Novel GC-MS Ionization Technique to Identify Unknown Compounds

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1. Introduction

Chemical ionization (CI) is commonly used with gas chromatography mass spectrometry (GC-MS) to confirm the molecular weight of compounds. Although EI, electron ionization, is a primary method and generates unique fragmentations which help to identify compounds, this typically causes a loss in molecular weight information. CI compensates this missing point. However, CI requires a compressed flammable gas cylinder, such as methane and isobutane. These kinds of gases are expensive and not allowed in some laboratories due to their safety policy.

To address these situations, Shimadzu has developed new chemical ionization technique using organic solvent which is being called solvent mediated chemical ionization (SMCI). In this study, the mechanism of this new technique and its use in unknown compound data will be presented.

2. Method

2-1. System Optimization

Solvent amount going into the MS was optimized by changing gas pressures and gas controller conditions, while transitions of response and S/N ratio of benzophenone (BZP, 100pg/uL) were monitored.

Methanol (HPLC grade), was sealed in a glass bottle and pressurized inside with nitrogen gas (UPC grade). Vapored methanol in the headspace of the glass bottle was introduced to the mass spectrometer through a gas controller (Figure 1).

The GCMS-TQ8040 NX was used with SH-Rxi-5MS column (30m x 0.25mmI.D., df=0.25µm) and AOC-20i/s (Shimadzu). The dedicated ion source for CI was used.

