Application Note

Instrument: Pegasus® BT

Deadly Counterfeit Pills: Untargeted Analysis of Fentanyl-Laced Drugs

LECO Corporation; Saint Joseph, Michigan USA

Key Words: Counterfeit Pills, Fentanyl, GC-MS

Introduction

Counterfeit pills are fake medications that have different formulations than legitimate medications produced by pharmaceutical companies.[1] They are often made to resemble actual medication but are laced with adulterants such as methamphetamine or fentanyl which can cause severe harm or death. These counterfeit pills are tied to the increase in the illegal use of fentanyl in the United States over the past few years. Opioids such as fentanyl have been used for decades to treat pain during surgical procedures and to manage chronic pain for patients globally.[2] Fentanyl was introduced as an analgesic in the 1960s and was widely used because of its very fast and strong action.[1] It is a powerful synthetic opioid that is 50 times more potent than morphine. Unfortunately, as with other medications, fentanyl use may be deadly if not used with proper dosing under the care of a physician. In many cases, fake medications are cut with mixtures of less expensive compounds to increase profits for illegal drug manufacturers and dealers. This has become extremely dangerous since these clandestine chemists have started using fentanyl as part of their formulations. The potency of fentanyl and its many derivatives, together with their unknown concentration in pills results in disastrous consequences for the end-user. In this application note, untargeted analysis of counterfeit medications was conducted using high-performance time-of-flight mass spectrometry. Pills were comprehensively characterized using state-of-the-art processing software, large spectral libraries, and formulae confirmation.

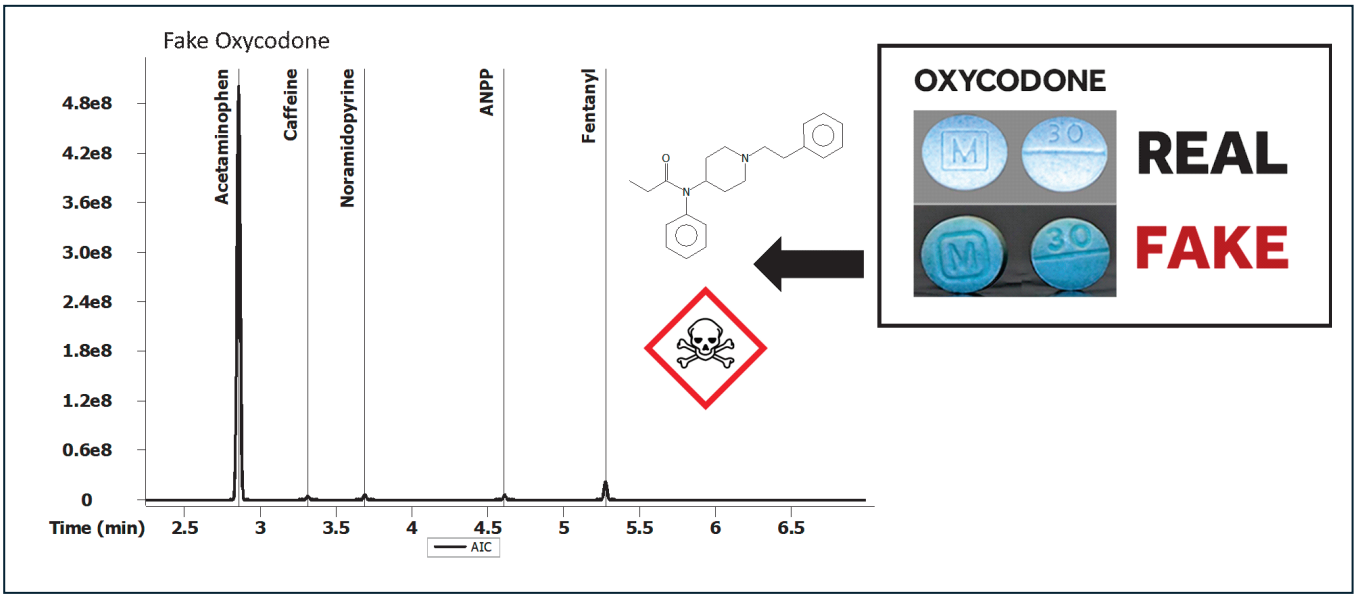


Figure 1. Analytical Ion Chromatogram (AIC) of Fake Oxycodone Tablet Extract and Image Comparing Real vs. Fake Oxycodone tablets, https://www.dea.gov/sites/default/files/2021-09/DEA_Fact_Sheet-Counterfeit_Pills.pdf. Accessed 12/7/21.

