Identification Of Fentanyl-Type Opioids Using GC-MS Fragmentation Data

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Abstract

Predictive fragments and fragmentation patterns were determined after analyzing the mass spectral data of 60+ fentanyl-like substances *via* GC-MS. Differences in retention times between structurally related items and positional isomers were also noted. This data can assist in the identification of novel opioids of the fentanyl structural class when standards or library matches are unavailable.

Introduction

- Fentanyl is a powerful synthetic opioid developed by Janssen Pharmaceutica in 1959 that has roughly one hundred times the potency of morphine.
- Structurally, fentanyl can be described as a 4-anilidopiperidine. Extensive structure-activity relationship (SAR) studies are well documented in scientific and patent literature.[1-3] Some additional 4-anilidopiperidines are safely used in human and/or veterinary medicine (such as alfentanil, sufentanil, and remifentanil[4,5]) but most, however, have never advanced to clinical trials.
- Until recently, only a handful of 4-anilidopiperidines were found on the illicit market for recreational drug use within the U.S. and overseas.[6,7]
- The rapid proliferation on the gray market of new molecular entities containing the fentanyl scaffold—designed to skirt existing regulations—has certainly made the identification of new psychoactive substances a challenge and a necessity.

Results

- The majority of the new fentanyl varieties have been modified at the areas defined in the figure as R_1, R_2, R_3, and R_4 (Figure 1).

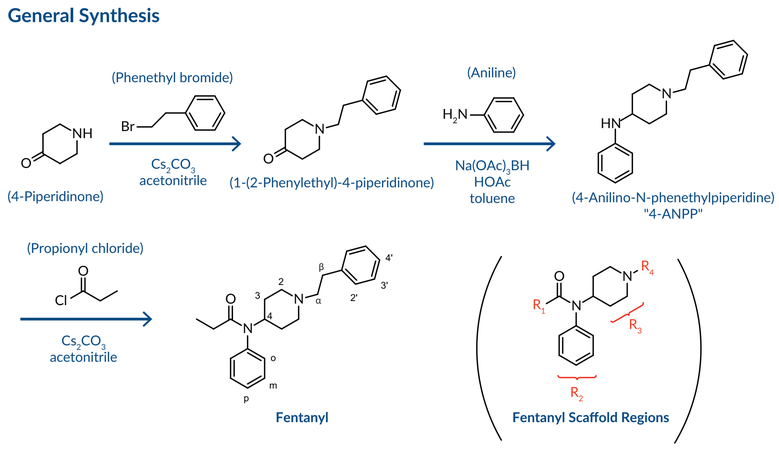
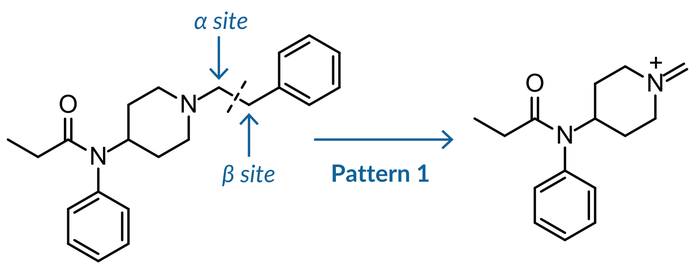


Figure 1 – General synthesis and comparison of fentanyl and the four different areas on the fentanyl scaffold that have been modified in new fentanyl varieties.

- Instrumentation:
  - Agilent 6890 Gas Chromatograph with a 5973 Mass Selective Detector (GC-MSD)
  - Restek Rtx-5MS (30 m x 0.32 mm x 0.5 µm) column, helium as carrier gas, flow at 2 ml per minute, and a split injection at a 15:1 ratio.
  - The temperature ramp: 50°C with a 1 minute hold, from 50°C to 300°C at 30°C per minute, holding at 300°C for 10 minutes. The inlet and transfer line temperatures are at 300°C. The MSD is tuned using Agilent's standard tune (S-Tune).
- *As new forensic materials go through the quality control process, the GC-MS data are added to Cayman's GC-MS Spectral library.*
- *Four major predictive patterns have emerged that provide clues to help in identifying unknown cases.*

Pattern 1:

Fentanyl-like compounds cleave between the α and β carbons of the ethyl heterocyclic linker, which results in the base peak (BP) value.



- Often with fentanyl compounds, the molecular weight (MW) ion is not generally detected in the spectra.
- In such cases, the BP corresponds to the ***primary cleavage*** of the compound, which occurs ***between the α/β site*** on the phenethyl moiety or similar chain.
- The presence of 91, the tropylium ion, in the spectra of fentanyl-like compounds strongly suggests the presence of a phenethyl group.
- A noteworthy exception to this first pattern occurs when the phenethyl moiety is replaced with a N-benzyl or N-methyl moiety, as with benzyl fentanyl.[8]
- In the case of benzyl fentanyl, the MW ion (322) is noticeable along with the BP of 91 resulting from the cleavage between the piperidine ring and the benzyl group (Figure 2).

