

Detection of para, ortho, meta-fluorofentanyl by surface-enhanced Raman spectroscopy

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ABSTRACT

The abuse of fentanyl and fentanyl analogs is becoming a global problem, with over 70,000 deaths reported in the USA, and there is evidence of its abuse beginning to appear in Europe and other areas. Fentanyl is 100 times more potent than morphine, and there are fentanyl analogs that are even more dangerous. Therefore, detecting and differentiating fentanyl isomers is critical in tracking this epidemic of drug use. Surface-enhanced Raman spectroscopy (SERS) is a useful method for detecting fentanyl isomers due to its capability of yielding spectroscopic fingerprints of the analytes. In this study, theoretical and experimental methods were compared in order to distinguish para fluorofentanyl, which is one of the most common fentanyl analogues in illicit mixtures, and its isomers ortho fluorofentanyl and meta fluorofentanyl. First, density functional theory (DFT) calculations were performed to define which vibrational peaks can be utilized in the identity of these analogs. The spectra obtained from the theoretical calculations were then compared with the spectra obtained from the Normal Raman and SERS experiments. To enhance sensitivity, bimetallic gold/silver nanostars (Au/Ag NS) were synthesized to provide the SERS enhancement along with magnesium chloride or potassium bromide aggregating agents with limits of detection in the low ng/mL range. The LOD value for para-fluorofentanyl is 3 ng/mL. The obtained results show that SERS is a successful technique to detect isomers of fentanyl analogs.

1. Introduction

The illegal use of drugs and stimulants and the resulting deaths from drug overdose are increasing rapidly all over the world, leading to an international crisis. While the number of opioid users worldwide was 26–36 million in 2010, this amount has doubled to 61 million in 2020 [1]. Due to their high potency, synthetic opioids such as fentanyl and fentanyl analogs cause more deadly results, with 70,000 people dying of synthetic opioid overdose in the USA in 2021. As a result, there is increasing concern that the abuse of this drug may spread to opioid users across the world [2].

In addition to fentanyl, the number of reports showing deaths due to overdose caused by fentanyl analogs is also increasing. At least one type of fentanyl analog was found in approximately 20 % of drug overdose deaths reported in the USA between 2016 and 2018 [3]. According to the reports of NFLIS, fluorofentanyl, and its isomers are among the analogs whose use is increasing rapidly. While the first report on the use of fluorofentanyl was recorded in 2016, this number increased to 167 in 2020. The use of fluorofentanyl and its isomers increased dramatically in

2021, with 10,340 reports registered in the NFLIS [4]. Ulens et al. have reported that para fluorofentanyl is more effective than fentanyl [5]. According to another study, the toxic effects of fentanyl analog isomers differ according to para, ortho, and meta positions (shown in Fig. 1). The results of Kanamori et al. have shown that fluorinated analogs in the ortho position are more likely to cause overdose deaths than the para and meta positions [6]. These findings highlight the importance of detecting and differentiating these isomers.

There are various methods for detecting fentanyl and fentanyl analogs. However, not every method is suitable for the determination of isomers. GC–MS and LC–MS techniques, which are the most commonly used methods in the detection of fentanyl and fentanyl analogs, are not always successful in separating isomers that have the same mass number due to similarity in fragmentation patterns and retention times. On the other hand, spectroscopic methods such as IR and Raman spectroscopy have significant advantages in detecting fentanyl and fentanyl analogs, based on their ability to detect subtle vibrational differences due to molecular structure. Furthermore, the analysis time is very short, and there is minimal sample preparation. Unfortunately, standard Raman

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spectroscopy suffers from poor sensitivity. However, implementation of SERS protocols permits sensitivity enhancements up to 7 orders of magnitude, making it possible to detect samples at the sub-part per billion level [7]. Recently, SERS-based methods have been used to detect fentanyl and fentanyl analogs at low concentrations in illicit drug mixtures [8–11].

This study aims to qualitatively distinguish para fluorofentanyl, and its isomers, ortho fluorofentanyl and meta fluorofentanyl, by comparing theoretical and experimental methods using surface-enhanced Raman spectroscopy.

2. Materials and methods

2.1. DFT calculations

Although B3LYP [12] and 6-311G** [13] is the most commonly used method and basis set duo in DFT, the calculation of Raman spectra of fentanyl analogs utilized hybrid variation-correlation functional method wB97XD [14] and the cc-pVTZ [15] basis set. Wang et al. compared these two methods and showed that the second method gave better results in the calculation of fentanyl and fentanyl analog spectra [16]. This is presumably due to the presence of multiple Van der Waals interactions on the fentanyl and fentanyl analogs' structure. B3LYP cannot calculate dispersion forces such as hydrogen bonds and dipole–dipole interactions as well as wB97XD. Also, cc-pvtz can calculate the correlation energy better than the 6-311G**. In this work, Raman frequencies and activities were converted to simulated spectra by GaussView 6.1. Optimized geometry and vibration modes were also visualized by GaussView 6.1 [17].

2.2. Normal Raman experiments

The fentanyl analogs used, para-fluorofentanyl (PFF), ortho-fluorofentanyl (OFF), and meta-fluorofentanyl (MFF) were included in the 'Fentanyl Analog Screening (FAS) Kits' purchased from Cayman Chemical. These analogs were dissolved in methanol (MeOH) purchased from Sigma Aldrich. Solutions of 0.4 mg/mL fentanyl analogs were prepared using 500 μ L of MeOH. In order for the analogs to form droplets, 20 μ L of each fentanyl analog solution was mixed with 20 μ L of water at a ratio of 1:1. 1 μ L of this prepared diluted mixture of the fentanyl analogs was added to a 3 cm² section of aluminum foil. After the added drop evaporated, 1 μ L of the mixture was added to the same spot. This process continued until the analogs formed a solid form on the aluminum foil.

