

Distinguishing isomers of fentanyl analogs using FT-Raman

Authors

Shea A Schleman,¹ Manuel Gomez,¹
Suja Sukumaran,² Rui Chen²

¹Albuquerque Police Department,
Albuquerque, NM

²Thermo Fisher Scientific, Madison, WI

Key words

FT-Raman spectroscopy, fentanyl,
fentanyl analogs, constitutional isomers,
geometric isomers, library search

Thermo Fisher solutions

Thermo Scientific™ Nicolet™ iS50 FTIR
Spectrometer, Thermo Scientific™
Nicolet™ iS50 FT-Raman module,
Thermo Scientific™ OMNIC™ Software.

Abstract

A pair of constitutional fentanyl analog (FA) isomers: butyryl and isobutyryl fentanyl, and a pair of geometric FA isomers: *cis*- and *trans*-3-methyl fentanyl were analyzed by FT-Raman. Upon examination of the FT-Raman spectra of fentanyl and the isomers, it is found that the fingerprint region is dominated by the features from the vibration modes of the two terminal benzene rings. These features are less discriminating for the FA isomers where the structural variations reside in the amide and piperidine moieties of the fentanyl structural scaffold. Instead, the spectral features in the low frequency region (250–400 cm⁻¹) from the δ (C-C) of aliphatic chains are the key differentiators between the isomers. The minute structural variations

between isomers results in significant differences in the Raman spectra allowing for positive identification and effective discrimination of the FA isomers using FT-Raman.

Introduction

Illicitly manufactured fentanyl and fentanyl analogs (FAs) is becoming a major driver of opioid overdose. Since 2013, an unprecedented surge in fentanyl overdose deaths has been caused by heroin laced with illicitly produced fentanyl and/or FAs sold as heroin. The US Drug Enforcement Agency's National Forensic Laboratory Information System reported a >300% increase in fentanyl.^{1,2} In addition to the high potency of fentanyl and many FAs, the law enforcement agencies are also constantly on their toes to keep up with the rapid and continued emergence of new FAs. Minor modifications to the fentanyl's structural scaffold by illicit drug manufacturers could yield novel and potent FAs that outpace the list of sanctioned compounds.

The challenges in the screening, detection, and identification of synthetic opioids warrant the use of multiple analytical techniques. For example, The CDC advises two-tiered testing, an enzyme-linked immunosorbent assay (ELISA) screen followed by gas chromatography/mass spectrometry (GC/MS) to identify the fentanyl compound in blood and urine in the cases of suspected overdose. Liquid

chromatography-mass spectrometry (LC-MS), liquid chromatography tandem mass spectrometry (LC-MS/MS), and ion mobility spectrometry (IMS) have also been used for screening and confirmation of fentanyl and FAs in case samples.³ However, unequivocal identification of the suspected FA can be complicated by isomeric compounds. Due to their structural similarity, some FA isomers have identical accurate mass, electron impact (EI), and electrospray ionization (ESI) fragmentation patterns, as well as close retention times on most gas and liquid chromatography methods.¹

Previously, we have demonstrated that FT-Raman spectroscopy is a safe, fast, and complementary technique for the detection and identification of powders and other apprehended materials including fentanyl.⁴ Raman analysis requires little to no sample preparation and allows for direct measurements through glass vials and evidence bags, greatly reducing the risk of lab personnel's accidental exposure to high potency fentanyl and FAs. It is nondestructive and allows the custody chain to be maintained from its seizure until the sample is placed in the evidence file.⁴

In this note, two examples of distinguishing FA isomers using FT-Raman spectroscopy are described. A pair of constitutional isomers: butyryl fentanyl and isobutyryl fentanyl, and a pair of geometric isomers: *cis*- and *trans*-3-methyl fentanyl were analyzed by FT-Raman. It is demonstrated that in addition to the fingerprint region, the Raman peaks in the low frequency region (250–400 cm^{-1}) resulting from the $\delta(\text{C-C})$ aliphatic chains are the key differentiators between the isomers.