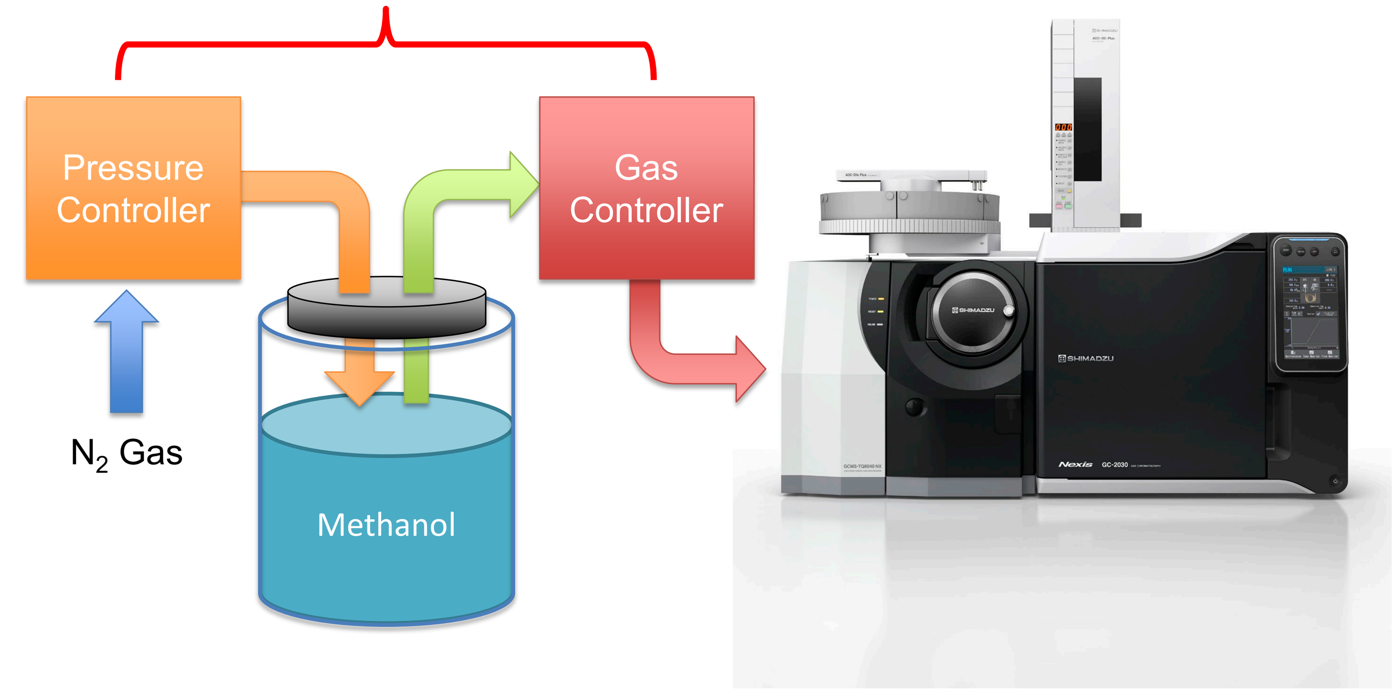

Figure 1 Solvent introduction system and GCMS-TQ8040 NX

2-2. Unknown Compounds Analysis

A drug analysis was performed with the same system configuration as described in 2-1. Ten compounds were selected and obtained from Sigma Aldrich. Human serum was obtained from Golden West Diagnostics and extracted with chloroform/isopropanol (3:1). Then, the extraction was dried down by a gentle flow of nitrogen gas and reconstituted with methanol. Drug standard solutions were spiked at 1µg/mL. Analytical conditions are described below.

[GC]

Injection Temperature:	260℃
Injection Mode:	Splitless (High Press. Injection, 250kPa, 1.5min)
Flow Control:	Constant Linear Velocity (45.6cm/sec)
Oven Temperature:	60℃ (2min), 10℃/min to 320℃ (10min)
Injection Volume:	1µL
Carrier Gas:	Helium

[MS]

Interface Temperature:	280℃
Ion Source Temperature:	200℃
Ionization Mode:	CI (Methane, Isobutane), SMCI (MeOH, N2)
Acquisition Mode:	Scan (*m/z* 80 - 700)
Event Time:	0.3sec (2,500u/sec)

3. Results

3-1. Optimization of Solvent Amount

Optimum solvent amount was evaluated by protonation ratio (M to MH^+) and S/N ratio of benzophenone. Four different conditions on the gas controller and various nitrogen pressures were verified. As a result, the setting in Condition 4 (see Figure 2) showed the most protonation ratio and better S/N ratio. The mass spectrum is shown in Figure 3.