Normal Raman measurements were taken with a benchtop Jasco

NRS 4100 Confocal Raman Microscope with a CCD detector. Spectra were obtained using a single accumulation in 120 s with 20 $\times$ and 50 $\times$ lenses and a 33 mW laser source at a wavelength of 785 nm. The baselines of these spectra were corrected in the Origin2021b program, and the signal/noise ratio was reduced. Baseline correction was done by subtracting the baseline in user-defined mode, and the signal/noise ratio was reduced by smoothing the data using the Savitzky-Golay method in the software.

2.3. Synthesis of bimetallic (Au/Ag) nanostars

Bimetallic Au/Ag nanostars were synthesized by the method described by He et al. [18] and modified by Wang et al. [16] Chlorauric acid (HAuCl₄) used for nanostar synthesis was purchased from Acros Organics, silver nitrate (AgNO₃) and L-ascorbic acid (L-AA) were purchased from Fisher Chemical, and sodium carbonate monohydrate (Na₂CO₃·H₂O) was purchased from Thermo Scientific.

10^{−2} M 36 μ L HAuCl₄ and 10^{−2} M 2 μ L AgNO₃ were added to 1 mL of water and vortexed for 10 s. Next, 6 μ L of 10^{−1} M L-AA was added as a reducing agent and vortexed for 20 s. At this time, it was observed that the solution turned blue. Finally, 1 M 1 μ L Na₂CO₃ was added, and the sample was vortexed for another 5 s to stabilize the nanostars [19]. The pH of the nanostars solution was 6.

2.4. SERS experiments

Among the inorganic salts used for aggregation in SERS experiments, magnesium chloride (MgCl₂) was purchased from Fisher Chemical, and potassium bromide (KBr) was purchased from Sigma Aldrich. SERS spectra were taken using the ThermoScientific First Defender RM device. This device also has a laser source of 100 mW at a wavelength of 785 nm, and software that automatically determines the measurement time and accumulation.

Two different protocols were examined. The first, described by Wang et al., involved dilution of fentanyl analog solutions with water to a concentration of 10 μ g/mL. 2.5 μ L of 1.67 M MgCl₂ was added to 245 μ L of previously synthesized nanostars and let sit for 5 min to ensure the nanostars were aggregated. Afterwards, 2.5 μ L of fentanyl analog solution was added and let sit for an additional 5 min. Then, the solution was transferred into a quartz cuvette for measurement with the portable Raman spectrometer [16].

In the second protocol, we modified a method developed by Kimani et al. Fentanyl analog solutions were diluted with water to a concentration of 100 μ g/mL. 20 μ L of this solution was added to 180 μ L of water, and after vortexing, 180 μ L of nanostars were added to the

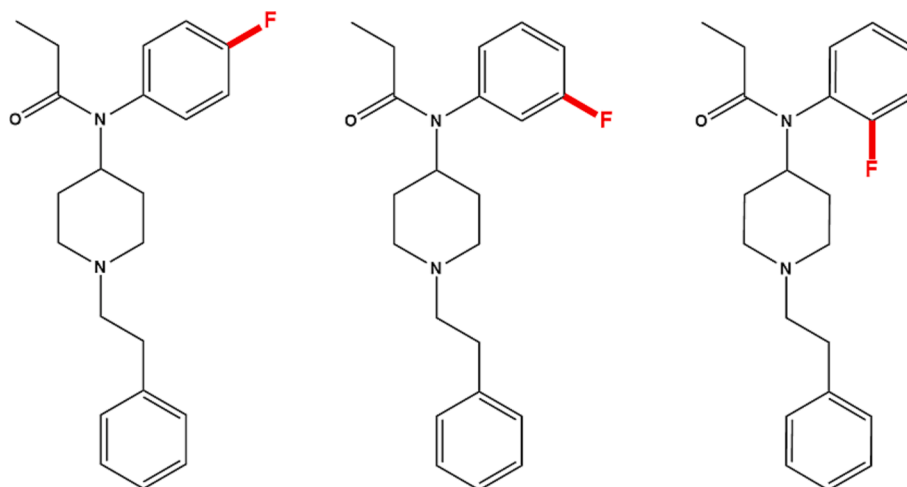


Fig. 1. Para, ortho, meta position of fluorofentanyl, respectively.

solution. After adding 5 μL of 3 M KBr, the solution was again vortexed and then was transferred to a quartz cuvette for measurement with the portable Ramans Spectrometer [20].

The KBr procedure, which provided better signal amplification, was used to determine the limit of detection (LOD) for para-fluorofentanyl. Para-fluorofentanyl solutions were prepared at various concentrations between 10 $\mu\text{g/mL}$ and 1 ng/mL . Using the relationship between para fluorofentanyl concentrations and Raman intensity at 823 cm^{-1} , a calibration curve was created, and the LOD and coefficient of determination (R^2) were calculated.

3. Results and discussion

3.1. DFT calculations and vibrational modes assignment

Theoretical spectra of fentanyl analogs were obtained by Density Functional Theory (DFT) calculations using the wB97XD method and the cc-pVTZ basis set. The Raman frequency calculations were done after performing an optimization of molecular geometries. DFT neglects non-harmonic motion in the vibrational frequencies of molecules. Thus, the theoretically obtained frequencies are larger than the experimentally observed fundamental frequencies. A multiplier, the scaling factor, is used to align the results of the DFT calculations with the experimental results. The scaling factor was calculated with the formula $\lambda = \nu_{\text{experimental}} / \omega_{\text{calculated}}$. Detailed scaling factor calculations for each fentanyl analog are shown in Table 1. The fact that the scaling factor is over 0.962 for all three analogs indicates that the applied DFT method gives results close to the experimental results. As Wang et al. [16] indicate, the wB97XD/cc-pVTZ method can be used to obtain theoretical Raman spectra of fentanyl analogs that have not been experimentally studied before.

