

Application Data Set from Shimadzu

- **Chromatographic Analysis LCMS-QTOF-002**

Quantitative Determination of an Unknown Component from a Suspected Explosive Sample Using Liquid Chromatography and Quadrupole Time-of-Flight Mass Spectrometry

Abstract: In this study, the composition of a suspected explosive sample was qualitatively analyzed using a Shimadzu liquid chromatography-quadrupole time-of-flight mass spectrometry (LC-Q-TOF-MS) system. The appearance time of an unknown component present in the sample was determined by obtaining an ultraviolet (UV) chromatogram. The primary high-resolution MS information of the corresponding time was obtained, and Formula Predictor was used to predict the component's possible molecular formula. This parameter was then imported into the ChemSpider database for compound retrieval, resulting in the unknown component being hypothesized to consist of HMX. The structural formula of HMX was subsequently imported into ACD/Labs software to analyze the structure of the high-abundance fragments in the secondary high-resolution mass spectrum and to deduce the possible fragmentation patterns. The results of this analysis further confirmed the unknown component in the sample to be HMX.

Keywords: liquid chromatography-quadrupole time-of-flight mass spectrometry; Q-TOF; explosive; HMX.

Explosions are the main means of terrorist attacks, and techniques for the detection and analysis of suspected explosives and explosive residues are currently being developed. Many

methods currently exist for detecting explosives, including canine identification, IMS, liquid chromatography–mass spectrometry (Lcms) analysis, and gas chromatography–mass spectrometry (GC-MS) analysis. Owing to the small size of the detection equipment and the speed at which analyte detection is performed, IMS has been widely used in the context of airport security. The principle on which IMS analysis is based is to detect ions with different mobilities subjected to the force exerted on them by an electric field and to determine the corresponding ion flight time. The downside of using IMS is that this technique is less effective

at separating closely related substances and at identifying explosives that are not in its database. With the increase in the variety of explosives being used, to even include some homemade and mixed explosives, the technology for explosive detection is facing new challenges. In this study, the Lcms system Shimazu high-resolution LCMS-9030 was used to rapidly lock possible compounds with a high accuracy and ChemSpider database. The identities of the observed compounds were further confirmed using secondary spectrum information and combined with ACD/Labs software.

1. Experiments

1.1. Apparatus

A Shimadzu UPLC Nexera system and a quadrupole time-of-flight mass spectrometer LCMS-9030 were used. The Nexera system included LC-30AD×2 (a pump), DGU-20A₅ (an online degasser), SIL-30AC (an autosampler), CTO-20AC (a column oven), SPD-M20A (a diode array detector), and CBM-20A (a system controller). Data acquisition and analysis were performed using the LabSolutions Ver. 5.95 workstation. Mass spectrum structure analysis was performed using ACD/Labs Ver. 2012 software.

1.2. Analysis Conditions

Liquid chromatography conditions

Column: Shim-pack GIST 2.1 mm I.D. × 100 mm L, 2.0 μm

Mobile phase: phase A (5 mM ammonium acetate aqueous solution), phase B (methanol)

Flow rate: 0.5 mL/min

Column temperature: 40 °C

Injection volume: 2 μL

Detection wavelength: 190–800 nm

Elution mode: gradient elution included a 5% initial concentration of the mobile phase B; see the details of the program utilized in Table 1.

Table 1: Gradient elution program.

Time (min)	Module	Command	Value
1.0	Pumps	Pump B Conc.	5
8.0	Pumps	Pump B Conc.	60
12.0	Pumps	Pump B Conc.	60
12.1	Pumps	Pump B Conc.	5
15.0	Controller	Stop	

Mass spectrometry conditions

Analytical apparatus: LCMS-9030

Ionization mode: ESI(-)

Heating gas: air, 10.0 L/min

Nebulizing gas: nitrogen, 3.0 L/min

Drying gas: nitrogen, 10.0 L/min

Collision gas: argon

Interface temperature: 300 °C

DL temperature: 250 °C

Heater block temperature: 400 °C

Scan mode: MS full scan m/z : 100–800

MSMS(DDA) m/z : 100–500

CE: 30 ± 10 V

Loop time: 0.2 s

1.3. Sample Pretreatment

10 mg of the sample of the suspected explosive was weighed and then subjected to ultrasonication for 10 min with 10 mL of methanol. The sample was then subjected to centrifugation at 3,000 rpm for 10 min. The supernatant was then collected and filtered through a 0.22 μm polytetrafluoroethylene membrane and then subsequently pipetted into an injection vial that was later utilized to perform the assay.