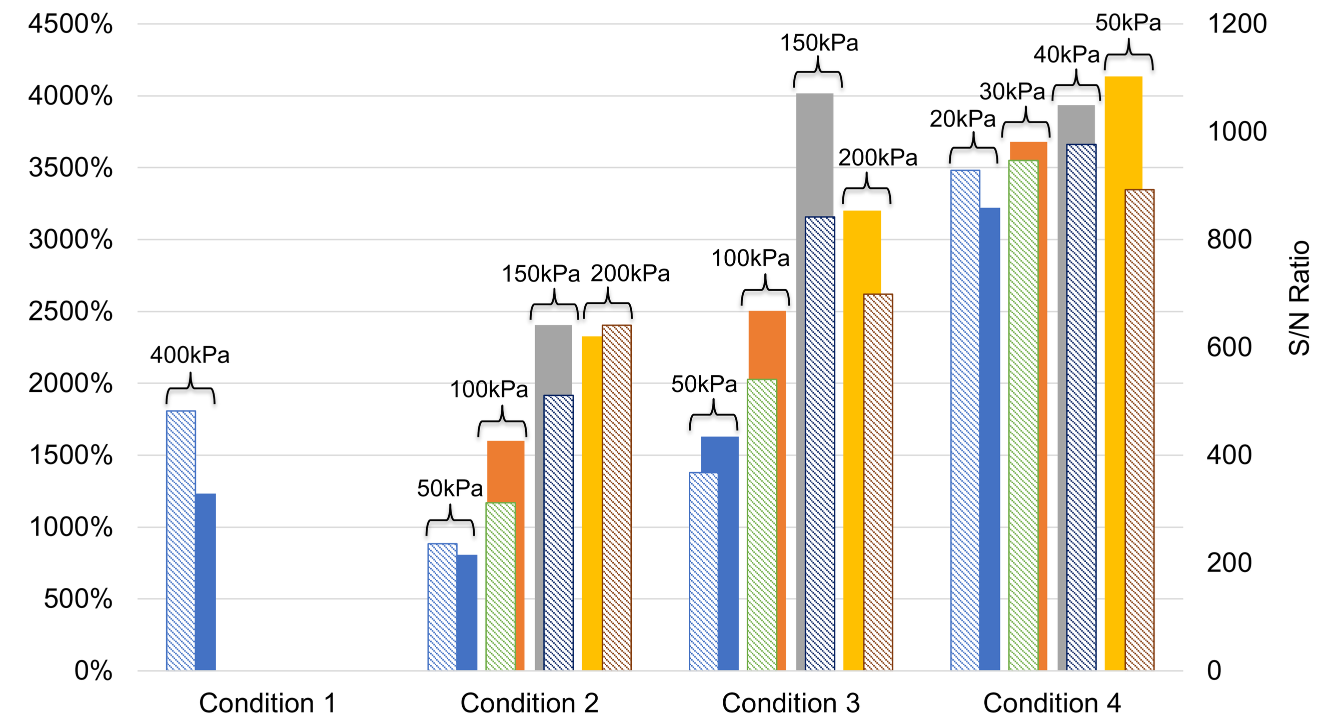

Figure 2 Solvent introduction system and GCMS-TQ8040 NX
Solid bar: protonation ratio (intensity ratio of *m/z* 183 and 182)
Slash bar: S/N ratio (no smoothing, noise calculation: RMS 1min)