Raman frequencies and activities were converted to simulated spectra by GaussView 6.1. The spectra of fentanyl analogs before applying the scaling factor are shown in Fig. 2-a-c. In Fig. 2-d, a comparison of theoretical Raman spectra of three analogs is given after

applying the scaling factor. In Fig. 2, the intensity is scaled to 100 %. It was concluded that these three isomers can be separated from each other theoretically by DFT calculations.

DFT calculations are a frequently used method to predict molecular vibrational modes. The vibrational modes of the molecules visualized by GaussView 6.1 were determined. Various symbols are used on the fentanyl molecule in Fig. 3 to avoid confusion in explaining vibrational modes.

As seen in Fig. 3, there are two non-interacting monosubstituted benzene rings at both ends of the fentanyl molecule and a benzene ring substituted with a piperidine ring in the middle. One of the nitrogen molecules (N_2) in its structure is bonded to the piperidine ring from one end, to the benzyl ring of B_2 from one end, and to the carbonyl ($\text{C}=\text{O}$) group from the other end to form an amide. The binding of the fluorine atom to the B_2 benzyl ring in the para, meta, and ortho positions constitutes the structure of the fentanyl analogs studied in this research.

The scaling factor was calculated from the common peaks observed in the computational and experimental spectra. In Table 2, common peaks of fentanyl analogs in DFT, NR, and SERS spectra are given, and vibrational modes of molecules at these peaks are determined. The symbols used when describing vibration modes are as follows: ν : stretching, δ : in-plane bending, σ : scissoring, ρ : rocking, γ : out-of-plane bending, τ : twisting, ω : wagging.

In the following descriptions, the given frequencies represent the DFT value with the scaling factor in Table 2. Since isomers have similar molecular structures and similar spectra, many molecular vibrational modes are also the same. Examining the DFT spectra by adding the scaling factor, there is a strong peak at about 1624 cm^{-1} for all three molecules. This peak is the result of a strong stretching within the carbon-carbon double bonds ($\text{C}=\text{C}$) in the B_1 and B_2 benzene rings. The peak occurring in the range of $991\text{--}996\text{ cm}^{-1}$ is one of the most important peaks for the detection of fentanyl and fentanyl analogues in the NR and SERS spectra. This peak is the result of in-plane bending vibration movements between $\text{C}=\text{C}$ bonds in B_1 and B_2 benzene rings. Another important peak in detecting fentanyl and fentanyl analogs

Table 1
Calculation of the scaling factor (Λ) for para, ortho and meta fluorofentanyl.

Analog	DFT	NR	NR*DFT	Sum of the experimental	DFT*DFT	Sum of the Calculated	Λ ($\nu^{\text{exp}}/\omega^{\text{cal}}$)
Para Fluorofentanyl	1687	1598	2,691,032	11,740,035	2,835,856	12,167,057	0,9649
	1506	1463	2,200,352		2,262,016		
	1238	1205	1,491,790		1,532,644		
	1210	1160	1,403,600		1,464,100		
	1076	1044	1,123,344		1,157,776		
	1066	1032	1,100,112		1,136,356		
	1030	1001	1,031,030		1,060,900		
	847	825	698,775		717,409		
Ortho Fluorofentanyl	1686	1604	2,704,344	15,917,465	2,842,596	16,545,590	0,9620
	1507	1463	2,204,741		2,271,049		
	1337	1299	1,736,763		1,787,569		
	1325	1258	1,666,850		1,755,625		
	1238	1202	1,488,076		1,532,644		
	1208	1155	1,395,240		1,459,264		
	1150	1092	1,255,800		1,322,500		
	1074	1042	1,119,108		1,153,476		
	1069	1030	1,101,070		1,142,761		
	859	827	710,393		737,881		
	735	728	535,080		540,225		
Meta Fluorofentanyl	1683	1600	2,692,800	11,668,410	2,832,489	12,090,792	0,9651
	1495	1455	2,175,225		2,235,025		
	1238	1199	1,484,362		1,532,644		
	1185	1154	1,367,490		1,404,225		
	1076	1038	1,116,888		1,157,776		
	1066	1030	1,097,980		1,136,356		
	1031	999	1,029,969		1,062,961		
	854	824	703,696		729,316		

