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 - EI/FI 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.

The JMS-T2000GC, an GC-HRMS system which supports both electron ionization (EI) and field ionization (FI), incorporates a common ion source capable of switching between the EI and FI modes while keeping the system in a vacuum. msFineAnalysis AI is designed to analyze the data acquired by the 2 ionization methods, and estimate chemical structures as well as compositions, which helps determine NMR measurement methods and examine the resulting NMR data. GC separation can also verify if a sample is made of a single component or a mixture of multiple components. JEOL's ROYAL HFX PROBE™, an accessory for the JNM-ECZ600 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 the chemical composition and structure of an organic synthetic compound using a JMS-T2000GC with msFineAnalysis AI and JNM-ECZ600 with ROYALPROBE™ HFX.

Measurement

A commercial fluoxetine was used as a sample. An analyte, a 1mg/mL solution in methanol, was prepared for MS analysis. Another analyte, a 10mg/0.6mL solution in deuterated DMSO, was prepared for NMR analysis. Table 1 shows the GC-HRMS and NMR measurement conditions.

Table 1. Measurement conditions

GC-HRMS	JMS-T2000GC (JEOL Ltd.)
GC inlet mode	Split 50:1
GC inlet temperature	280 °C
GC Column	ZB-5MS, 30m x 0.25mm, 0.25μm (Phenomenex. Inc.)
GC Oven	50°C (1min) →20°C/min →300°C (5min)
Carrier gas	He, 1.0mL/min
MS Ionization	EI+: 70eV, 300μA
	FI+: -10 kV, 40 mA
MS Monitor ion range	*m/z* 10-800
Analysis software	msFineAnalysis AI (JEOL Ltd.)

NMR	JNM-ECZ 600 (JEOL Ltd.)
Proton observed frequency	600 MHz
Probe	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.)