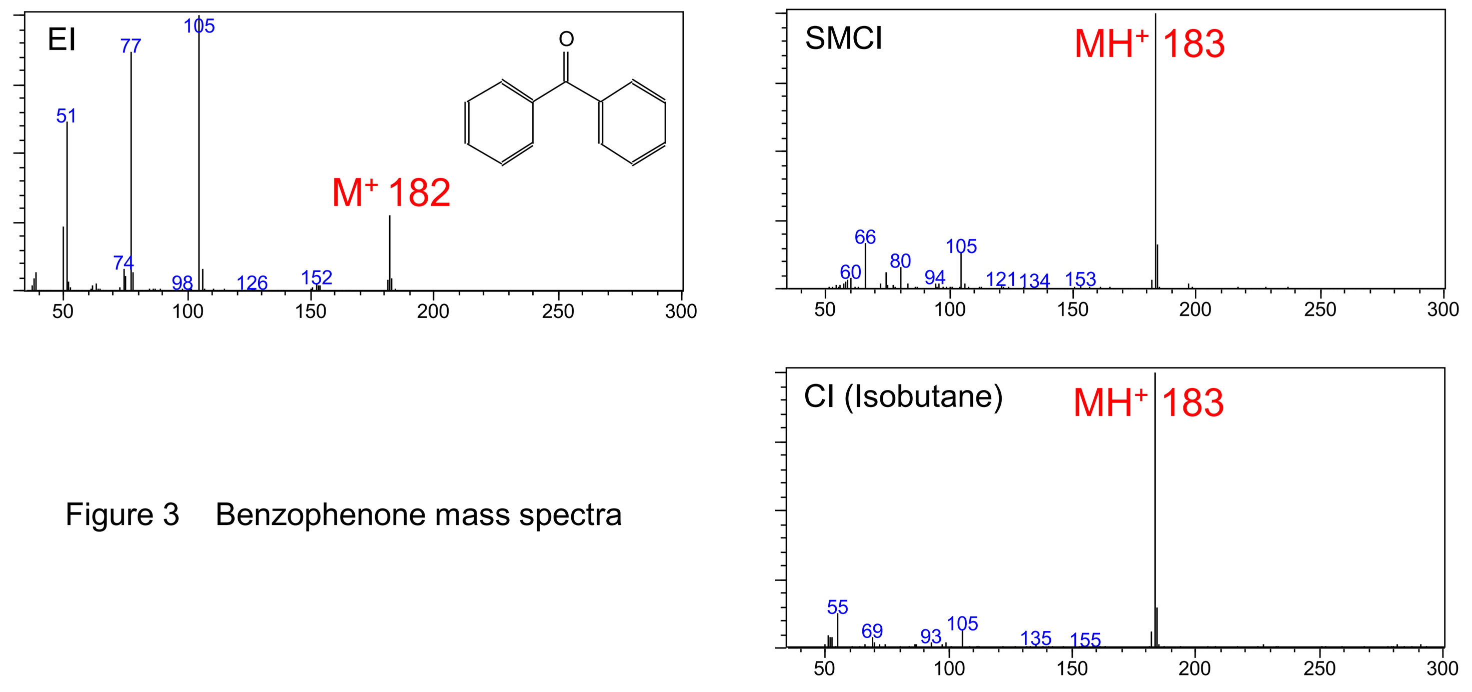

Figure 3 Benzophenone mass spectra

3-2. Identification of Unknown Compounds

At first, one unknown peak was detected in the standard solution data and assumed to be diethyl phthalate from EI mass spectrum (see Figure 4). Then SMCI mass spectrum was acquired to confirmed what phthalate ester it was.

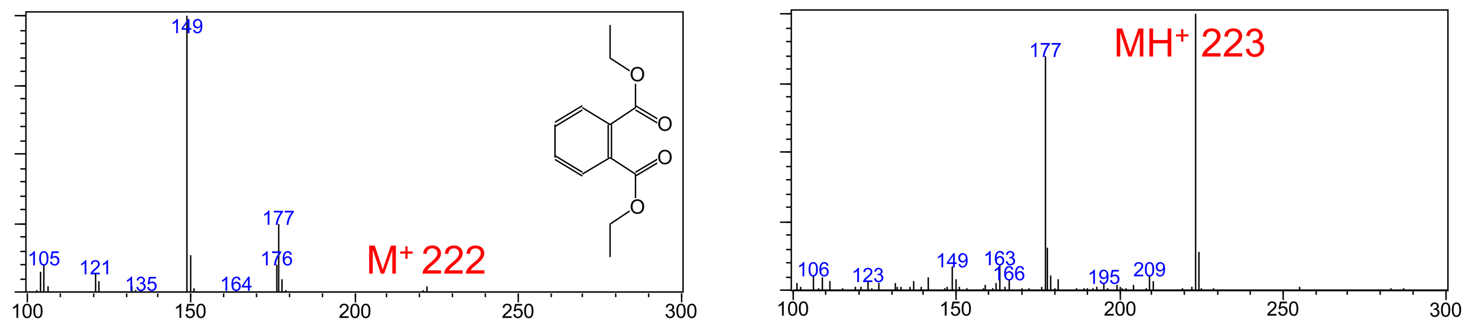

Figure 4 Diethyl phthalate mass spectra

Figure 5 and 6 show the mass spectra of drugs using EI and SMCI. It's important to check molecular weight for unknown compounds; however, these EI mass spectra didn't show any clues due to fragmentation by EI. On the other hand, SMCI mass spectra showed the protonated molecular ion as a base peak and it was comparable result with standard CI using isobutane.