Materials and methods

Fentanyl and FA standards were procured from Cayman Chemicals (Ann Arbor, Michigan) and analyzed as received. All fentanyl and regulated materials used in this study were analyzed at the Albuquerque Police Department Crime Laboratory. Samples were analyzed in double bags or glass vials and handled in compliance with the Scheduled I chemical handling protocols.

A Nicolet iS50 FTIR Spectrometer equipped with a calcium fluoride beam splitter and a Nicolet iS50 Raman module was used for all analyses. A 1064 nm laser with the power

set at 0.5 W was used as the excitation source. A total of 128 scans were co-added for each spectrum at 4 cm^{-1} resolution, with a total acquisition time of ~130s. The spectra were analyzed using OMNIC Software.

Results and discussion

Fentanyl and FAs are in the phenylpiperidine class of synthetic opioids. Fentanyl, specifically, has a phenyl propanamide group attached to the phenylpiperidine (Figure 1). Figure 2 shows the FT-Raman spectrum of fentanyl, with the major peaks annotated by their respective wavenumbers. Detailed Raman peak assignments of fentanyl using density function theory (DFT) calculation were reported by Leonard *et al.*⁵ In the fingerprint region (400–1600 cm^{-1}), a majority of the spectral features (e.g., 622, 743, 829, 1001, 1075, 1160, 1455, 1585, 1598 cm^{-1}) originate from the vibrations of the two terminal benzene rings. While strong in intensity, those peaks are undiscriminating for the FA isomers in the current study. It is noted that $\delta(\text{C-C})$ aliphatic chains produce strong Raman signals in the spectral region of 250–400 cm^{-1} (e.g. 267 cm^{-1} and 308 cm^{-1} in Figure 2).

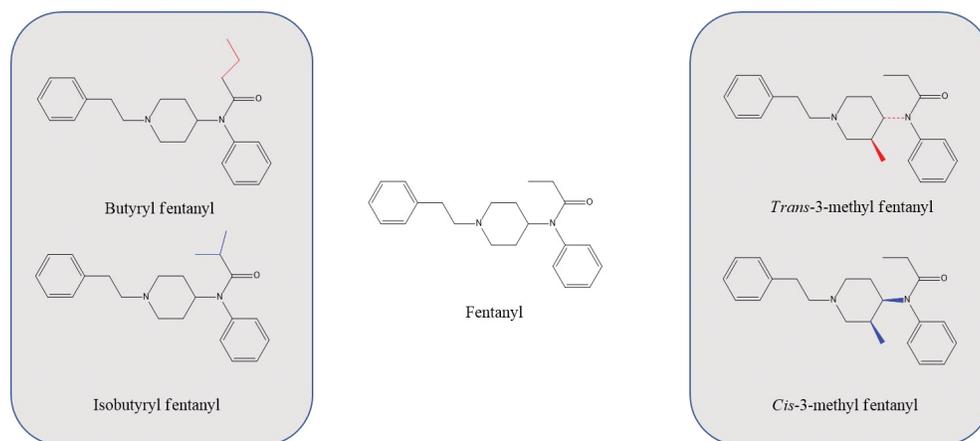


Figure 1. Structures of fentanyl and two pairs of FA isomers in this study.