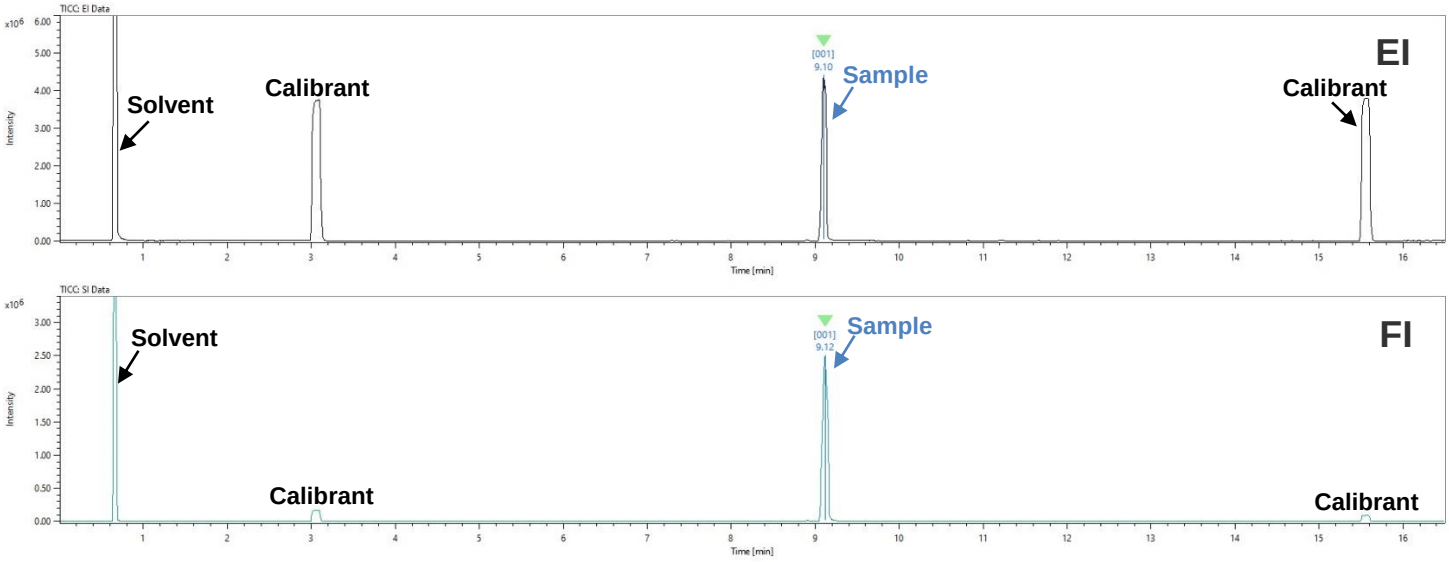

Figure 1. TICCs (top: EI, bottom: FI)

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Results and Discussion

Figure 1 shows the total ion current chromatograms (TICCs) of EI and FI. A sample peak was detected at the same retention time in the EI and FI TICCs respectively. Because no other chromatogram peak was detected, it was verified that the sample was made of a single component. Figure 2 shows the results acquired by msFineAnalysis AI. The data indicated fluoxetine was the top candidate. The chemical composition of the molecular ion in the FI mass spectrum was estimated to be $C_{17}H_{18}F_3NO$. The isotope pattern of the molecular ion was in good agreement with that of estimated $C_{17}H_{18}F_3NO$. The chemical composition of the EI fragment ions were also consistent to $C_{17}H_{18}F_3NO$. Moreover, NIST database search identified fluoxetine as having the highest degree of similarity.