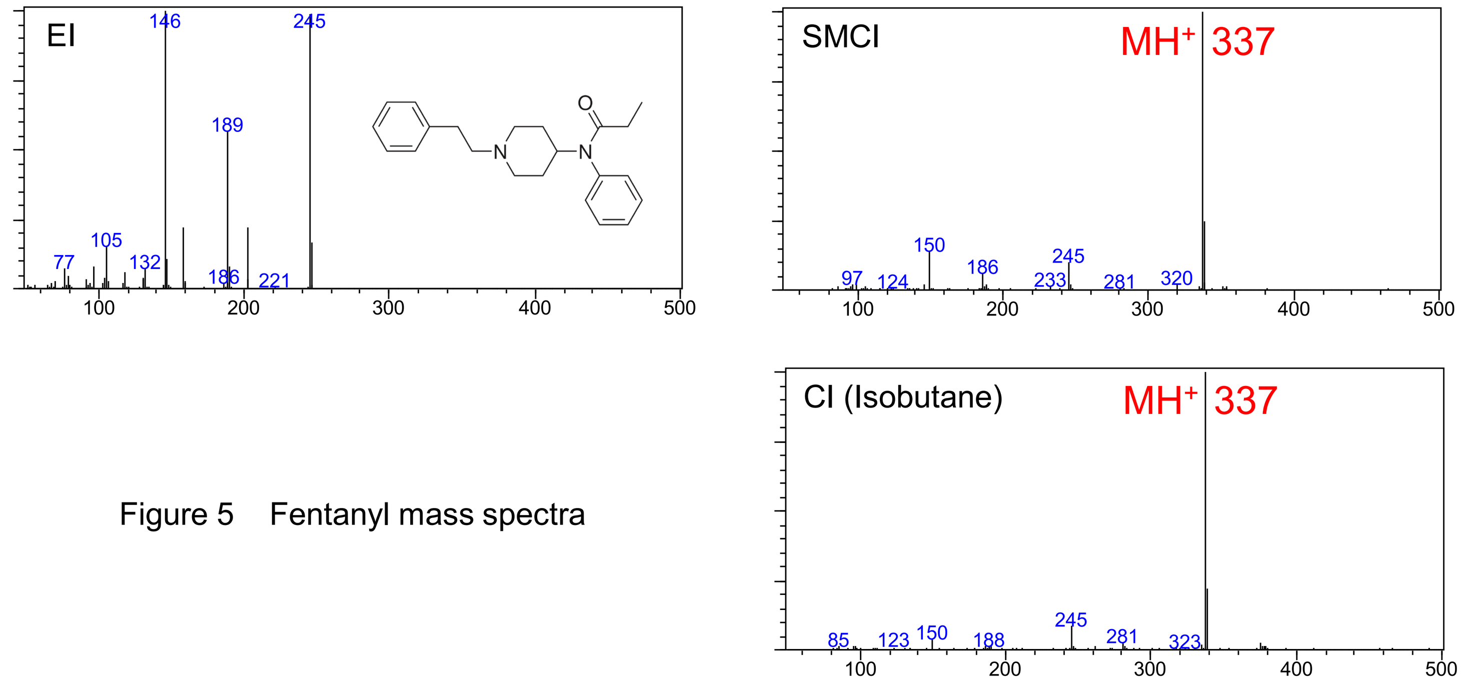

Figure 5 Fentanyl mass spectra

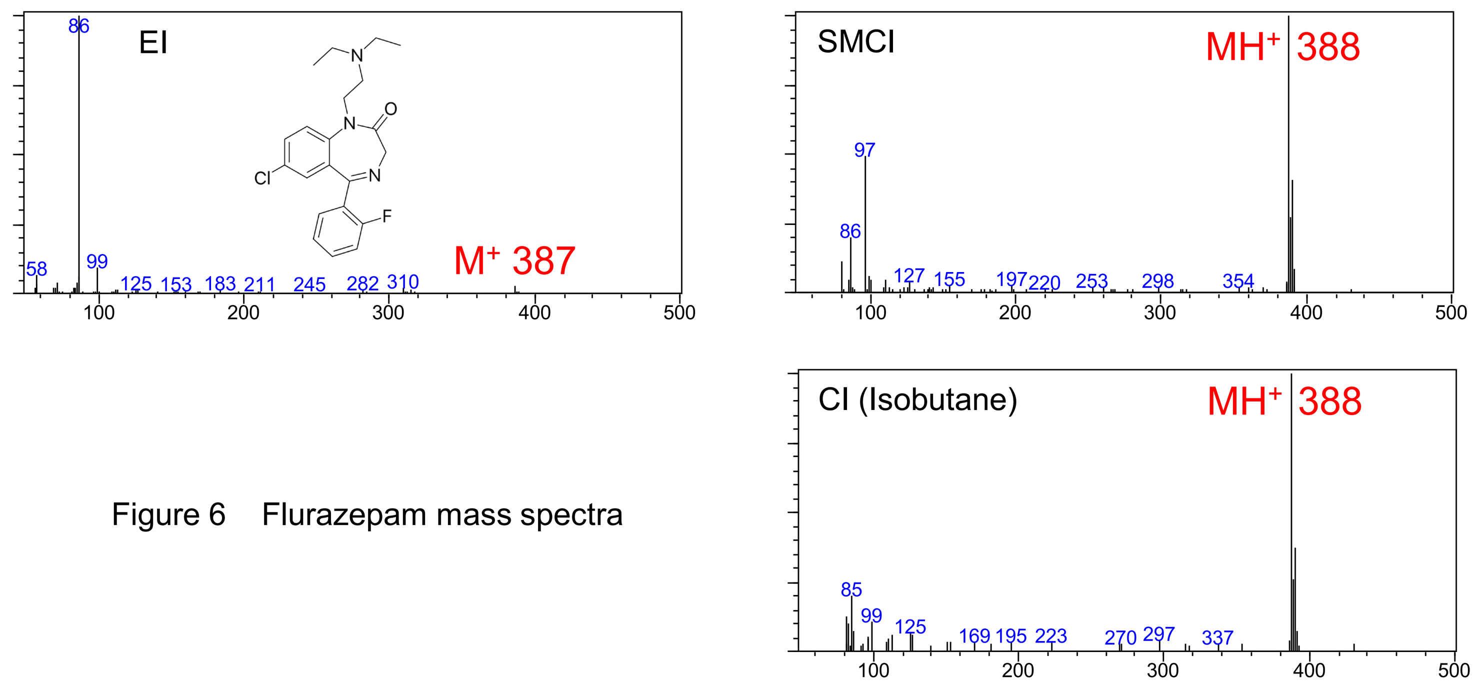

Figure 6 Flurazepam mass spectra

S/N ratios of SMCI showed better sensitivity for some compounds as can be seen in Table 1. Nitrogen gas helped to lower the base line since small molecules were exhausted effectively by the turbo molecular pump in mass spectrometer.

Table 1 S/N ratios

Ionization	SMCI	CI (Isobtane)	CI (Methane)
Compounds	S/N	S/N	S/N
Caffeine	845	492	661
Pentylone	47	91	18
Diazepam	253	495	237
Desalkylflurazepam	164	28	113
Midazolam	239	58	112
Nimetazepam	81	26	48
Fentanyl	207	210	116
Flurazepam	239	221	172
Etizolam	12	13	22
Triazolam	11	15	22

Noise calculation: Peak to Peak, 0.1min

4. Conclusions

SMCI allows an easy and safe method for molecular weight confirmation. Its data quality is sufficient and highly sensitive in comparison with standard CI using methane and isobutane. In addition, SMCI can reduce running cost of CI analysis by more than 80% due to the change of gases and the use of small amount of organic solvent (Table 2).

Table 2 Running cost of SMCI and CI

Ionization	SMCI	CI
Items	Nitrogen or Argon gas, Methanol	Isobutane or Methane gas
Cost per a year	155 USD	785 USD

Note

This solvent introduction system has not been commercialized yet. If you are interested in the system, please contact Shimadzu sales representatives.