Experimental

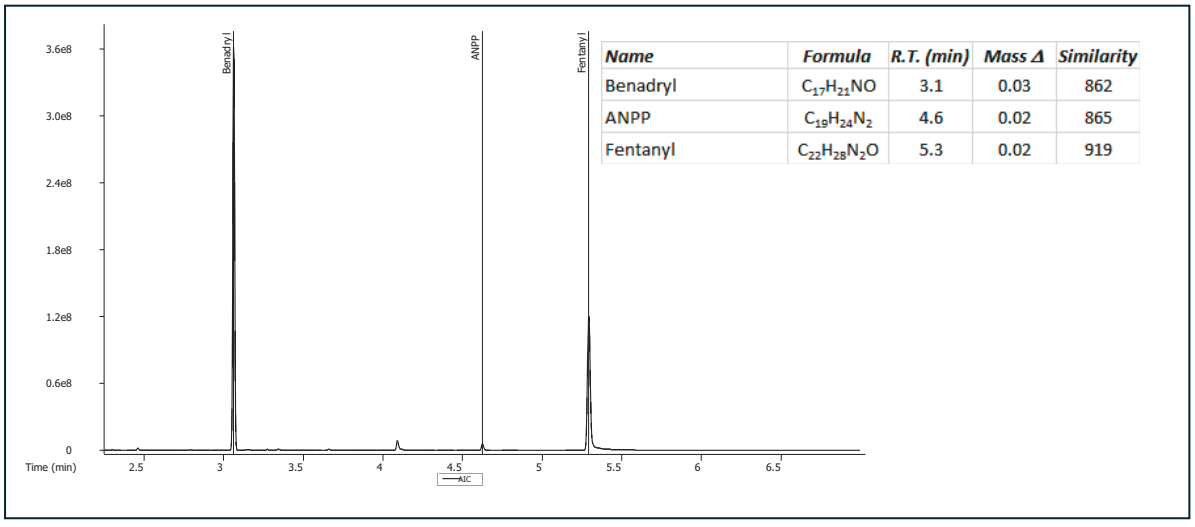
Counterfeit medications were analyzed with the assistance of a collaborating forensic laboratory. Counterfeit medication analyzed in this study included fake oxycodone, fake Benadryl, and an unknown pill. Pills were crushed using a mortar and pestle. The resulting powdery materials were transferred to 20 mL scintillation vials, weighed, mixed with 5mL of 8:1:1 chloroform, methanol, and isopropanol, and extracted via sonication for 20 minutes. The heterogeneous mixtures were filtered through syringe filters and 100 µL aliquots were transferred to GC vials with 300 µL inserts. The LECO Pegasus® BT GC-MS and ChromaTOF® brand software were used for pill extract analysis. Untargeted data processing included peak finding, library searches, and formula mass error or delta calculations ($M\Delta = M_{obs} - M_{calc}$). The instrumental analysis parameters are outlined in Table 1.

Table 1. GC-High Performance TOFMS (*Pegasus* BT) Conditions

Gas Chromatograph	Agilent 7890 with L-PAL3 Autosampler
Injection	1µL, Split 100:1 @ 250 °C
Carrier Gas	He @ 1.4 mL/min, Constant Flow
Column	Rxi-35 Sil MS, 15 m x 0.25 mm i.d. x 0.25 µm (Restek, Bellefonte, PA, USA)
Temperature Program	110 °C to 300 °C @ 40 °C/min (4 min)
Mass Spectrometer	**LECO *Pegasus* BT**
Transfer Line	290 °C
Ion Source Temperature	250 °C
Ionization Mode	EI
Mass Range (m/z)	45-600
Acquisition Rate	10 spectra/s

Results and Discussion

The analysis methodology resulted in the robust characterization of pill components, including adulterants such as fentanyl and derivatives in under 8 minutes. Comprehensive analysis of pills using a combination of time-of-flight mass spectrometry and *ChromaTOF* software processing resulted in rapid identification of compounds using spectral similarity searches and formulae mass error determinations. For example, counterfeit Benadryl tablets were analyzed and found to contain not only Benadryl, but also fentanyl, and 4-aminophenyl-1-phenethylpiperidine (ANPP) as shown in Figure 2. ANPP is also known as despropionyl fentanyl and is a precursor to fentanyl and its derivatives. The Analytical Ion Chromatogram (AIC) and inset table lists the formula, retention times, MΔ values, and spectral similarity search results for the major components of this counterfeit pill.