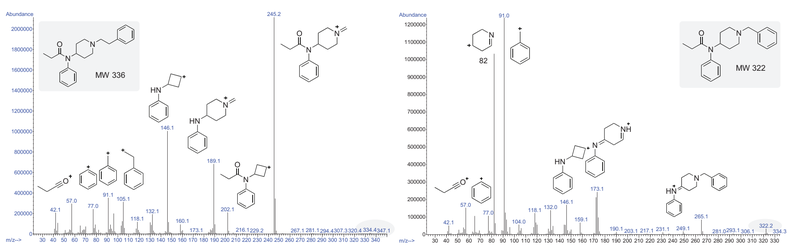
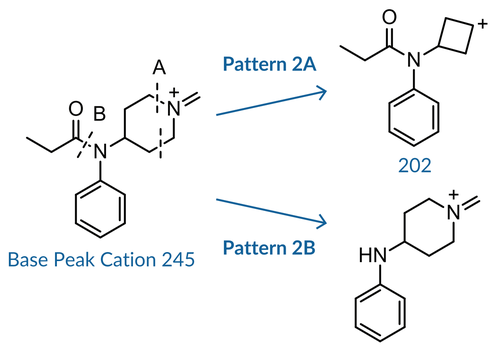


Figure 2 – A comparison of the GC-MS of fentanyl with its corresponding cation fragments (left) and the GC-MS of benzyl fentanyl (right).

Pattern 2:

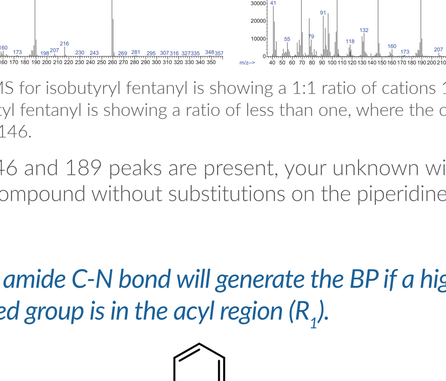
Additional cleavage of the BP cation occurs along the piperidine ring and at the amide C-N bond.



- The BP cation undergoes ***additional cleavage in two areas.***
- The first is ***along the piperidine ring***, Pattern 2A, eliminating the nitrogen and two carbons to form a cyclobutyl cation (mass 202 for fentanyl).[8,10]
- The second cleavage occurs along the ***C-N amide bond*** to produce a peak with an abundance of 30-60% of the BP (mass 189 for fentanyl), Pattern 2B.[8]

Pattern 3:

Subsequent cleavage at either the piperidine ring or the amide C-N bond of the secondary fragments results in a third characteristic fragment.



- The third characteristic fragment results from ***cleavage along the amide bond and additional cleavage within the piperidine ring.***
- The ratio between 146 and 189 is almost always greater than one unless there is a branched alkyl group or a closed alkyl ring system as seen with isobutyryl fentanyl and cyclopentyl fentanyl (Figure 3).

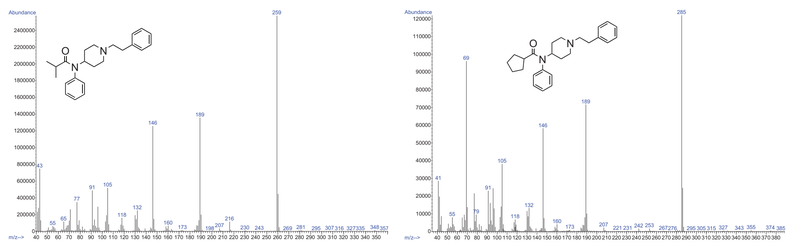
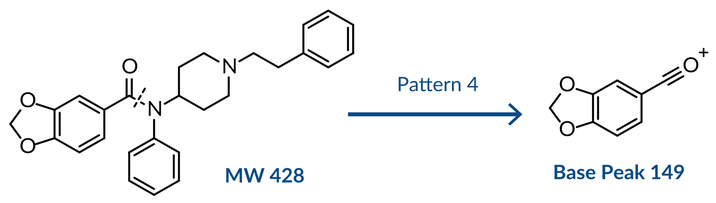


Figure 3 – The GC-MS for isobutyryl fentanyl is showing a 1:1 ratio of cations 146 to 189, while the GC-MS for cyclopentyl fentanyl is showing a ratio of less than one, where the cation 189 is in larger abundance than the 146.

- Thus, if the 146 and 189 peaks are present, your unknown will most likely be a fentanyl-like compound without substitutions on the piperidine or aniline groups.