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

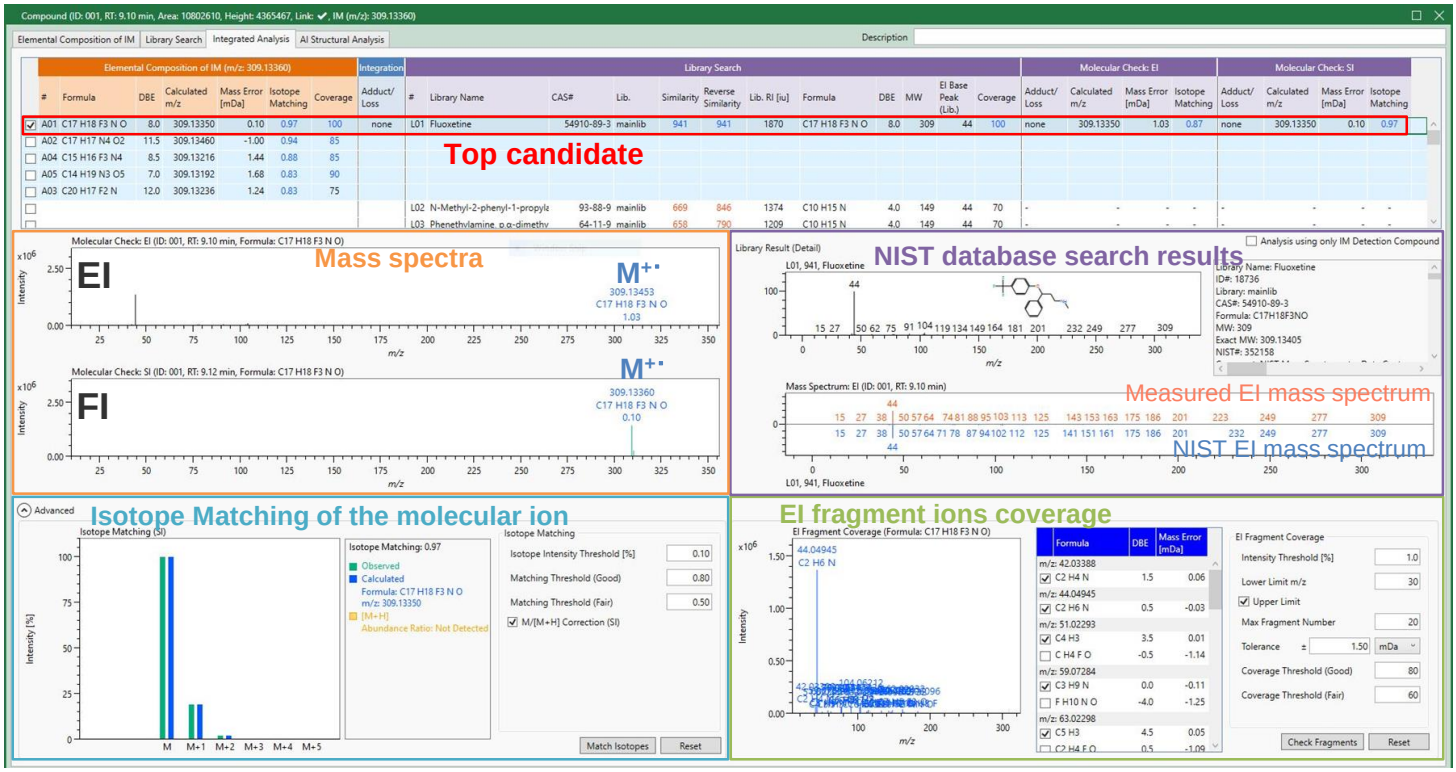

Figure 2. msFineAnalysis AI results

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).

Fig. 3 shows the NMR spectra acquired. The figure shows important identifications in red, which were determined by the synthesis route of typical fluoxetine described in Reference 1. The figure also suggested the chemical structure of fluoxetine shown in Figure 4 including other NMR signals.