Name	Formula	R.T. (min)	Mass Δ	Similarity
Benadryl	$C_{17}H_{21}NO$	3.1	0.03	862
ANPP	$C_{19}H_{24}N_2$	4.6	0.02	865
Fentanyl	$C_{22}H_{28}N_2O$	5.3	0.02	919

Figure 2. AIC for Counterfeit Benadryl.

Spectral similarity values for ANPP and fentanyl were 862 and 919/1000 respectively. Furthermore, MΔ for ANPP's molecular ion was 0.02 Da (Figure 3). The molecular ion for fentanyl is not present in the spectra; however, the fragment ions in the Peak True spectrum correlate well with those in the library match. In addition, the MΔ values ranged from 0.01 to 0.04 Da for the fragment ions A-D (Figure 4).

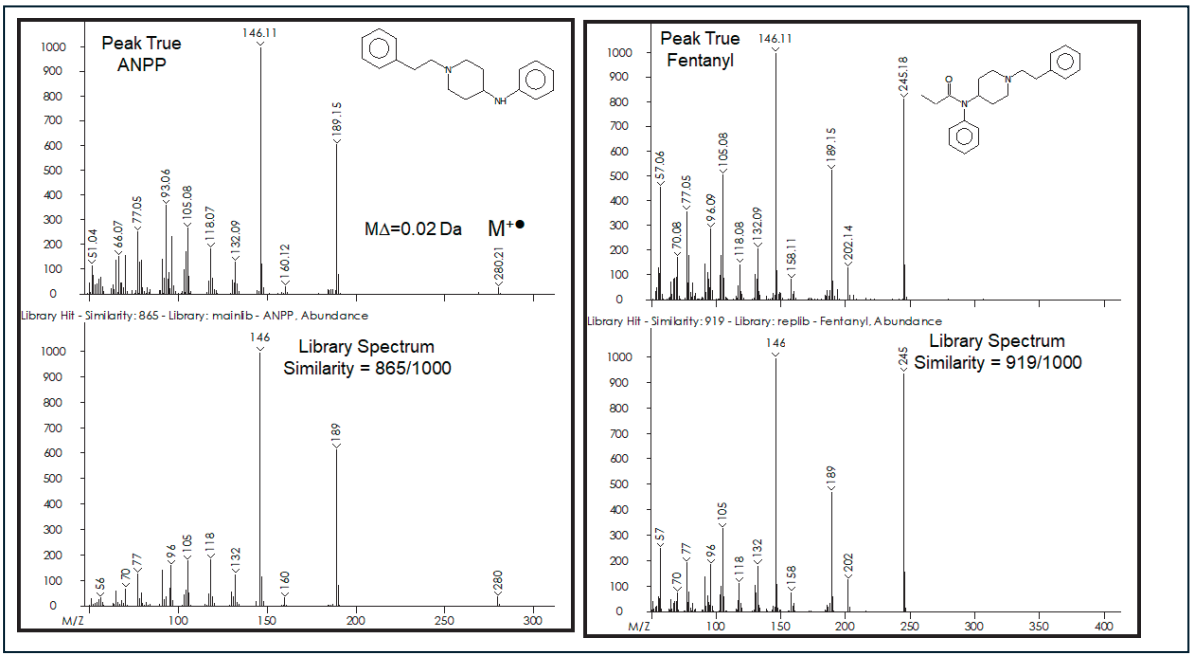
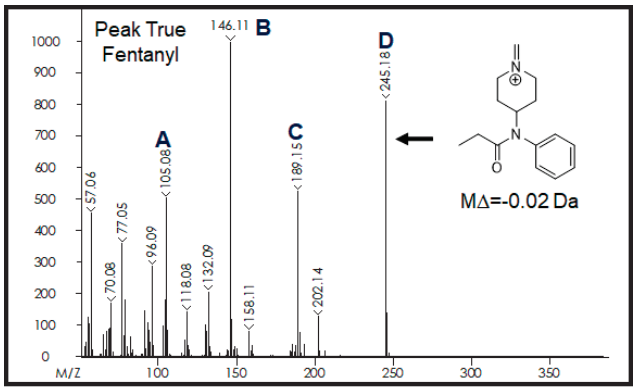


