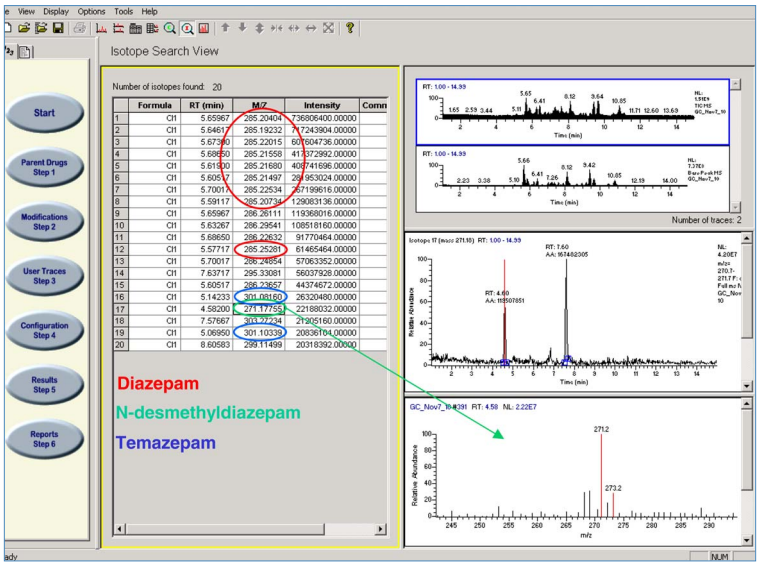

Figure 3: Isotope search results for diazepam.

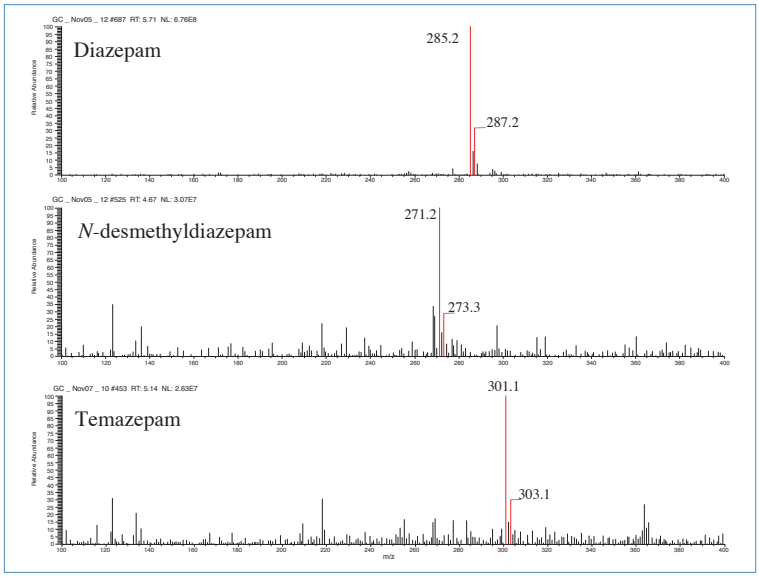

Figure 4: Isotopic pattern of diazepam and its metabolites.

Authored by: Gargi Choudhary and Tania A.Sasaki, Thermo Fisher Scientific