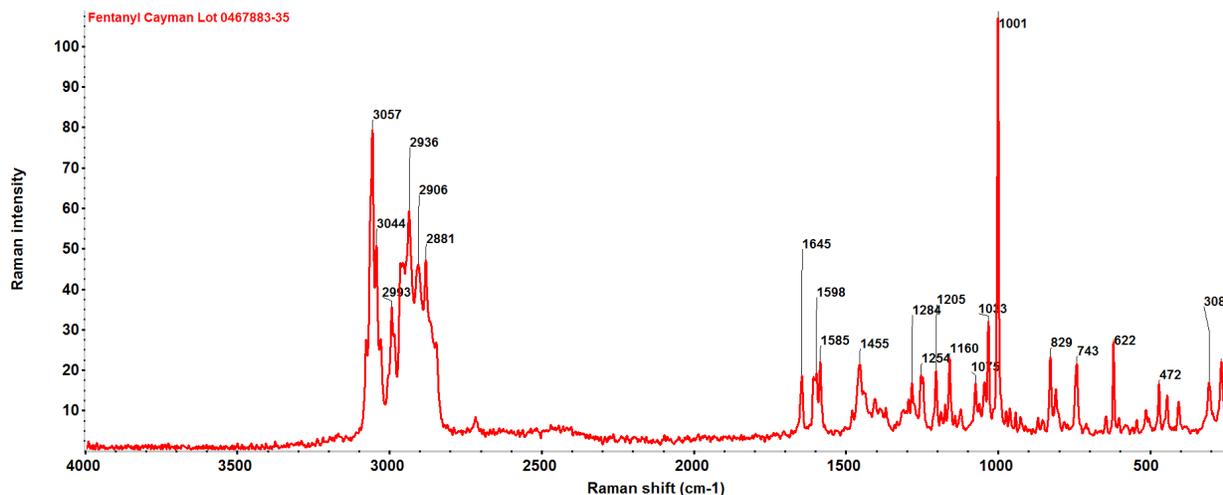


Figure 2. FT Raman spectrum of fentanyl.

Butyryl fentanyl and isobutyryl fentanyl are a pair of constitutional isomers that differ in the terminal groups attached to the amide moiety of the fentanyl scaffold (Figure 1). They are short-acting FAs approximately 30 times less potent than fentanyl. However, at least 40 confirmed overdose deaths involving butyryl fentanyl abuse were reported by the US DEA.¹ Butyryl fentanyl and isobutyryl fentanyl were classified as Schedule I narcotics in 2016 and 2018, respectively. Figure 3 shows the FT-Raman spectra of butyryl fentanyl and isobutyryl fentanyl. The peak at 735 cm⁻¹ resulting from the C-H symmetric bend is unique to isobutyryl fentanyl. Due to the different terminal groups attached to the carbonyl carbon, the positions of the carbonyl C-O stretch peaks are different between the isomers: 1655 cm⁻¹ for isobutyryl fentanyl and 1646 cm⁻¹ for butyryl fentanyl. In the low frequency region of 250–400 cm⁻¹, there are unique peaks at 323 and 372 cm⁻¹ for isobutyryl fentanyl, and 282 cm⁻¹ for butyryl fentanyl, although the exact assignments of these low frequency peaks are difficult to ascertain. The Raman spectra of butyryl fentanyl and isobutyryl fentanyl nonetheless exhibit sufficient variance to distinguish this pair of constitutional isomers.

Classified as Schedule I narcotics, 3-methylfentanyl (3-MF) emerged in 1984 in Allegheny County, Pennsylvania and was responsible for 16 fatal overdose cases.² 3-MF exists as a diastereomeric species: (±)-*cis*-3-MF and (±)-*trans*-3-MF. The *cis*- and *trans*- isomers contrast in the orientation of the methyl group attached to the piperidine ring (Figure 1). The two isomeric species has markedly different potencies: (±)-*trans*-3-MF has a similar potency to fentanyl and (±)-*cis*-3-MF is 8 times more potent than fentanyl.⁶ Figure 4 shows the FT-Raman spectra of *cis*-3-MF and *trans*-3-MF in the spectral range of 250–1800 cm⁻¹. While there are subtle differences between the isomers in the spectral region of 1200–1300 cm⁻¹, where the peaks are mainly from the C-N stretch and C-H twist of piperidine, more pronounced differences are observed in the low frequency region of 250–500 cm⁻¹. There are a group of peaks (265, 307, 409, 478 cm⁻¹) that are unique to *cis*-3-MF but noticeably missing for *trans*-3-MF.

Figure 3. Raman spectra of butyryl fentanyl and isobutyryl fentanyl in the spectral range of 250–1800 cm⁻¹. The shaded region in blue color highlights the spectral difference between the two compounds.