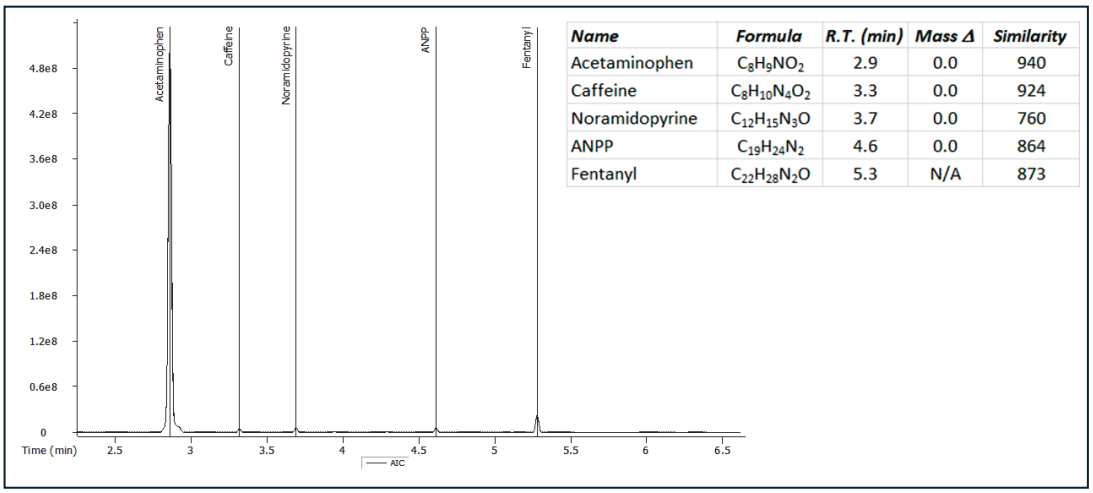
Figure 3. Peak True and Library Match Spectra for ANPP and Fentanyl.



Peak	Formula	m/z_{obs}	m/z_{calc}	Mass Δ
A	C_7H_7	105.08	105.07	0.01
B	$C_{10}H_{12}N$	146.11	146.10	0.01
C	$C_{12}H_{15}NO$	189.15	189.11	0.04
D	$C_{15}H_{21}N_2O$	245.18	245.16	0.02

Figure 4. Peak True Mass Spectrum for Fentanyl with Tabulated Mass Δ Values (0.01 – 0.04 Da) for the Major Fragments (A – D).

The AIC for the counterfeit oxycodone pill is displayed in Figure 5. The table inset lists formulas, retention times, MΔ values, and spectral similarities for acetaminophen, caffeine, noramidopyrine, ANPP, and fentanyl. Oxycodone was not detected in the fake oxycodone pill extract. The mass spectra for acetaminophen and caffeine are shown in Figure 6. The spectral similarities for these compounds were 760 and 924/100. Both of the compounds exhibit strong molecular ions with excellent MΔ values (0.00 Da) in their mass spectra. Noramidopyrine, also known as metamizole, is an analgesic and antipyretic. The spectral similarity value for noraminopyrine was marginal at 760/100 (Figure 7); however, the MΔ values for fragment ions A-D were excellent (-0.03 to 0.02 Da).



Name	Formula	R.T. (min)	Mass Δ	Similarity
Acetaminophen	$C_8H_9NO_2$	2.9	0.0	940
Caffeine	$C_8H_{10}N_4O_2$	3.3	0.0	924
Noramidopyrine	$C_{12}H_{15}N_3O$	3.7	0.0	760
ANPP	$C_{19}H_{24}N_2$	4.6	0.0	864
Fentanyl	$C_{22}H_{28}N_2O$	5.3	N/A	873

Figure 5. AIC for Counterfeit Oxycodone.