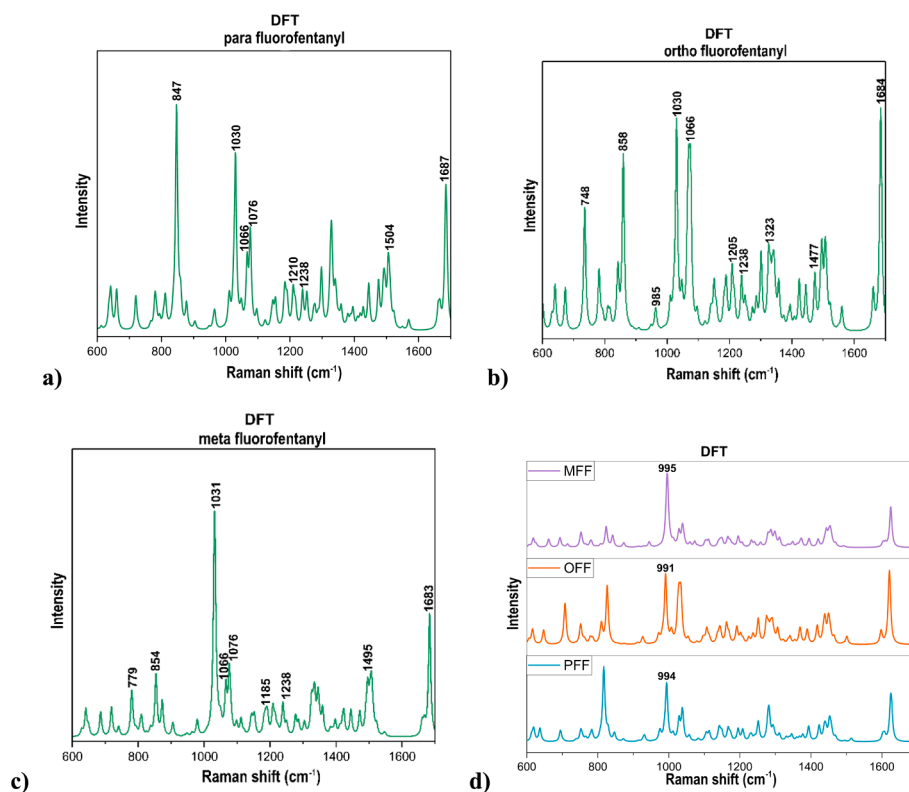


Fig. 2. DFT spectrum of a) para-fluorofentanylyl b) ortho-fluorofentanylyl c) meta-fluorofentanylyl without scaling factor, d) DFT spectra comparison of para, ortho, meta-fluorofentanylyl with scaling factor. The theoretical spectra were used to assign vibrational frequencies of each molecule.

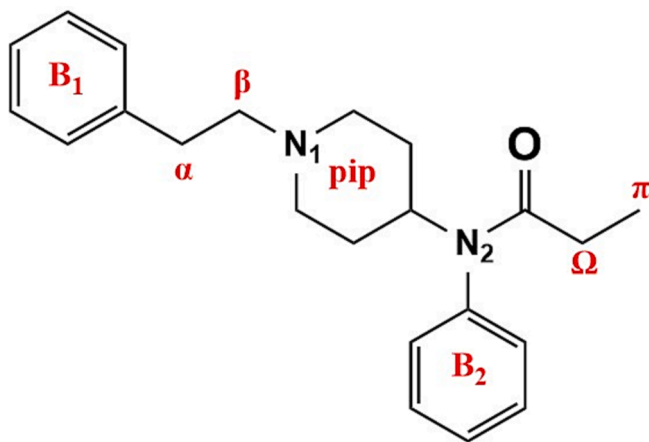


Fig. 3. Fentanyl molecule structure used to determine molecular vibrational modes.

occurs at 1028 cm^{-1} . It is formed due to stretching vibration movements between C=C bonds in B₁ and B₂ benzene rings. Another common vibrational mode occurred at 1194 cm^{-1} . Here, stretching is observed in the bond connecting the C_α and B₁ benzene rings, while wagging has occurred in the hydrogen atoms attached to C_α. In addition, there is stretching between the carbon atoms in the piperidine ring at the same wavenumber. Finally, a peak was observed at $1162\text{--}1167\text{ cm}^{-1}$ thanks to the twisting occurring in the CH₂s in the α, β, and piperidine rings and the out-of-plane bending occurring in the bond between the N₁ atom and the β carbon.

One of the most diagnostic vibrational modes of para fluorofentanylyl occurs in the molecule at 817 cm^{-1} . A strong stretching occurs in the bond between the B₂ benzene ring and the para-position fluorine atom,

causing the movement of the whole molecule. There is scissoring at the CH₂ groups in the α, β, and piperidine ring, causing a peak at 1450 cm^{-1} . Twisting also occurs in the CH₂s in the α, β, and piperidine rings, producing a characteristic peak at 1037 cm^{-1} .

When the vibration modes of meta fluorofentanylyl were examined, it was observed that the binding of the fluorine atom to the B₂ benzene ring at the meta position did not greatly affect the molecule. However, thanks to the stretching formed in the bond between the B₁ benzene ring and the α carbon, the whole molecule moved, causing the peak at 823 cm^{-1} to occur. The peak at 1442 cm^{-1} was formed by scissoring in the α, β, and Ω CH₂s and the in-plane bending of CH₃. Finally, the peak observed at 1038 cm^{-1} was formed by stretching between carbons in the piperidine ring and the strong stretching between the α and β carbons.

More vibration modes were detected in ortho fluorofentanylyl compared to other isomers. In addition to the common peaks mentioned above, the peaks that contribute to the vibration modes are as follows: The peak occurring at 1449 cm^{-1} is due to the scissoring of hydrogens in the methyl group at the tip of the molecule, the piperidine ring and the hydrogens attached to the α carbon. The wagging of hydrogens attached to the Ω carbon, the stretching between the carbon and N₂ where the B₂ benzene ring is attached to N₂, and the rocking vibrations in the B₂ benzene ring connected to it, away from the fluorine atom caused the peak at 1286 cm^{-1} . The wagging of hydrogens attached to the α carbon and the twisting of the hydrogens attached to the β carbon and in the piperidine ring formed another peak at 1274 cm^{-1} . It is formed by stretching between the carbon and N₂ to which it is attached. The rocking of hydrogen bonded to Ω and π carbons and stretching vibrations between α and β carbons formed a characteristic peak at 1033 cm^{-1} . The peak at 826 cm^{-1} is due to stretching vibrations in the bond between the B₁ benzene ring and the α carbon, which causes the movement of the whole molecule. Finally, the stretching between the carbon in the carboxyl group and the Ω carbon and the rocking vibrations of the hydrogens in the piperidine ring caused the observation at 707 cm^{-1} .

