Utilizing msFineAnalysis AI to Verify an Organic Synthetic Compound Structure Based on the Data Acquired by Gas Chromatography-High Resolution Mass Spectrometry and Nuclear Magnetic Resonance - Direct MS Probe Measurement -

Product: Mass spectrometer (MS), nuclear magnetic resonance system (NM)

General

Gas chromatography-high resolution mass spectrometry (GC-HRMS) combined with nuclear magnetic resonance spectroscopy (NMR) is widely used for identifying chemical compositions and structures of organic synthetic compounds.

If a target sample is a high boiling point compound, which does not pass through GC, direct MS measurement is more suitable than GC-MS. The JMS-T2000GC, a GC-HRMS system, supports three different direct MS probes: 1) DIP (Direct Insert Probe) to heat a gas capillary containing a sample; 2) DEP (Direct Exposure Probe) with a filament; and 3) FDP (Field Desorption Probe) designed for field desorption, a soft ionization method. msFineAnalysis AI, designed to predict chemical compositions and formula, is effective in analyzing results acquired by direct MS as well as GC-MS, to determine desired NMR measurement methods and analyze the resulting NMR data.

JEOL's ROYALPROBE™ HFX, an accessory for the JNM-ECZ600R NMR system, is capable of tuning on the HF side (^{1}H,^{19}F) for single and double resonance, allowing the operator to select one desired for the experiment. Combined with the LF side (^{13}C, etc.), the probe supports simultaneous tuning for triple resonance, rendering itself as a powerful tool to determine chemical structures of organic synthetic compounds made of C, H, and F.

In this work, we examined a high boiling point compound in a JMS-T2000GC using a DEP, EI (DEI: Desorption Electron Ionization), FD (Field Desorption), and msFineAnalysis AI, and analyzed the resulting data in a JNM-ECZ600R with ROYALPROBE™ HFX to determine its chemical composition and structure.

Measurement

Ciprofloxacin, having a high boiling point of 581.8°C, was used as a sample. The sample was prepared into a 1mg/mL methanol solution for MS analysis. For NMR analysis, 10 mg of the sample was dissolved in 0.6 mL of a 50:1 solution of deuterated water and acetic acid-d4. Table 1 shows the HRMS and NMR measurement conditions.

Table 1. Measurement and analysis conditions

HRMS	JMS-T2000GC (JEOL Ltd.)
Ionization	DEI+: 70eV, 300μA FD+: -10kV
DEP current program	0A →5.12A/min →1A
FD emitter current program	0mA →51.2mA/min →40mA
Monitor ion range	*m/z* 10-800
Analysis software	msFineAnalysis AI (JEOL Ltd.)
NMR	**JNM-ECZ 600R (JEOL Ltd.)**
Proton observed frequency Probe	600 MHz ROYALPROBE™ HFX (JEOL Ltd.)
Method	^{1}H, ^{19}F, $^{13}C\{^{1}H,^{19}F\}$, ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC
Analysis software	NMR software Delta 5.3.3 (JEOL Ltd.)

Measured Results

Figure 1 shows the results acquired by msFineAnalysis AI from the DEI and FD mass spectra. The data indicated ciprofluoxetine was the top candidate. The chemical composition of the molecular ion in the FD mass spectrum was estimated to be $C_{17}H_{18}FN_3O_3$. The isotope pattern of the molecular ion was in good agreement with that of estimated $C_{17}H_{18}FN_3O_3$. The chemical composition of the EI fragment ion was also consistent to $C_{17}H_{18}FN_3O_3$. Moreover, NIST data base search identified ciprofluoxetine as having the highest degree of similarity.

Next, we used NMR to verify the MS results. Based on the data acquired by msFineAnalysis, we determined the nuclei and methods for NMR measurement.