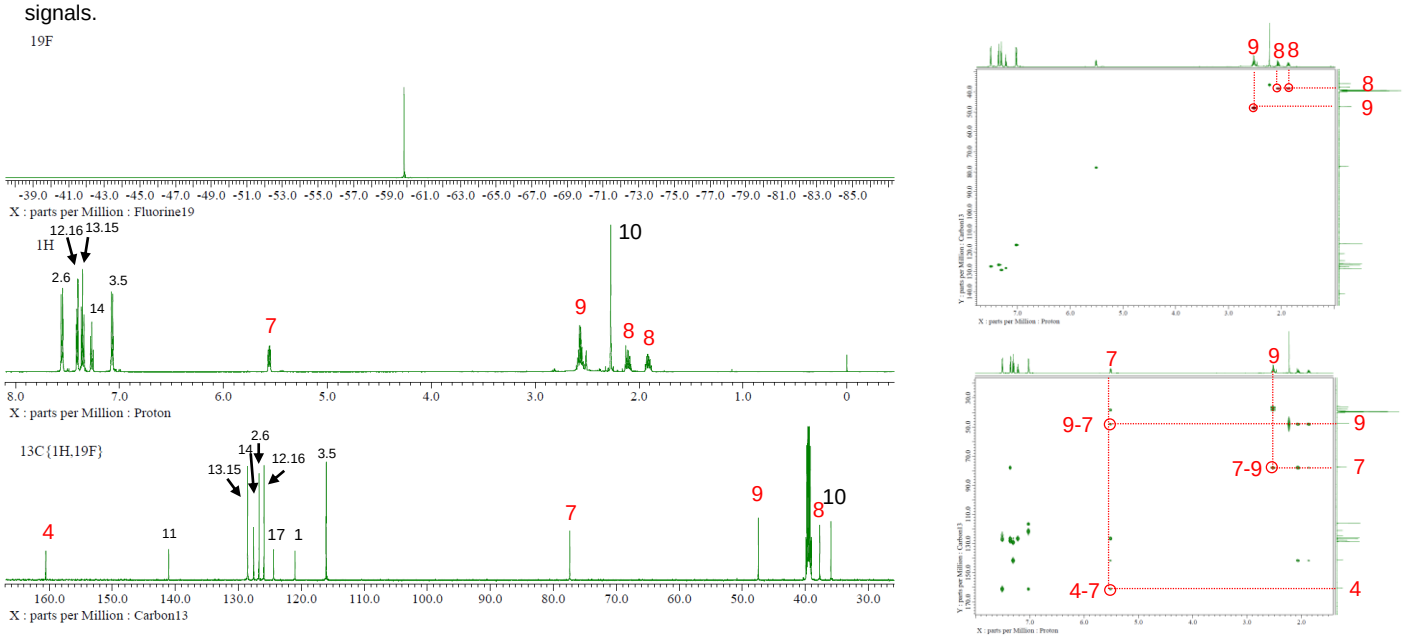

Figure 3. NMR spectra
Left: ^{19}F spectrum (top), ^{1}H spectrum (middle), $^{13}C\{^{1}H, ^{19}F\}$ (bottom)
Right: HSQC (top), HMBC (bottom)

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Position	^{13}C / ppm	^{1}H / ppm	$J_{F\text{-}C}$ / Hz
1	121.6		35
2	127.3	7.55	6
3	116.6	7.06	
4	161.1		
5	116.6	7.06	
6	127.3	7.55	6
7	77.9	5.55	
8	38.3	1.91/2.10	
9	48.1	2.56	
10	36.6	2.27	
11	141.6		
12	126.5	7.41	
13	129.1	7.35	
14	128.1	7.27	
15	129.1	7.35	
16	126.5	7.41	
17	125.0		271

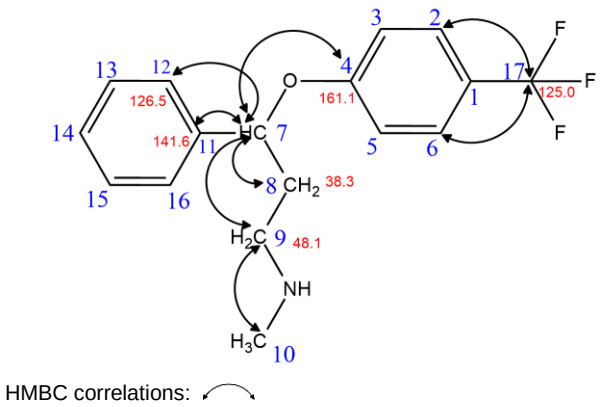

Figure 4. Chemical shift table and HMBC correlations on the chemical structure

Summary

Figure 5 shows the information acquired from the measurements using MS and NMR. The GC-HRMS data from EI and FI confirmed that the sample is made of a single compound and estimated the chemical composition and structure of the compound. Next, the NMR experiment, based on the chemical composition estimated by GC-HRMS, acquired the skeletal structure of the compound and the location of its functional groups. msFIneAnalysis AI correctly estimated the sample to be fluoxetine from the chemical structure, which was verified by the NMR results.

The JMS-T2000GC + msFineAnalysis AI combined with the JNM-ECZ600 + ROYALPROBE™ HFX, 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.

1. JMS-T2000GC with EI/FI and msFineAnalysis AI

- Examine if the sample consists of a single component or a mixture of multiple components.
- Estimate the chemical composition from the exact mass of the molecular ion.
- Check the isotope pattern matching and the EI fragment ion pattern matching.
- Examine the double bond equivalent (DBE).
- Predict the chemical structure.

2. JNM-ECZ600 with ROYALPROBE™ HFX

- Measure each detectable element of the estimated chemical formula.
- Narrow down the predictable compounds while assigning NMR spectra.
- Determine the skeletal structure and functional groups.
- Examine the enantiomeric purity.

Figure 5. Measurement and analysis procedures of synthetic compounds

References

1) Cody J. Wenthur, Megan R. Bennett, and Craig W. Lindsley., ACS Chem Neurosci. 2014 Jan 15; 5(1): 14–23. doi: 10.1021/cn400186j

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