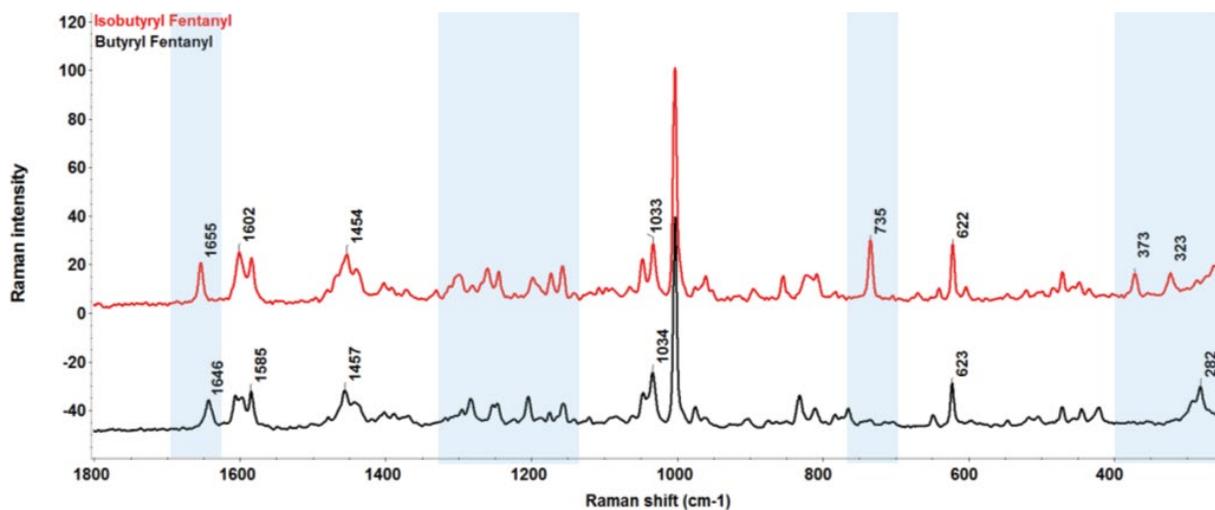
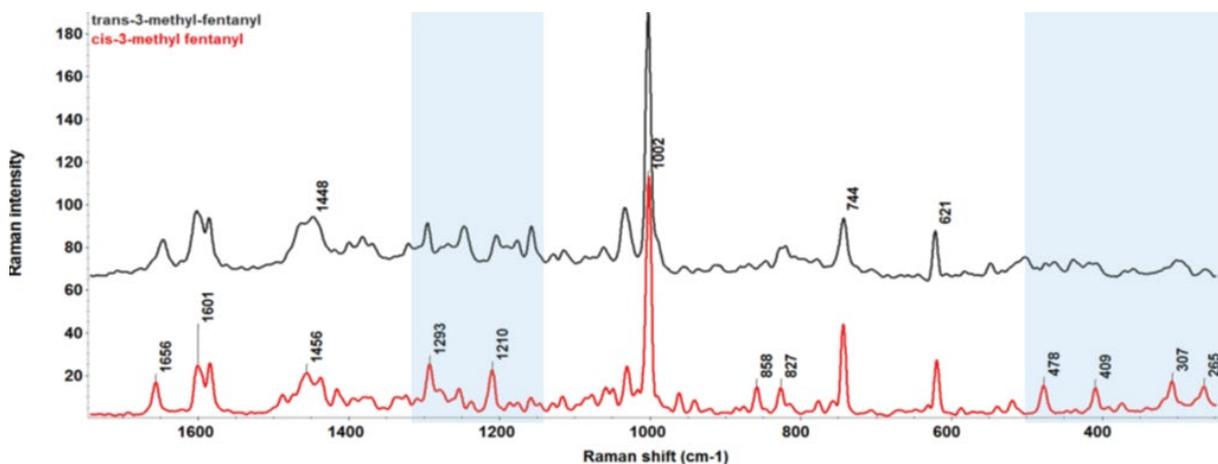


Figure 4. Raman spectra of *cis*-3-MF (red line) and *trans*-3-MF (black line) in the spectral range of 250–1800 cm⁻¹. The shaded region in blue color highlights the spectral difference between the two compounds.