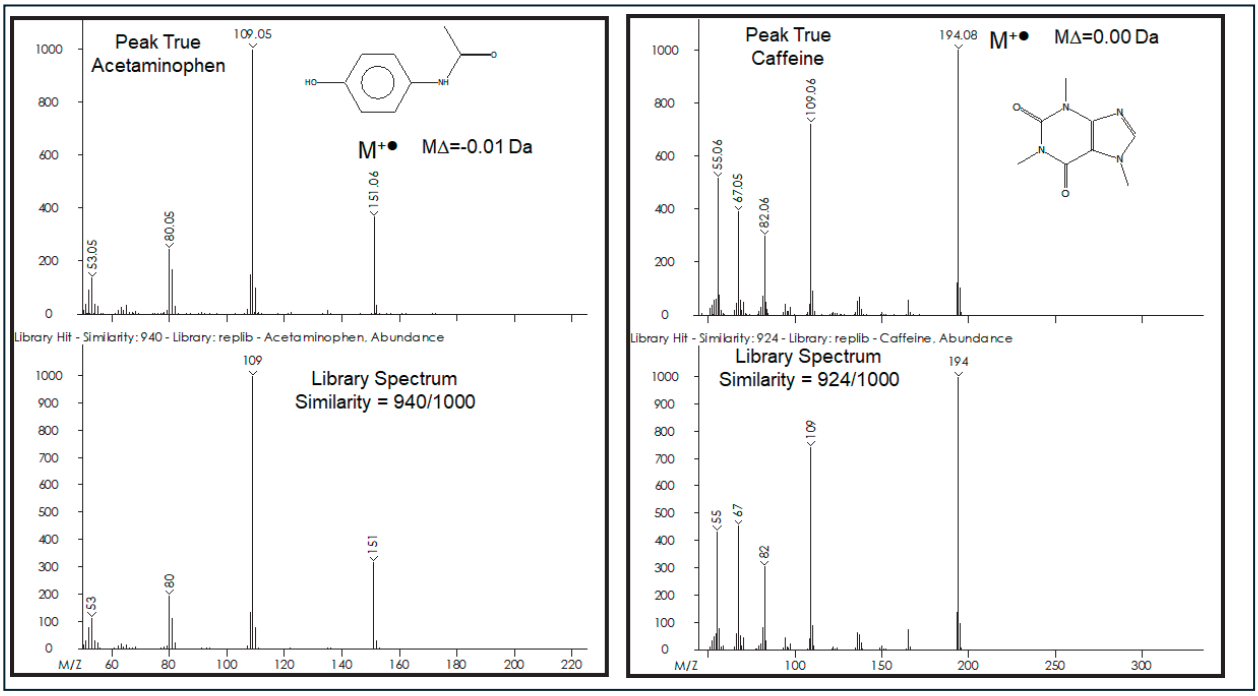
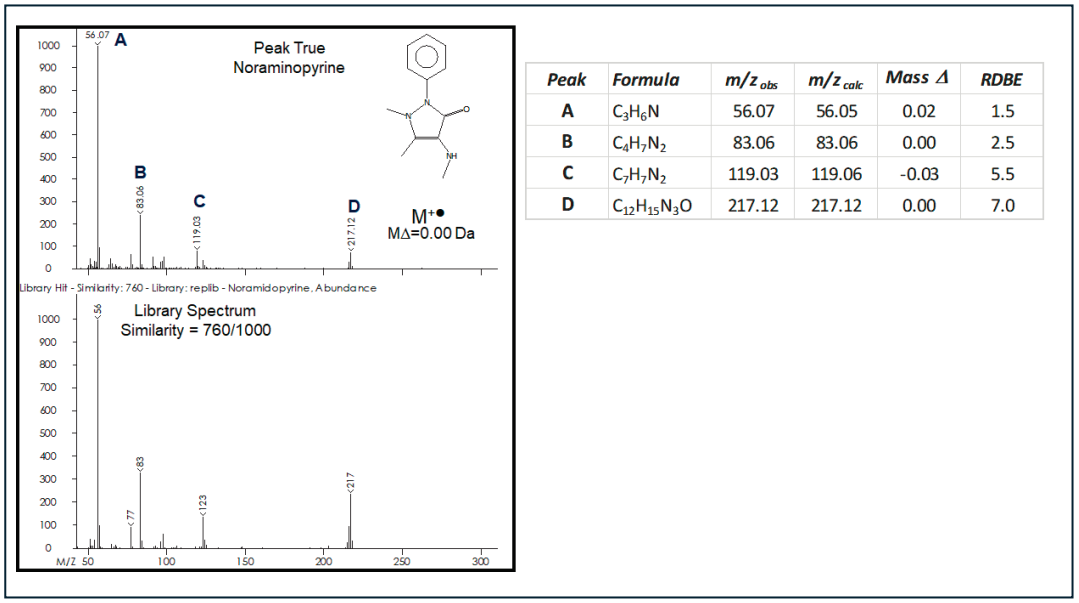


Figure 6. Peak True and Library Match Spectra for Acetaminophen and Caffeine.