2. Results and Discussion

2.1. Ultraviolet (UV) Chromatogram of the Suspected Explosive Sample

The UV chromatogram of the suspected explosive sample at 254 nm is shown in Figure 1. An obvious chromatographic peak appears at the 4.72 min point, which may be due to the unknown explosive.

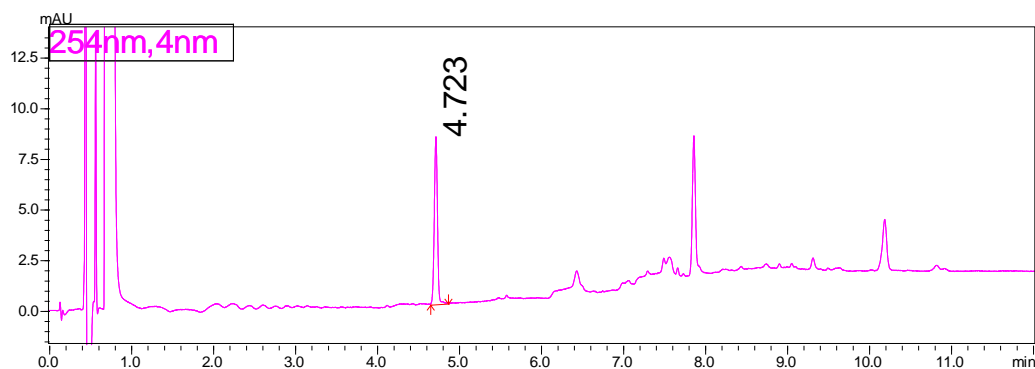


Figure 1: UV chromatogram of the suspected explosive sample.

2.2. Mass Spectrometry Analysis and Molecular Formula Prediction of RT 4.72 Unknown Composition

Figure 2 shows the mass spectrometry BPC chromatogram of the suspected explosive sample. An obvious signal at 4.77 min is observed, which corresponds to the substance associated with the peak at 4.72 min observed in the UV chromatogram. The unknown composition of RT 4.72 will be further analyzed.

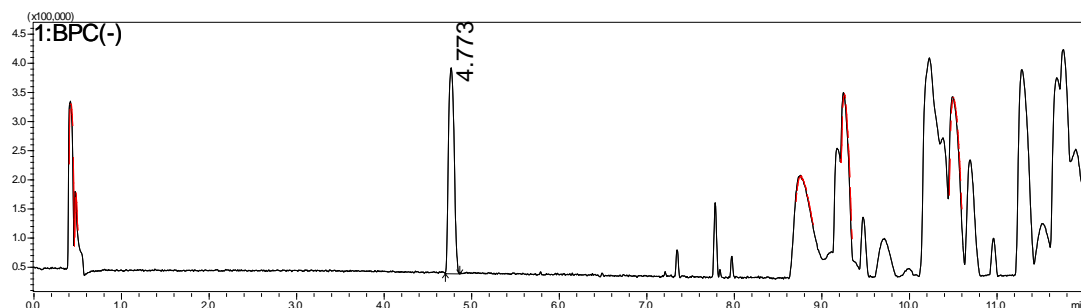


Figure 2: BPC chromatogram of the suspected explosive sample.

Figure 3 shows the primary mass spectrum and an enlarged view of RT 4.72. RT 4.72 was subjected to high-resolution mass spectrometry analysis, and the results of this experiment indicated that the intensity ratio of the peak at m/z 331.0156 to that at m/z 333.0120 was 3 : 1. It was predicted as $[M+Cl]^-$. Taking advantage of the high accuracy of the results obtained using the Shimadzu LCMS-9030 system, the mass axis accuracy of the internal standard method was less than 1 ppm/h. The molecular formula of the compound with RT 4.72 was hypothesized using Formula Predictor, with the maximum number of atoms of elements C, H, O, N, and Cl present in the molecule set being 150, 300, 12, 10, and 1, respectively. The predicted structure of the compound associated with the peak at m/z 331.0156 is reported in Figure 5. Based on the results of Formula Predictor, the only possible formula for the target compound was $C_4H_8N_8O_8$, and the mass number deviation was -0.90 ppm. Figure 4 shows the ion extraction flow diagrams of the peaks at m/z 331.0156 and 341.0447, both of which are characterized by the same appearance time and most likely are due to the same compound. The value of m/z 341.0447 was predicted, and the prediction results are shown in Figure 6. This peak was comprehensively determined to be due to $[M+HCOO]^-$, with a mass number deviation of -0.11 ppm, ranking first in the prediction list.