Pattern 4:

Cleavage at the amide C-N bond will generate the BP if a highly stabilized or highly substituted group is in the acyl region (R_1).



- This is the case for furanyl fentanyl (95) and benzodioxole fentanyl (149).
- Incidentally, cleavage also occurs at the α/β site for furanyl fentanyl, benzodioxole fentanyl, phenyl fentanyl, and tetramethyl cyclopropyl fentanyl, generating less abundant fragments 283, 337, 293, and 313, respectively (Figure 4).

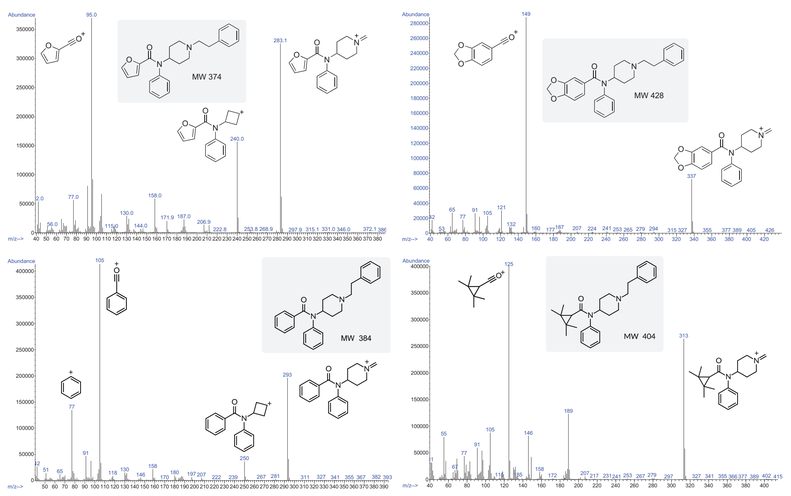


Figure 4 – Cleavage at the amide C-N bond will generate the BP if a highly stabilized group has been substituted in the acyl region (R_1). The primary cleavage of the highly resonance-stabilized cation gives the BP fragments shown at 95 and 149 for furanyl fentanyl (top left), benzodioxole fentanyl (top right), phenyl fentanyl (bottom left), and tetramethyl cyclopropyl fentanyl (bottom right).

References

1. Huang, B., Deutsche, K.H., Lalinde, N.L., *et al.* European Patent EP 0160422 B1 (1985).
2. Vardanyan, R.S. and Hruby, V.J. *Future Med. Chem.* **6(4)**, 385-412 (2014).
3. Vuckovic, S., Prostran, M., Ivanovic, M., *et al. Curr. Med. Chem.* **16(19)**, 2468-2474 (2009).
4. Wang, L. and Bernert, J.T. *J. Anal. Toxicol.* **30(5)**, 335-341 (2006).
5. Valaer, A.K., Huber, T., Andurkar, S.V., *et al. J. Chromatogr. Sci.* **35(10)**, 461-466 (1997).
6. Higashikawa, Y. and Suzuki, S. *Forensic Toxicol.* **26(1)**, 1-5 (2008).
7. Katselou, M., Papoutsis, I., Nikolaou, P., *et al. Forensic Toxicol.* **34(2)**, 201-212 (2016).
8. Ohta, H., Suzuki, S., and Ogasawara, K.J. *Anal. Toxicol.* **23(4)**, 280-285 (1999).
9. Breindahl, T., Kimergard, A., Andreasen, M.F., *et al. Drug Test. Anal.* **9(3)**, 415-422 (2017).
10. Goromaru, T., Katashima, M., Matsuura, H., *et al. Chem. Pharm. Bull.* **33(9)**, 3922-3928 (1985).

Exceptions

While most fentanyl-type unknowns may be discerned using these predictive patterns, there are always a few exceptions to the rules. For instance, when there is a substitution in the 4-position within the R_3 region on the piperidine ring, such as carfentanil. Pattern 1 still applies because the BP is shown at 303, yet the functional group in the 4-position is either eliminated during fragmentation or is retained, causing rearrangement leading to other fragments. Further investigation is needed for compounds similar to carfentanil that have substitutions in the 4-position on the piperidine ring (Figure 5).

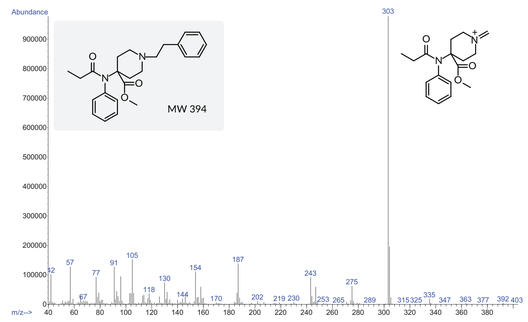


Figure 5 – GC-MS of carfentanil.