The Raman spectra of fentanyl and the isomeric pairs were searched against a fentanyl library which contains fentanyl and 13 FAs. Details of this library will be published elsewhere. The search results are summarized in Table 1. As expected, all compounds were correctly identified. Interestingly, for each isomer, the 2nd best match is not its isomeric counterpart. For example, the 2nd best match for both butyryl fentanyl and isobutyryl fentanyl is cyclopentyl fentanyl. These results suggest that while the structural difference between isomers is minute, the spectral difference is significant enough for an effective discrimination of the isomers.

Conclusions

In this application note, fentanyl, a pair of constitutional FA isomers (butyryl and isobutyryl fentanyl), and a pair of geometric FA isomers: (*cis*- and *trans*-3-methyl fentanyl) were analyzed by FT-Raman. The fingerprint region of the Raman spectra is dominated by the features from the vibration modes of the two terminal benzene rings. These features, however, are less discriminating for the isomeric pairs. The spectral features in the low frequency region (250–400 cm⁻¹) from the δ (C-C) of aliphatic chains, on the other hand, are the key differentiators between the isomers. Library searches against a fentanyl library provided strong correlations for all compounds. More importantly, the 2nd best match for each isomer from the library search is not its isomeric counterpart. The minute structural variations between isomers result in significant differences in the Raman spectra, allowing for positive identification and effective discrimination of the isomers using FT-Raman. FT-Raman spectroscopy is a safe, fast, and discriminating analytical technique for the detection and identification of fentanyl and fentanyl analogs.



Table 1. Search results against the fentanyl library.

Compound	Top match	2 nd best match
Butyryl fentanyl	Butyryl fentanyl	Cyclopentyl fentanyl
Isobutyryl fentanyl	Isobutyryl fentanyl	Cyclopentyl fentanyl
Trans-3-methyl fentanyl	Trans-3-methyl fentanyl	Butyryl fentanyl
Cis-3-methyl fentanyl	Cis-3-methyl fentanyl	Fentanyl
Fentanyl	Fentanyl	Acetyl fentanyl

References

- Paul J. Jannetto, Anders Helander, Uttam Garg, Gregory C. Janis, Bruce Goldberger, Hemamalini Ketha, *The Fentanyl Epidemic and Evolution of Fentanyl Analogs in the United States and the European Union*, *Clinical Chemistry*, 2019, 65(2), 242–253.
- Patil Armenian, Kathy T. Vo, Jill Barr-Walker, Kara L. Lynch, *Fentanyl, fentanyl analogs and novel synthetic opioids: A comprehensive review*, *Neuropharmacology*, 2018 May 15;134(Pt A):121–132.
- Edward Sisco, Jennifer Verkouteren, Jessica Staymates, Jeffrey Lawrence, *Rapid Detection of Fentanyl, Fentanyl Analogues, and Opioids for on-Site or Laboratory Based Drug Seizure Screening using Thermal Desorption DART-MS and Ion Mobility Spectrometry*, *Forensic Chemistry*, Volume 4, June 2017, 108–115.
- Suja Sukumaran, Rui Chen, Shea A Schleman, Laura J Bell, *FT-Raman: an invaluable addition to the forensic arsenal to combat the opioid epidemic*, APPLICATION NOTE AN52304, Thermo Fisher Scientific
- Jennifer Leonard, Abed Haddad, Omar Green, Ronald L. Birke, Thomas Kubic, Ali Kocak, John R. Lombardi, *SERS, Raman, and DFT analyses of fentanyl and carfentanyl: Toward detection of trace samples*, *J Raman Spectrosc.* 2017;48:1323–329.
- Melissa F. Fogarty, Donna M. Papsun, Barry K. Logan, *Analysis of cis and trans 3-methylfentanyl by liquid chromatography–high resolution mass spectrometry and findings in forensic toxicology casework*, *Drug Test Anal.* 2018;10:1474–1482.

Find out more at thermofisher.com/raman