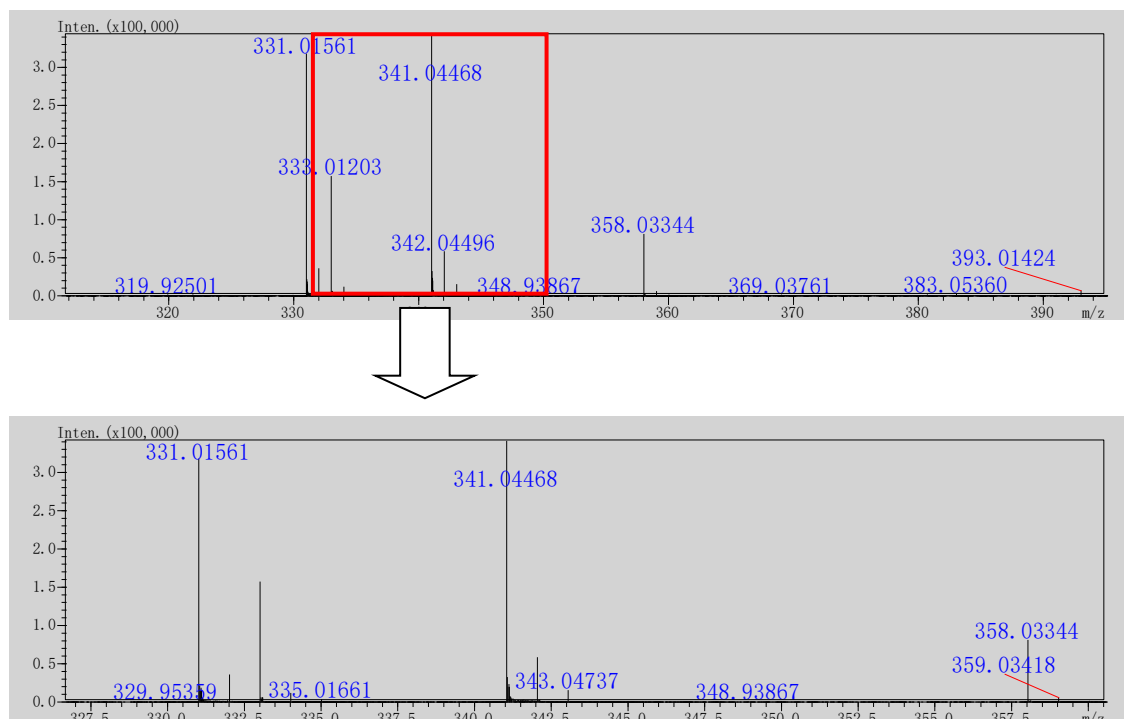


Figure 3: Primary mass spectrum and an enlarged view of RT 4.72.

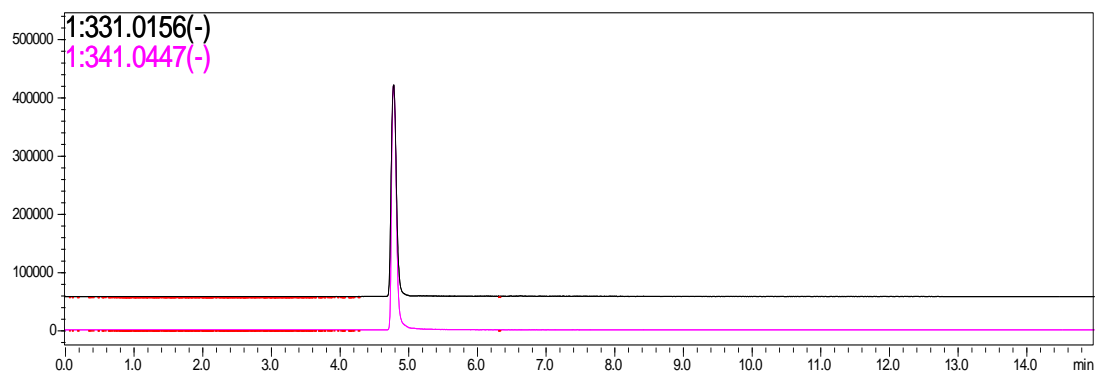


Figure 4: Ion extraction flow diagram of the peaks at m/z 331.0156 and m/z 341.0447 obtained in a high-resolution mass spectrometry experiment.