Table 2

Vibrational modes of para, ortho, meta-fluorofentanyl.

Para fluorofentanyl		CALCULATED			EXPERIMENTAL	
Description	Mode	DFT	DFTx 0,9649	NR	SERS	
ν (C=C) _{B1,B2}	124	1684	1624	1598	1600	
σ (CH ₂) _ω , σ (CH ₂) _β , σ (CH ₂) _{pip}	116	1506	1450	1460	1440	
ν (C _α -B ₁), ω (CH ₂) _α	85	1238	1194	1200	1204	
τ (CH ₂) _{pip} , τ (CH ₂) _{α,β} , γ (N ₁ -C _β)	83	1210	1167	1154	1156	
τ (CH ₂) _{pip} , τ (CH ₂) _{α,β}	78	1154	1037	1073	1102	
ν (C=C) _{B1}	70	1066	1028	1038	1033	
δ (C=C) _{B1}	66	1030	993	998	1002	
ν (F-C) _{B2} , δ (C=C) _{B2}	50	847	817	820	823	
Meta fluorofentanyl		CALCULATED			EXPERIMENTAL	
Description	Mode	DFT	DFTx 0,9651	NR	SERS	
ν (C=C) _{B1,B2}	125	1683	1624	1600	1599	
σ (CH ₂) _{ω,β} , σ (CH ₂) _Ω , δ (C _π H ₃)	110	1495	1442	1455	1442	
ν (C _α -B ₁), ω (CH ₂) _α	86	1238	1194	1199	1204	
τ (CH ₂) _{pip} , τ (CH ₂) _{α,β} , γ (N ₁ -C _β)	83	1208	1165	1154	1154	
ν (C _α -C _β), ν (C-C-C) _{pip}	71	1076	1038	1038	–	
ν (C=C) _{B1}	70	1065	1027	1030	1032	
δ (C=C) _{B1,B2}	66	1032	996	999	1003	
ν (C _α -B ₁)	51	853	823	824	827	
Orto fluorofentanyl		CALCULATED			EXPERIMENTAL	
Description	Mod	DFT	DFTx 0,9620	NR	SERS	
ν (C=C) _{B1,B2}	124	1684	1621	1604	1604	
σ (C _π H ₃), σ (CH ₂) _{pip} , σ (CH ₂) _α	115	1507	1449	1463	1442	
ω (CH ₂) _Ω , ν (N ₂ -C _{B2}), ρ (C-H) _{B2}	95	1337	1286	1299	1306	
ω (CH ₂) _ω , τ (CH ₂) _β , τ (CH ₂) _{pip}	93	1325	1274	1258	1262	
ν (C _α -B ₁), ω (CH ₂) _α , ν (C-C-C) _{pip}	85	1238	1191	1202	1204	
τ (CH ₂) _{pip} , τ (CH ₂) _{α,β} , γ (N ₁ -C _β)	83	1208	1162	1155	1154	
ν (N ₂ -C _{B2}), ρ (CH ₂)	77	1150	1106	1092	1089	
ν (C _α -C _β), τ (CH ₂) _Ω , τ (CH ₂) _π	71	1074	1033	1042	–	
ν (C=C) _{B1,B2}	70	1069	1028	1030	1030	
δ (C=C) _{B1}	66	1030	991	1001	1002	
ν (C _α -B ₁)	52	859	826	827	827	
ν (C-C _Ω), ρ (C-H) _{pip}	42	735	707	728	730	

Using this data, the spectra of fentanyl analogues can be predicted theoretically with DFT calculations, and the isomers can be separated from each other. In addition, although these very similar molecules have many peaks in common, the identification of specific vibrational modes can be used to separate the isomers.

3.2. Normal Raman and SERS results

After the theoretical calculations, normal Raman and SERS experiments were performed to determine the accuracy of the spectra. Using the Origin 2021 program (Origin(Pro), Version 2021b), baseline correction was performed to remove background signals found in the acquired averaged NR and SERS spectra to enable clearer and more accurate analysis of peaks. Also, smoothing was used to reduce noise and make the peaks more distinct.

Although the normal Raman and SERS spectra of fentanyl analogues generally matched, there was occasional shifts of a few cm⁻¹ between them. However, due to the low concentration of fentanyl analogues in the SERS experiments, some weak or moderate peaks observed in the NR

spectra could not be observed in the SERS spectra. On the other hand, some extra peaks not in the NR spectra appeared in the SERS spectra. This might be the result of the interaction of the nanoparticles used in the SERS experiments with the molecule on the surface.

As mentioned before, considering the molecular structure of para, meta, and ortho fluorofentanyl, these fentanyl analogues are structurally similar to each other, and the number of common peaks in Raman spectra is quite a few. However, even the change in the position of the fluorine atom in the benzene ring changes the molecular structure, so this change can be observed in Raman spectra.

The NR and SERS spectra of para fluorofentanyl are given in Fig. 4-a and -b, respectively. While defining the vibration modes, the presence of fluorine in the para position in the aniline ring affects the spectra, causing a very strong peak in the Raman spectrum. In the SERS spectrum, the intensity of this peak, which corresponds to 823 cm⁻¹, is almost the same as the intensity of the peak at 1002 cm⁻¹. This intense peak causes a stretching in the bond between the benzene ring and the fluorine atom attached in the para position to this benzene ring. The peaks occurring at 1038 cm⁻¹, 1102 cm⁻¹, 1154 cm⁻¹, 1200 cm⁻¹, and 1598 cm⁻¹ in normal Raman were also observed in the SERS spectrum with a maximum shift of 5 cm⁻¹. The peak at 1460 cm⁻¹ in NR shows a larger shift of 20 cm⁻¹, corresponding to 1440 cm⁻¹ in SERS. The peaks corresponding to 637 cm⁻¹, 716 cm⁻¹, 1073 cm⁻¹, 1257 cm⁻¹, and 1647 cm⁻¹ observed in the NR did not occur in the SERS spectrum. However, an extra peak was observed at 783 cm⁻¹ in the SERS spectrum. These effects are presumably due to the interactions between the nanoparticles and the analytes.

