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Application Data Sheet

No.45

LC-MS
Liquid Chromatograph Mass Spectrometer

Identification of Benzodiazepines and Their Metabolites Using Synchronized Survey Scan®

This report describes the analysis of benzodiazepines and their metabolites using ultra-high speed triple quadrupole mass spectrometry (LCMS-8030).

The LCMS-8030 has a Synchronized Survey Scan function that automatically performs an MS/MS scan when a desired threshold level is exceeded, thereby allowing for combined MRM and MRM-dependent product ion scans in a single analysis. The speed of the LCMS-8030 makes it possible to perform MRM for compounds at high sensitivity simultaneously with performing a product ion scan, and obtain an MS/MS spectra for each compound. Since the collision energy (CE) can be configured for each product scan, it is possible to choose an optimum CE for each compound.

This report shows the MRM mass chromatograms and library search results based on MS/MS spectra data for Etizolam, Triazolam, and their metabolites (alpha-Hydroxyetizolam, 8-Ethylhydroxyetizolam, alpha-Hydroxytriazolam and 4-Hydroxytriazolam).

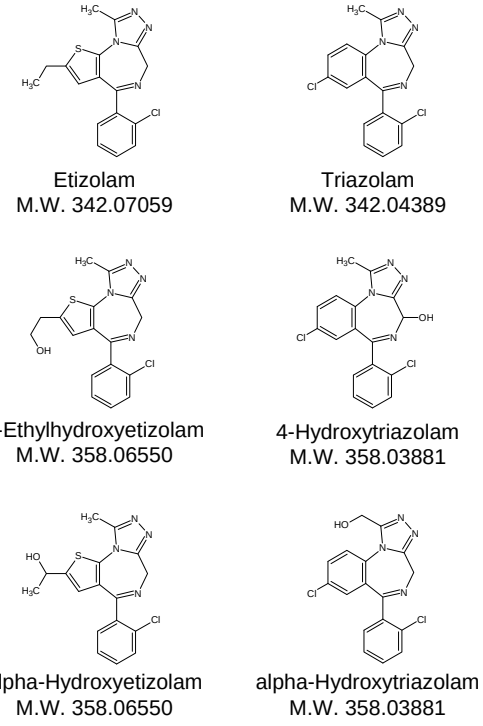

Figure 1: Structure of Etizolam, Triazolam and their metabolites

Table 1: Analytical Conditions

HPLC conditions (Prominence UFLC XR)

Column	:	YMC Triart C18, 1.9um, 12nm (2mmI.D. x 150mmL)
Mobile phase A	:	10mM Formic acid in water
Mobile phase B	:	10mM Formic acid in water / Acetonitrile (50/50)
Flow rate	:	0.3mL/min
Time program	:	B conc. 40%(0 min)-65%(40 min)-40%(40.01-60min)
Injection volume	:	1uL
Column temperature	:	40℃

MS conditions (LCMS-8030)

Ionization	:	ESI
Polarity	:	Positive
Measurement mode	:	MRM

Compounds	Quantitative	CE	Qualitative	CE
Etizolam	343.05>314.10	-28	343.05>138.15	-37
Triazolam	343.05>308.20	-24	343.05>315.00	-27
8-Hydroxyetizolam (M-III)	359.05>305.05	-24	359.05>315.25	-20
alpha-Hydroxyetizolam (M-VI)	359.05>286.20	-28	359.05>287.20	-27
alpha-Hydroxytriazolam	359.05>176.20	-27	359.05>341.15	-18
4-Hydroxytriazolam	359.05>341.10	-22	359.05>111.20	-39

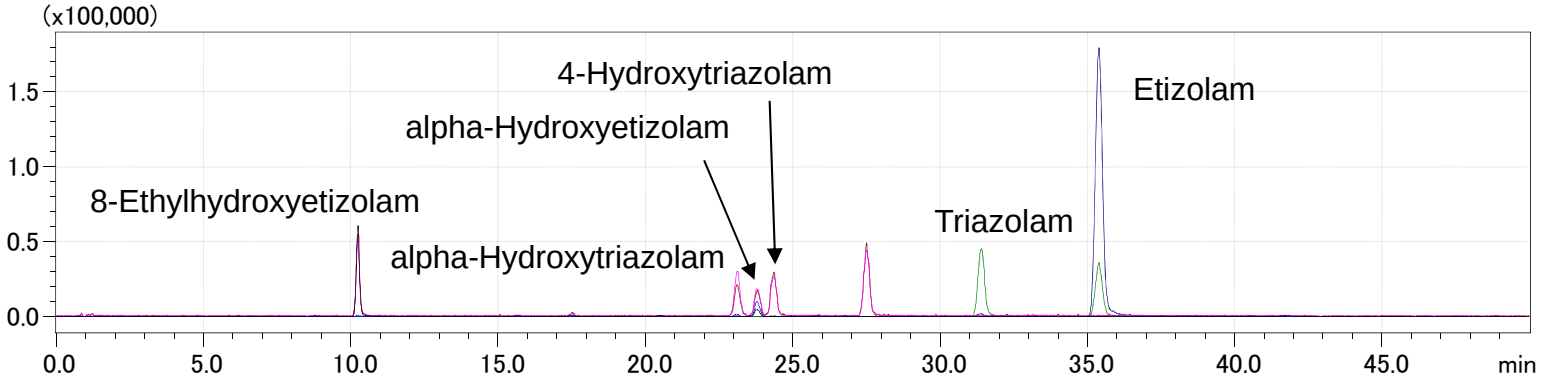

Figure 2: MRM chromatograms of Etizolam, Triazolam and their metabolites

Figure 2 shows the user Interface of the Library Search Parameter Setting of LabSolutions Software. One can obtain accurate results using precursor *m/z* and specific polarity in addition to information from MS/MS spectra (*m/z* and intensity).