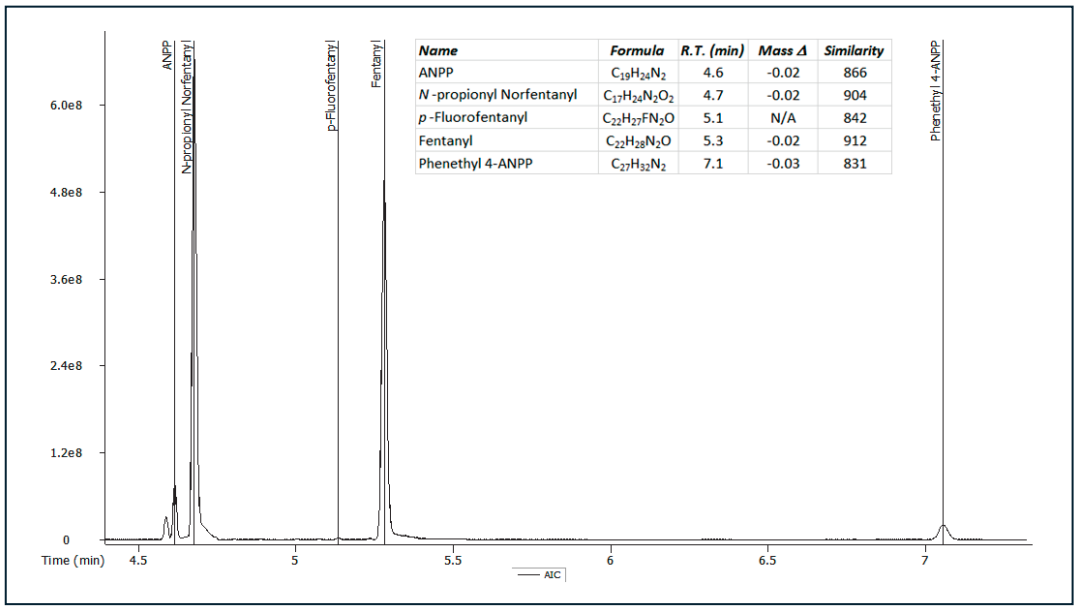


Peak	Formula	m/z_{obs}	m/z_{calc}	Mass Δ	RDBE
A	C_3H_6N	56.07	56.05	0.02	1.5
B	$C_4H_7N_2$	83.06	83.06	0.00	2.5
C	$C_7H_7N_2$	119.03	119.06	-0.03	5.5
D	$C_{12}H_{15}N_3O$	217.12	217.12	0.00	7.0

Figure 7. Peak True and Library Mass Spectra for Noraminopyrine with Tabulated Mass Values (-0.03 – 0.02 Da) and RDBEs for the Major Fragments (A-D).