Formula Predictor - 331.015614 - MS(ESI-) RT: 4.755 - 4.797 Scan#: 2656 - 2678

331.015614 Charge: 1

Error Margin: 2 ppm

Fixed

DBE Range: -2 - 1000

Electron Ions: Both configurations

Limit

HC Ratio: 0 - 3

Apply Nitrogen Rule

Max results: 500

#	Score	Pred. (M)	Pred. m/z	Meas. m/z	Diff. (mDa)	Formula (M)	Ion	Diff. (ppm)	Iso Score	DBE
1	41.55	296.046509	331.015911	331.015614	-0.297	C4 H8 N8 O8	[M+Cl]-	-0.8961	35.56	5.0

Figure 5: Molecular formula prediction results based on the mass spectrometry peak appearing at m/z 331.0156.

Formula Predictor - 341.044675 - MS(ESI-) RT: 4.755 - 4.797 Scan#: 2656 - 2678

341.044675 Charge: 1

Error Margin: 2 ppm

Fixed

DBE Range: -2 - 1000

Electron Ions: Both configurations

Limit

HC Ratio: 0 - 3

Apply Nitrogen Rule

Max results: 500

#	Score	Pred. (M)	Pred. m/z	Meas. m/z	Diff. (mDa)	Formula (M)	Ion	Diff. (ppm)	Iso Score	DBE
1	66.75	342.051989	341.044712	341.044675	-0.037	C5 H10 N8 O10	[M-H]-	-0.1091	63.08	5.0
2	66.74	296.046509	341.044712	341.044675	-0.037	C4 H8 N8 O8	[M+HCOO]-	-0.1091	63.08	5.0

Figure 6: Molecular formula prediction results based on the mass spectrometry peak appearing at m/z 341.0447.

2.3. ChemSpider Database Result Retrieval

ChemSpider (<http://www.chemspider.com/>) is a free-access searchable database of chemical compounds that integrates information on 68 million compounds collected from 253 platforms. It allows various retrieval methods to be employed, such as using the molecular formula, chemical name, trade name, or structural formula. This database is the most widely used database for the identification of unknown structures. The molecular formula $C_4H_8N_8O_8$ that was predicted in the experiment described in Section 2.2 was imported into ChemSpider for candidate compound retrieval, and the result of this procedure is reported in Figure 7. The only candidate compound

provided by ChemSpider was HMX, whose Chinese name is octogen (melting point: 282 °C, density: 1.96 g/cm³). HMX is a white crystalline solid at room temperature and is soluble in acetone, acetonitrile, and chloroform, as well as other solvents. Importantly, HMX is a powerful military explosive that is characterized by an explosive force equivalent to 1.5 times that of TNT.

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ChemSpider
Search and share chemistry

Search ChemSpider

Simple Structure Advanced History

Found 1 result
Search term: **C4H8N8O8** (Found by synonym)

HMX

Molecular Formula: C₄H₈N₈O₈
Average mass: 296.155 Da
Monoisotopic mass: 296.046509 Da
ChemSpider ID: 16636

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Figure 7: Retrieval results obtained from the ChemSpider database after using the molecular formula C₄H₈N₈O₈ as the input.

2.4. Speculative Results of RT 4.72 Secondary Mass Spectrum Combined with ACD/Labs Fragmentation Patterns

The secondary mass spectrum (Figure 8) corresponding to RT 4.72 and m/z 341.0447 was imported into the ACD/Labs software to perform a structure prediction on the high-abundance fragments combined with the possible structural formula predicted by 2.3. The prediction results are reported in Figure 9. The possible fragmentation patterns were thus inferred; see the results reported in Figure 10.

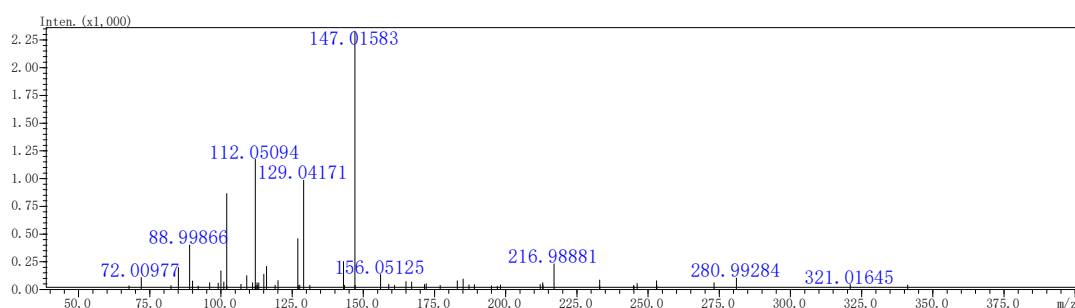


Figure 8: Secondary mass spectrum of the peak at m/z 341.0447 of RT 4.72.