Conclusion

In summary, by following the patterns outlined above (and taking into consideration notable exceptions) we hope that we have provided useful tips for interpreting the GC-MS of novel 4-anilidopiperidines.

Table 1:

Comprehensive list of fentanyl-like compounds with corresponding molecular weight (MW) and Base Peaks (BP).

Item No.	Product Name	MW (free base)	BP
22752	*para*-fluoro Tetrahydrofuran fentanyl HCl	396.5	305
19410	Carfentanil	394.5	303
22390	Cyclohexyl fentanyl HCl	390.5	299
20018	*para*-Chloroisobutyryl fentanyl HCl	384.9	293
18089	*para*-methoxy-Butyryl fentanyl HCl	380.5	289
20859	Tetrahydrofuran fentanyl HCl	378.5	287
22664	Tetrahydrofuran fentanyl 3-tetrahydro-furancarboxamide HCl	378.5	287
20439	Cyclopentyl fentanyl HCl	376.5	285
20034	*para*-Chlorofentanyl HCl	370.9	279
21740	*meta*-fluoro Methoxyacetyl fentanyl HCl	370.4	279
21741	*para*-fluoro Methoxyacetyl fentanyl HCl	370.4	279
18583	Ocfentanil HCl	370.4	279
19313	FIBF HCl	368.5	277
19566	*meta*-Fluorobutyryl fentanyl HCl	368.5	277
19567	*ortho*-Fluorobutyryl fentanyl HCl	368.5	277
17049	*para*-Fluorobutyryl fentanyl HCl	368.5	277
20206	*meta*-Fluoroisobutyryl fentanyl HCl	368.5	277
20207	*ortho*-Fluoroisobutyryl fentanyl HCl	368.5	277
22749	*para*-fluoro Cyclopropyl fentanyl HCl	366.4	275
20035	*para*-Methoxyfentanyl HCl	366.5	275
20818	α-methyl Butyryl fentanyl HCl	364.5	273
20817	(±)-*cis*-3-methyl Butyryl fentanyl HCl	364.5	273
18934	Valeryl fentanyl HCl	364.5	273
22389	Cyclobutyl fentanyl HCl	362.5	271
21952	3-Fluorofentanyl HCl	354.4	263
19424	*meta*-Fluorofentanyl HCl	354.4	263
19425	*ortho*-Fluorofentanyl HCl	354.4	263
15496	*para*-Fluorofentanyl HCl	354.4	263
21932	*ortho*-fluoro Acrylfentanyl HCl	352.4	261
21116	*para*-fluoro Acrylfentanyl	352.5	261
20782	Methoxyacetyl fentanyl HCl	352.4	261
20821	α-methyl Thiofentanyl HCl	356.5	259
14728	Butyryl fentanyl HCl	350.5	259
18280	α-methyl Fentanyl HCl	350.5	259
9002747	(±)-*cis*-3-methyl Fentanyl HCl	350.5	259
9002482	(±)-*trans*-3-methyl Fentanyl HCl	350.5	259
18584	Isobutyryl fentanyl HCl	350.5	259
22633	*meta*-Methylfentanyl HCl	350.5	259
22634	*ortho*-Methylfentanyl HCl	350.5	259
20038	*para*-Methylfentanyl HCl	350.5	259
21739	Cyclopropyl fentanyl HCl	348.4	257
17421	β-Hydroxythiofentanyl HCl	358.5	245
20789	β-hydroxy Fentanyl HCl	352.4	245
9002860	β-methyl Fentanyl HCl	350.5	245
21914	4'-methyl Fentanyl HCl	350.5	245
20786	Thiofentanyl HCl	342.5	245
20816	α-methyl Acetyl fentanyl HCl	336.4	245
14719	Fentanyl HCl	336.4	245
20819	Furanylethyl fentanyl HCl	326.4	245
19312	Acrylfentanyl HCl	334.4	243
9002271	4'-methyl Acetyl fentanyl HCl	336.4	231
ISO00128	Acetyl fentanyl HCl	322.4	231
20858	Benzodioxole fentanyl	428.5	149
22744	2,2,3,3-tetramethyl-Cyclopropyl fentanyl HCl	404.6	125
22551	Phenyl fentanyl HCl	384.5	105
20785	Thienyl fentanyl HCl	328.4	97
22779	*ortho*-methyl Furanyl fentanyl HCl	388.5	95
18705	Furanyl fentanyl HCl	374.4	95
21213	Furanyl fentanyl 3-furancarboxamide isomer HCl	374.4	95
20350	Benzyl Carfentanil HCl	380.4	91
19883	Benzyl fentanyl HCl	322.4	91
22660	Benzyl Acrylfentanyl HCl	320.4	91