NR and SERS spectra of meta fluorofentanyl shown in Fig. 4-c and -d show that the peak around 820 cm⁻¹, which is strong in para fluorofentanyl, occurred around 824 cm⁻¹ as a much weaker peak in these spectra. This time, the presence of a fluorine atom didn't affect this vibrational mode. This peak occurred as a result of stretching in the bond between the B₁ benzene ring and the α carbon. Two peaks of the same intensity were observed at 1030 cm⁻¹ and 1038 cm⁻¹ in NR. These peaks formed a more intense single peak at 1032 cm⁻¹ in the SERS spectrum.

The NR and SERS spectra of ortho fluorofentanyl are shown in Fig. 4-e and -f. The peak at 827 cm⁻¹ in NR occurred at moderate intensity. The peak at 728 cm⁻¹ observed in the NR was much weaker at 730 cm⁻¹ in the SERS spectrum. The peaks observed at 1030 cm⁻¹ and 1042 cm⁻¹ in NR are shifted in meta fluorofentanyl and formed a single peak at 1030 cm⁻¹ in the SERS spectrum. The peak at 1463 cm⁻¹ is observed at 1442 cm⁻¹, showing a larger shift as in para fluorofentanyl.

The NR and SERS spectra of para, meta, and ortho fluorofentanyl isomers were similar. The most important peak determining the separation of these isomers from each other is the peak observed around 820 cm⁻¹. The presence of a fluorine atom in the benzene ring caused this peak to occur in every isomer, but the para, meta, or ortho position of the fluorine atom greatly affected the intensity of this peak. In addition, in ortho-fluorofentanyl, the double peaks are formed as a single peak at 1030 cm⁻¹, which is also one of the distinguishing factors. Finally, the peak intensity observed at approximately 1156 cm⁻¹ is higher in para fluorofentanyl than in other isomers. A comparison of the NR and SERS spectra of these isomers separately is shown in Fig. 5-a and -b.

In Fig. 6-a-c the DFT, NR, and SERS spectra of para, meta, and ortho fluorofentanyl are given together, respectively. The scaling factor has been considered in the DFT spectra in these graphs. Overall, the theoretical and experimental generally match each other.

Finally, the spectra of para fluorofentanyl was used to compare two different SERS protocols. Although the concentration of para fluorofentanyl is the same, signal enhancement in experiments using KBr was much higher than in experiments using MgCl₂ (Fig. 7). This may result from the KBr treatment resulting in higher levels of aggregation and concomitantly more hotspots.