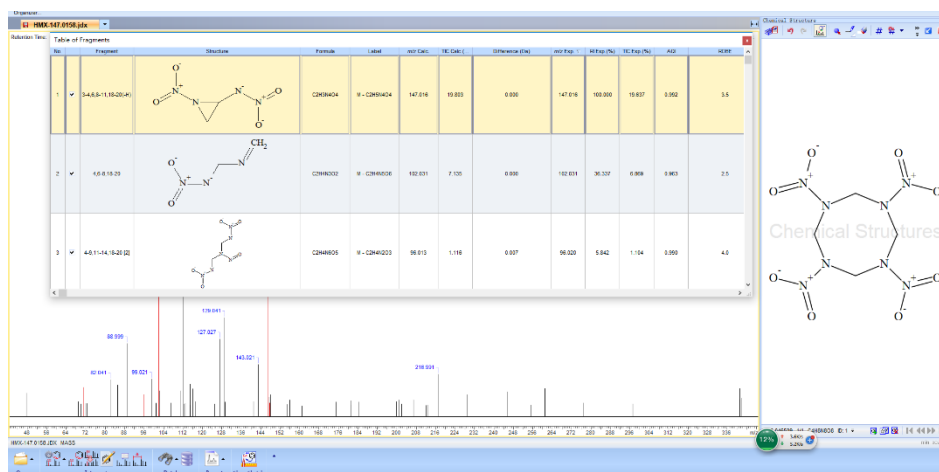


Figure 9: Fragment structure prediction results obtained by uploading to the ACD/Labs software the results of the secondary mass spectrometry experiment performed on the peak at m/z 341.0447.

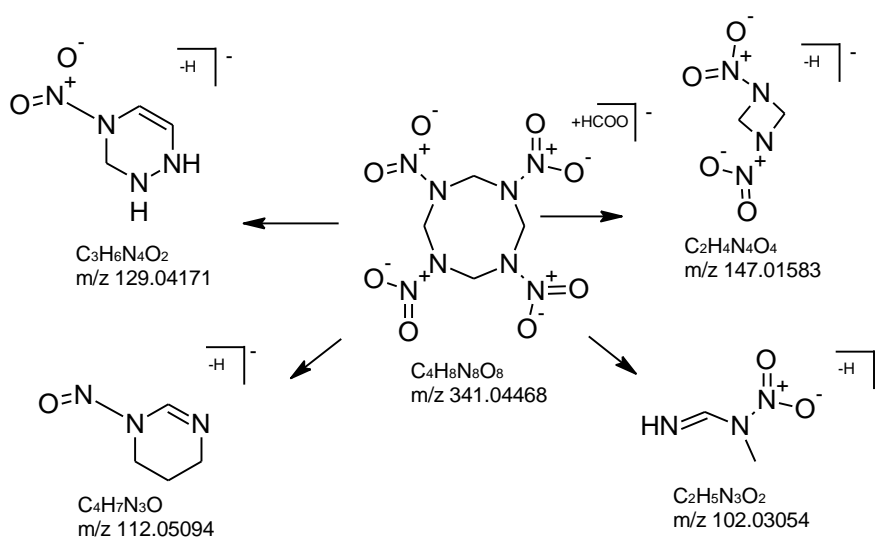


Figure 10: Possible secondary fragmentation pattern of the peak at m/z 341.0447.

3. Conclusion

In this study, the Lcms instrument Shimadzu LCMS-9030 was successfully used to identify an unknown component from a suspected explosive sample by relying on this system's advantages, such as high-resolution and high accuracy. Importantly, this analytical approach was utilized in combination with Formula Predictor, ChemSpider, ACD/Labs, and other auxiliary software packages and databases. The analysis results indicated that using LCMS-9030 was associated with sub-ppm errors. The results also indicated that LCMS-9030 is a powerful tool for predicting the molecular formulas and inferring the structures of the components of samples of unknown composition.