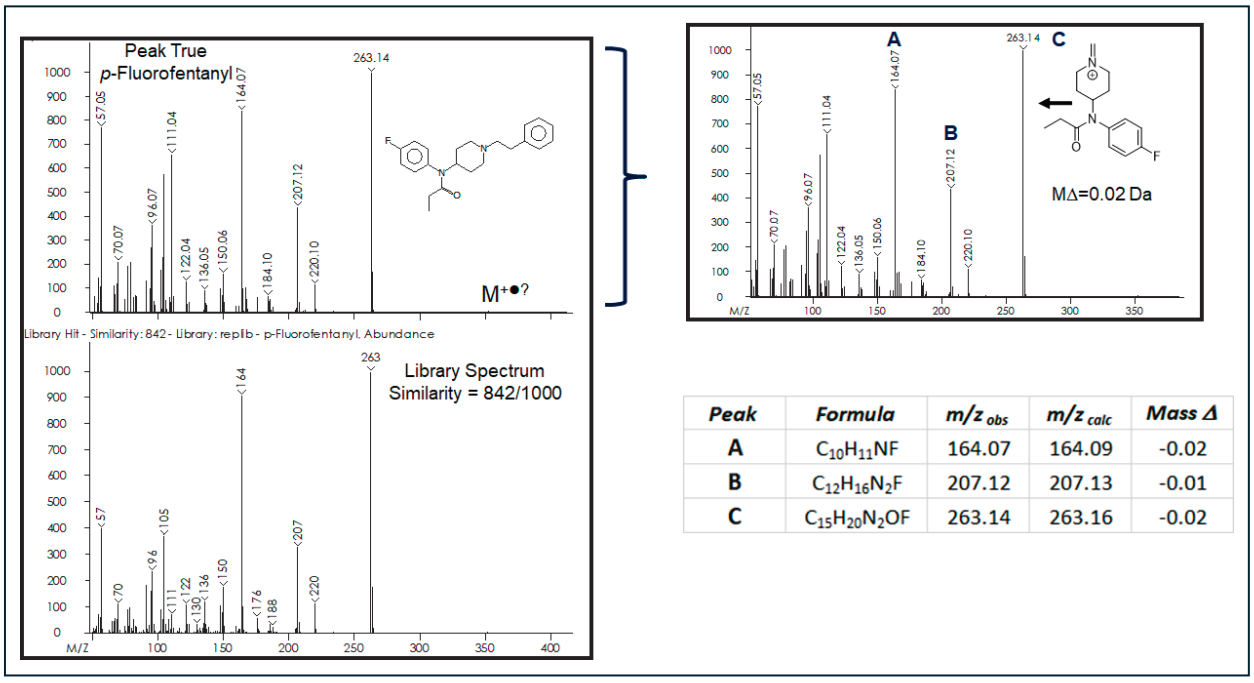
Often forensic laboratories receive confiscated pills with no discernable markings or that have been partially crushed into smaller pieces. This is the case with the unknown pill analyzed in this investigation. Fortunately, the methodology described in this application note were used to fully characterize the components of the pill and may provide some information about its origin based on its chemical content. The AIC and table insert in Figure 8 list some of the major contents in the pill including ANPP, *N*-propionyl Norfentanyl, *p*-fluorofentanyl, fentanyl, and phenethyl 4-ANPP.

The Peak True and library mass spectra for *p*-fluorofentanyl, a fentanyl analog, are shown in Figure 9. This compound does not have a molecular ion in its mass spectrum; however, the spectral similarity value (842/100) together with the MΔ values for its major fragment ions A-C (-0.02 to -0.01 Da) facilitated its identification in the unknown sample.



Name	Formula	R.T. (min)	Mass Δ	Similarity
ANPP	$C_{19}H_{24}N_2$	4.6	-0.02	866
N-propionyl Norfentanyl	$C_{17}H_{24}N_2O_2$	4.7	-0.02	904
p-Fluorofentanyl	$C_{22}H_{27}FN_2O$	5.1	N/A	842
Fentanyl	$C_{22}H_{28}N_2O$	5.3	-0.02	912
Phenethyl 4-ANPP	$C_{27}H_{32}N_2$	7.1	-0.03	831

Figure 8. AIC for an Unknown Sample.



Peak	Formula	m/z_{obs}	m/z_{calc}	Mass Δ
A	$C_{10}H_{11}NF$	164.07	164.09	-0.02
B	$C_{12}H_{16}N_2F$	207.12	207.13	-0.01
C	$C_{15}H_{20}N_2OF$	263.14	263.16	-0.02

Figure 9. Peak True Mass Spectrum for p-Fluorofentanyl with Tabulated Mass Δ Values (0.01 – 0.04 Da) for the Major Fragments (A-C).

The mass spectra for *N*-propionyl porfentanyl and phenethyl 4-ANPP are displayed in Figure 10. The spectral similarity and MΔ values were 904/1000 (MΔ = -0.02 Da) and 831/1000 (MΔ = -0.03 Da) respectively. Phenethyl 4-ANPP is a fentanyl precursor which may serve as a marker for alternative fentanyl synthesis routes.[3] The spectral similarity and MΔ values in its mass spectrum were 831/100 and -0.03 Da.