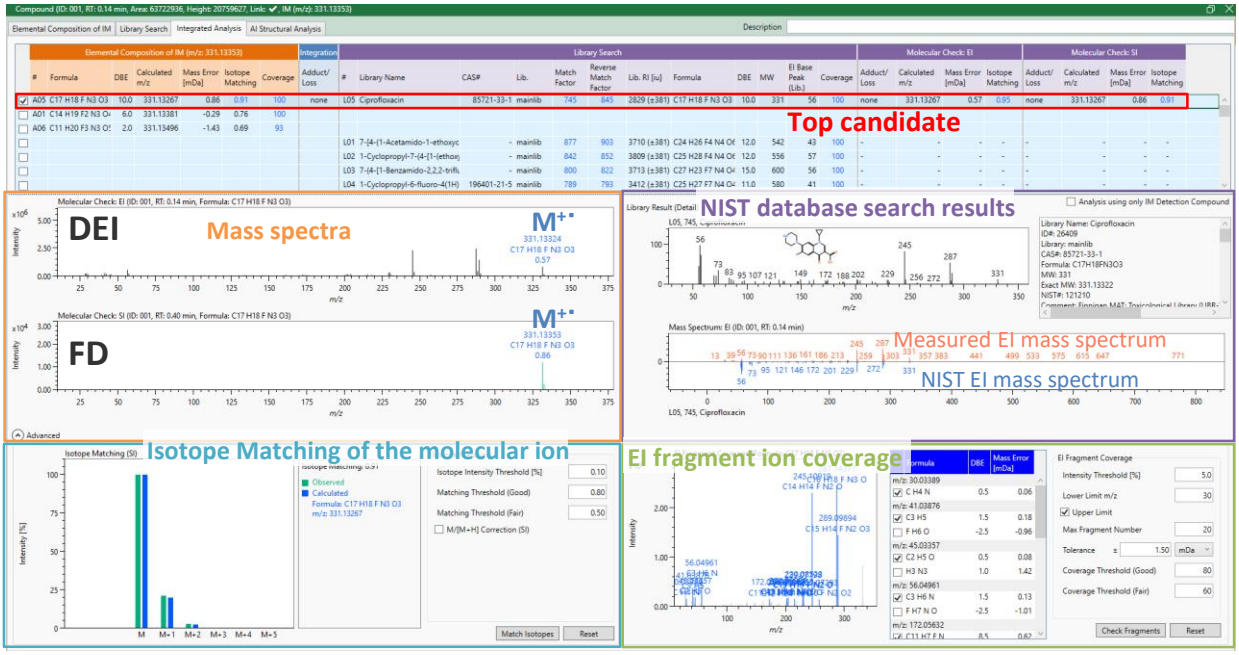

Figure 1. msFineAnalysis AI results

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Based on the data acquired by msFineAnalysis AI, we selected ^{19}F, ^{1}H, and ^{13}C NMR measurement . Specifically, we conducted one dimensional ^{19}F, ^{1}H, and $^{13}C\{^{1}H,^{19}F\}$, two dimensional HSQC (Heteronuclear Single Quantum Coherence), and HMBC (Heteronuclear Multiple Bond Correlation).

Figures 2 and 3 show the NMR spectra acquired. The figures show important identifications in red, which were determined by the synthesis route of ciprofluoxetine described in Reference 1. The figures suggest the chemical structure of ciprofluoxetine including other NMR signals.