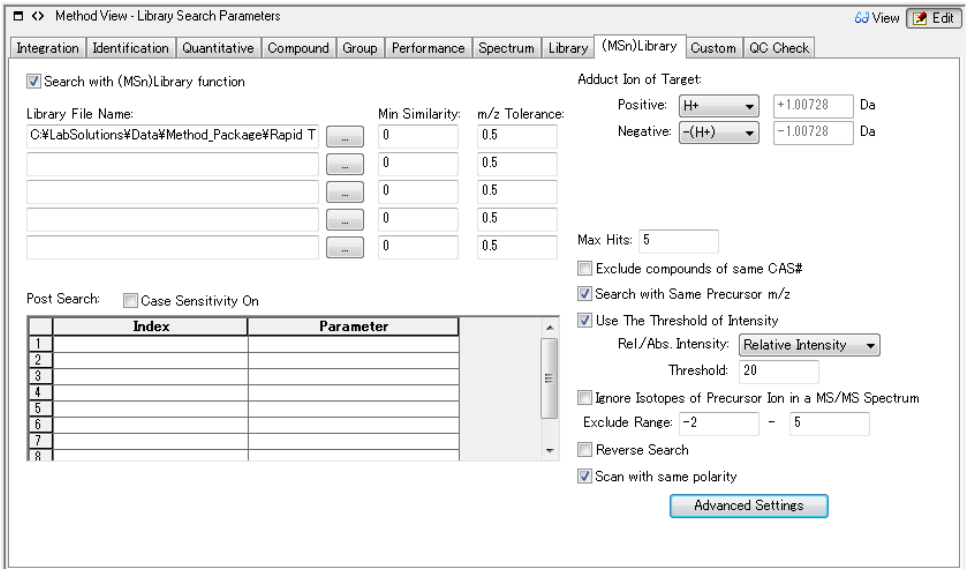

Figure 2: User Interface of Library Search Parameter Setting of LabSolutions Software

Using precursor *m/z* and polarity (positive or negative) is important for a library search because it is possible for inaccurate information to be displayed without these being specified (Figure 3 bottom).

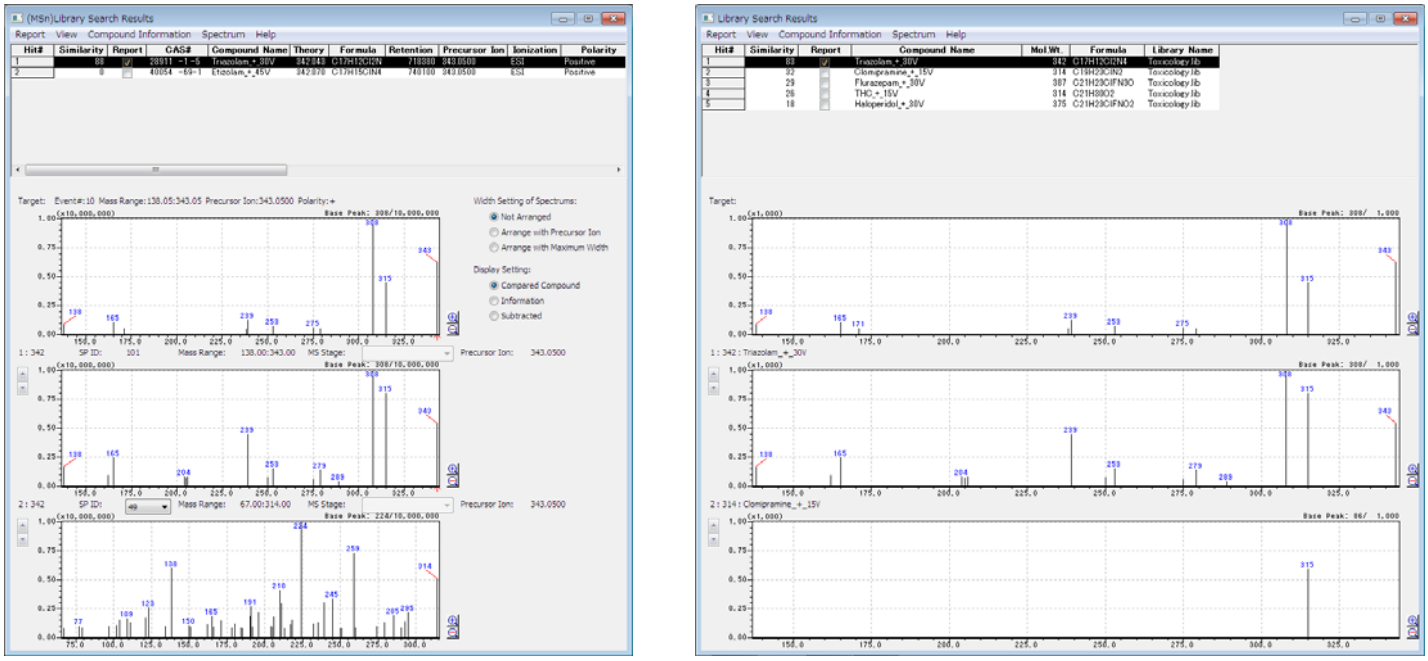

Figure 3: Library Search Results (upper : parameter ON, bottom : parameter OFF)

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