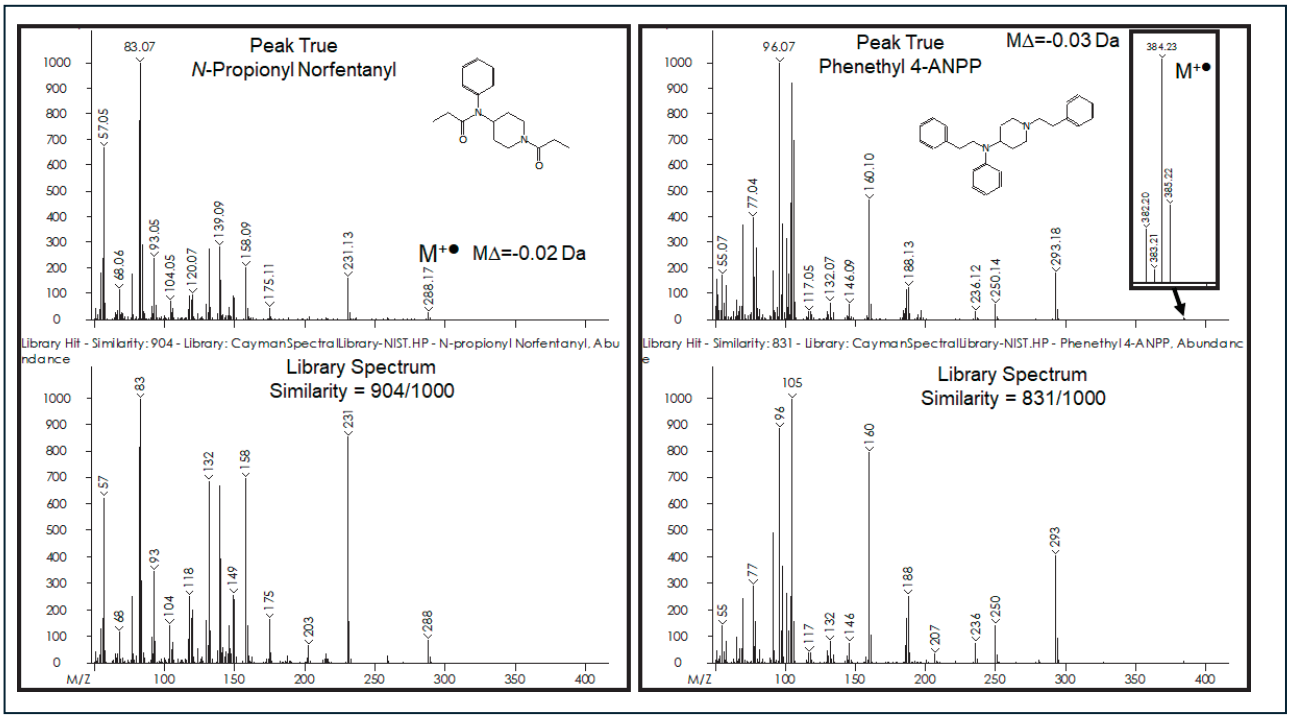


Figure 10. Peak True and Library Match Spectra for N-Propionyl Norfentanyl and Phenethyl 4-ANPP.

Conclusion

A simple sample preparation method was implemented for the extraction of counterfeit pill components. Analysis of the extracts was accomplished using the LECO *Pegasus* BT and *ChromaTOF* software. Untargeted analysis of pills using comprehensive, time-of-flight mass spectrometry with advanced processing facilitated the rapid identification of adulterants such as fentanyl and its derivatives through spectral similarity searches, and molecular formulae verifications based on excellent mass precision.

References

[1]"Fentanyl DrugFacts", Drug Facts, *National Institute on Drug Abuse*, June 2021.

[2]"Recommended Methods for the Identification and Analysis of Fentanyl and its Analogs in Biological Specimens" *United Nations Office on Drugs and Crime*, Vienna, 2017.

[3]Vandeputte M.M, Krotulski A.J., Hulpia F., Van Clengergh S. and Stove C.P., "Phenethyl-4-ANPP: A Marginally Active Byproduct Suggesting a Switch in Illicit Fentanyl Synthesis Routes", *Journal of Analytical Toxicology*, April 2021.

LECO Corporation | 3000 Lakeview Avenue | St. Joseph, MI 49085 | Phone: 800-292-6141 | 269-985-5496
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