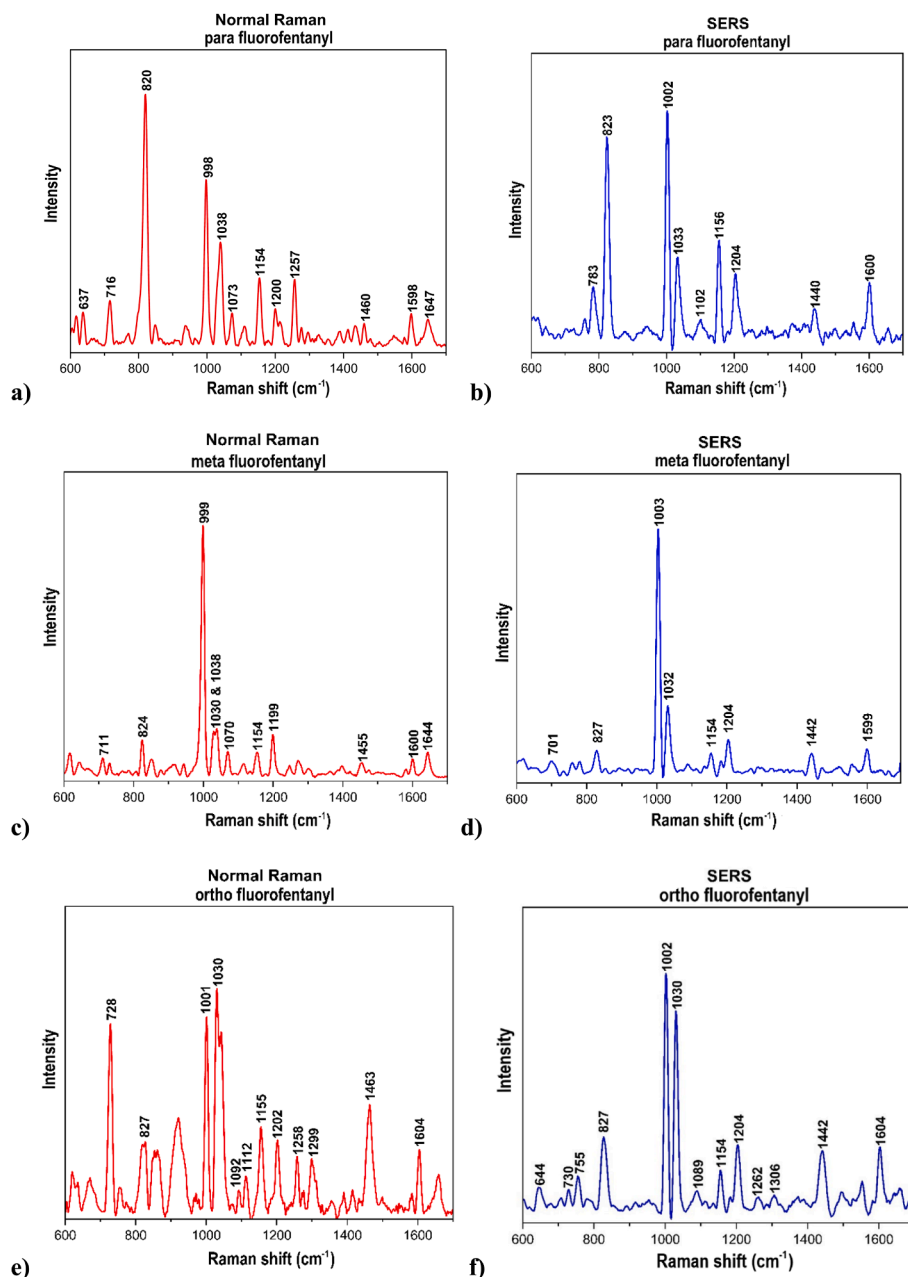


Fig. 4. Normal Raman and SERS spectra of para, meta, ortho fluorofentanyl.

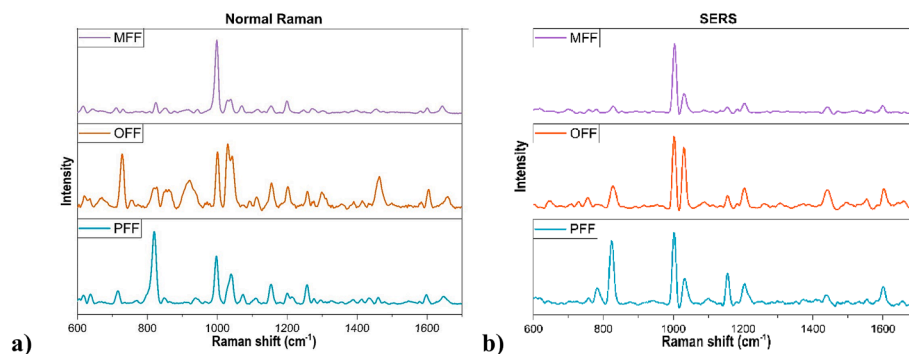


Fig. 5. Comparison of a) normal Raman and b) SERS spectra of para, ortho, meta-fluorofentanyl. These isomers can be separated from each other based on the differences at the peaks around 823 cm^{-1} , 1030 cm^{-1} , and 1154 cm^{-1} .

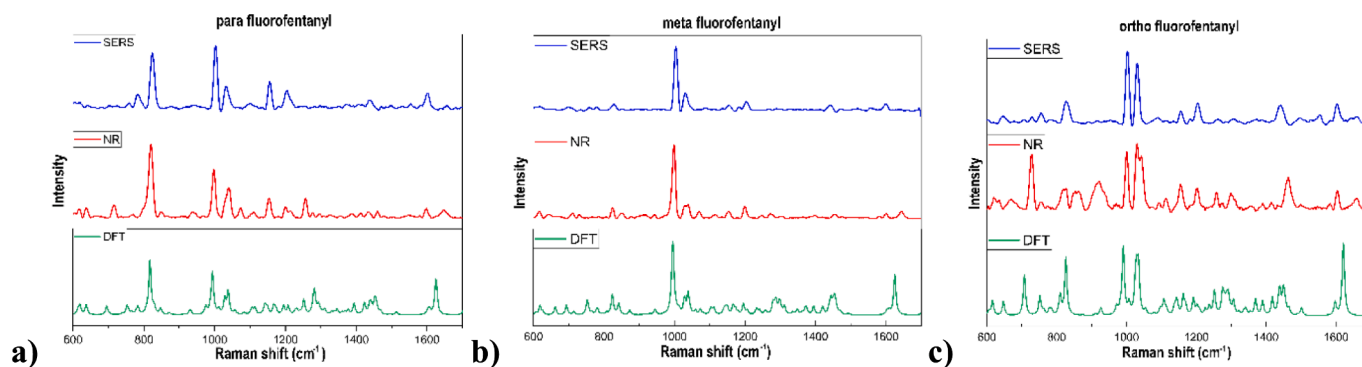


Fig. 6. DFT, NR, and SERS spectra of a) para-fluorofentanyl, b) meta-fluorofentanyl, c) ortho-fluorofentanyl. The results show the alignment between the theoretical spectra and the experimental results from normal Raman and SERS spectra.

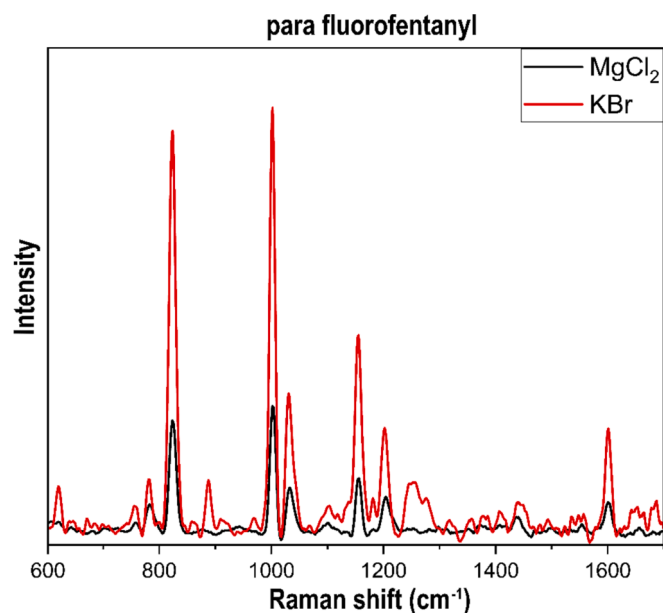


Fig. 7. Comparing two SERS protocols that utilize different inorganic salts. The KBr treatment is rapid (1 min), and provides a more enhanced spectra than the MgCl_2 protocol (10 min).