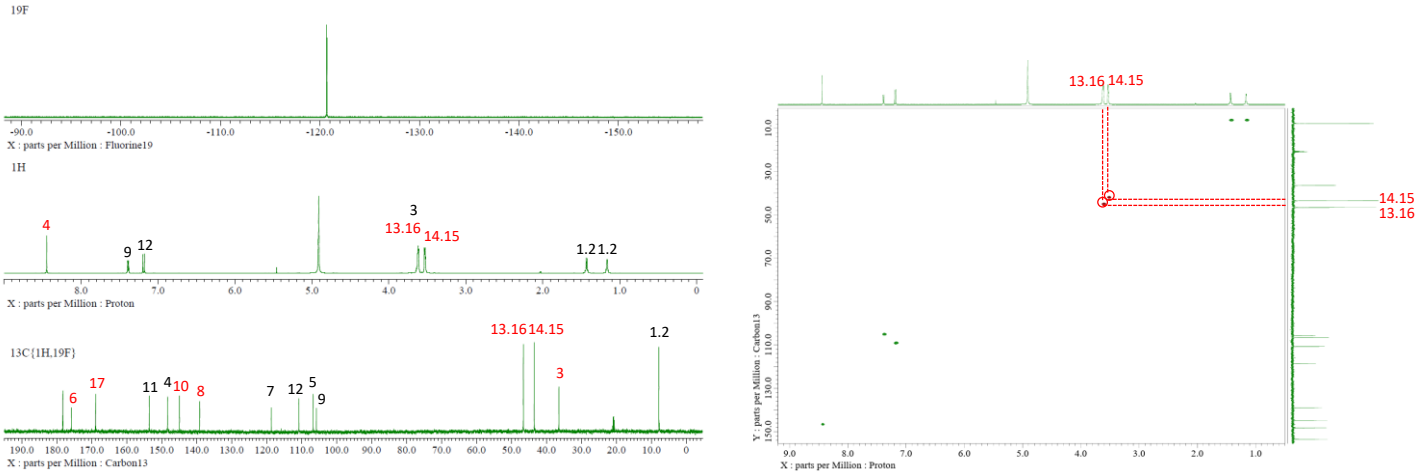
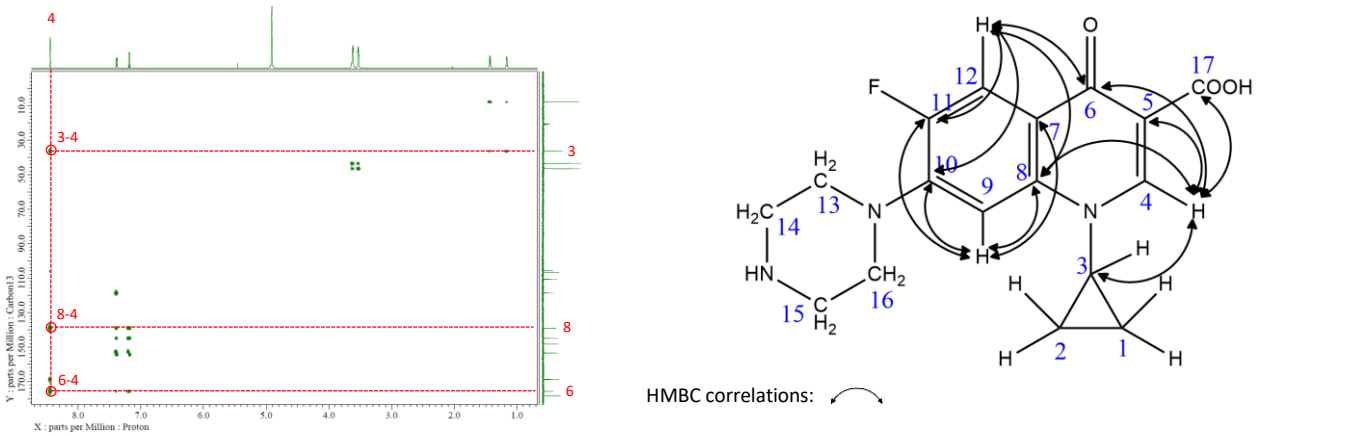


Figure 2. NMR spectra
Left: ^{19}F spectrum (top), ^{1}H spectrum (middle), $^{13}C\{^{1}H, ^{19}F\}$ (bottom)
Right: HSQC



Position	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
^{1}H / ppm	1.43/1.16	1.43/1.16	3.63	8.45					7.39			7.19	3.62	3.53	3.53	3.62	—
^{13}C / ppm	7.9	7.9	36.5	148.3	106.7	175.8	118.7	139.1	105.8	144.9	153.5	110.8	46.6	43.5	43.5	46.6	168.9
J_{F-C}/ Hz							9.2			10.7	253	23.8	4.4				

Figure 3. Chemical sift table, HMBC spectrum and HMBC correlations on the chemical structure

Summary

The HRMS data acquired from DEI and FD estimated the chemical composition and structure of the compound. The NMR experiment, based on the chemical composition estimated by HRMS, determined the skeletal structure of the compound and the location of its functional groups. msFIneAnalysis AI correctly estimated the sample to be ciprofluoxetine from the chemical structure, which was verified by the NMR results.

The JMS-T2000GC + msFineAnalysis AI combined with the JNM-ECZ600R + ROYALPROBE™ HFX, which was introduced here to identify the chemical structure of an organic synthetic compound, is expected to have a wide range of applications for organic synthetic compounds at all stages of their synthetic process.

References

1) N. Perrer Tosso, Bimbisar K. Desai, Eliseu De Oliveira, Juekun Wen, John Tomlin, and B. Frank Gupton, J. Org. Chem. 2019, 84, 3370−3376.
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