3.3. LOD and LOQ calculations

Fentanyl and its analogs can be found in very low concentrations in illicit drug mixtures. Therefore, the KBr procedure with greater signal enhancement was used to determine the limit of detection (LOD) and the limit of quantification (LOQ). Various concentrations of para fluorofentanyl between 10000 ng/mL and 1 ng/mL were prepared.

Since the peak corresponding to 823 cm^{-1} in para fluorofentanyl is the most distinctive and the strongest peak, a linear calibration curve was generated for this peak with Raman intensities at different concentrations (1 ng/mL–10 ng/mL) (Fig. 8). LOD and LOQ values were calculated by the equation of this curve, where the coefficient of determination (R^2) was 0.98. As a result, the LOD of para fluorofentanyl was determined as 3.3 ng/mL, whereas the LOQ value was 9.2 ng/mL.

The spectrum of para-fluorofentanyl at various concentrations is given in Fig. 9-a. The variation of Raman intensity depending on the concentration at 823 cm^{-1} is shown more closely in Fig. 9-b.

4. Conclusion

In this study, para fluorofentanyl, one of the most common fentanyl analogues in drug mixtures, and its isomers were detected by

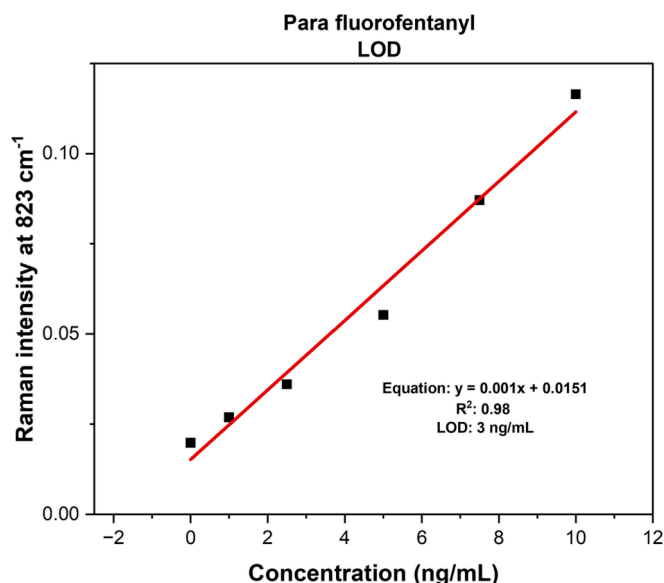


Fig. 8. Adsorption isotherm of para-fluorofentanyl and linear calibration curve plotting peak intensity at 823 cm^{-1} .

computational and experimental methods. The theoretical Raman spectra of these isomers were obtained using density functional theory (DFT) as a computational method. In DFT calculations, the wB97XD/cc-pVTZ couple was chosen as the method and basis set. As an experimental method, first normal Raman and then SERS spectra of the isomers were obtained. To obtain SERS spectra, Au/Ag nanostars, which provide strong interaction with fentanyl and its analogs on the surface, were synthesized [16,18,19]. Signal enhancements were compared by applying two different SERS protocols. Experimenting with the more sensitive KBr protocol at various concentrations, the LOD value of para fluorofentanyl was found to be 3 ng/mL, while the LOQ value was 9.2 ng/mL. When the DFT, NR, and SERS spectra of para, meta, and ortho fluorofentanyl were examined separately, it was concluded that these isomers could be separated from each other based on the differences at the peaks around 823 cm^{-1} , 1030 cm^{-1} , 1154 cm^{-1} .

CRediT authorship contribution statement

Sevde Dogruer Erkok: Investigation, Methodology, Validation, Writing – original draft. **Tufan Guray:** Investigation, Resources, Supervision, Writing – review & editing. **Bruce McCord:** .

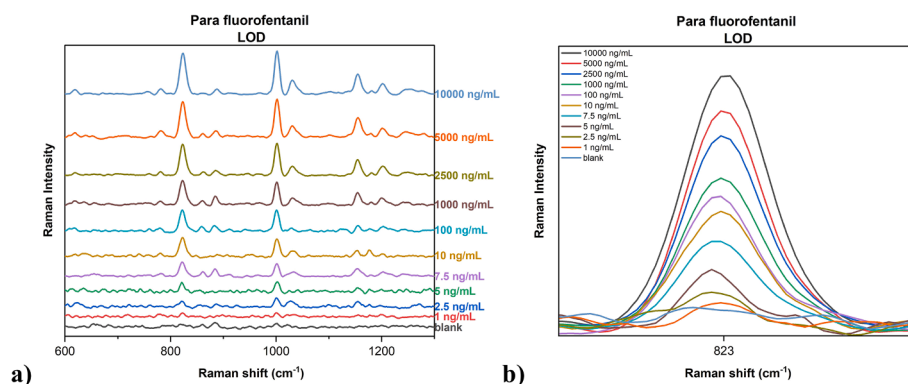


Fig. 9. a) Spectra of para-fluorofentanyl at different concentrations b) Raman intensity at 823 cm^{-1} of para-fluorofentanyl at different concentrations.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Sevde Dogruer Erkok reports financial support was provided by Scientific and Technological Research Council of Turkey (TUBITAK).

Data availability

Data will be made available on request.

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