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Differentiation and identification of fentanyl analogues using GC-IRD

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HIGHLIGHTS

- Demonstration of the differentiation and identification of fentanyls using GC-IRD.
- Optimization of GC-IRD parameters including chromatographic and spectral acquisition.
- Reporting of limitations in sensitivity for casework samples.
- Case study description involving the use of GC-IRD for analysis of a polydrug mixture.

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ABSTRACT

Fentanyl analogues and their positional isomers have similar chemical structural configurations making them difficult to identify and differentiate. Gas chromatography coupled to a gas-phase infrared detector (GC-IRD) is a useful and powerful tool for the unambiguous identification of fentanyl compounds where traditional analytical techniques such as gas chromatography–mass spectrometry (GC–MS) offer limited information for this class of compounds. In this study, we demonstrate the utility of GC-IRD and show how this complementary information enables the identification of fentanyl analogues (2- and 3- furanylfentanyl, 2-furanylbenzylfentanyl, crotonylfentanyl, cyclopropylfentanyl, methoxyacetylfentanyl, carfentanil, *meta*-fluoroisobutyryl fentanyl, *para*-fluoroisobutyryl fentanyl and *ortho*-fluoroisobutyryl fentanyl) when combined with GC–MS data. A description of the operating conditions including how the optimization of GC-IRD parameters can enhance the spectral resolution and unambiguous identification of these fentanyl analogues is presented, for the first time. In particular, the effects of light pipe temperatures, acquisition resolution, the use of a programmed temperature vaporizing (PTV) inlet, and the analytical concentration of the sample were evaluated. A real-world case example illustrates the current challenges frequently encountered in casework and how the implementation of GC-IRD may overcome some of these challenges in fentanyl differentiation and identification.

1. Introduction

The United States declared a national public health emergency in October of 2017 in response to the Opioids Crisis [1]. Since 2014 there has been a significant increase of drug related deaths attributed to the abuse of opioids, with over 60,000 deaths in 2017 [2]. Fentanyl and related substances are among the opioids having the most impact on drug overdose deaths [2]. Following the trend of fentanyl-related overdoses, there has been a dramatic increase of fentanyl and fentanyl isomer seizures submitted and analyzed in crime laboratories from 978 in 2013 to over 30,000 fentanyl identifications alone in 2018 [3]. Rapid detection, identification, and reporting of these novel fentanyl substances is vital to the assessment and understanding of the illicit drug markets in specific locations and to the disruption of distribution networks. While there have been recent advancements to address analytical capability gaps, such as novel search algorithms [4] and the enhancement of communication and information sharing, such as the Drug Enforcement Administration (DEA)'s Synth-Opioids Real Time Communication Network [5], there are still opportunities for the improvement of technology and methodology geared to overcoming existing analytical challenges in seized drug evidence samples. The chemical structure of fentanyl is manipulated in clandestine laboratories creating new variations of the substance that have resulted in a long list of new analogues, whose structural similarity makes it difficult to detect and identify using laboratory traditional analytical schemes [4].

Forensic scientists routinely utilize electron impact gas chromatography-mass spectrometry (EI-GC–MS) for the separation and identification of substances in drug samples; however, the use of GC–MS for the

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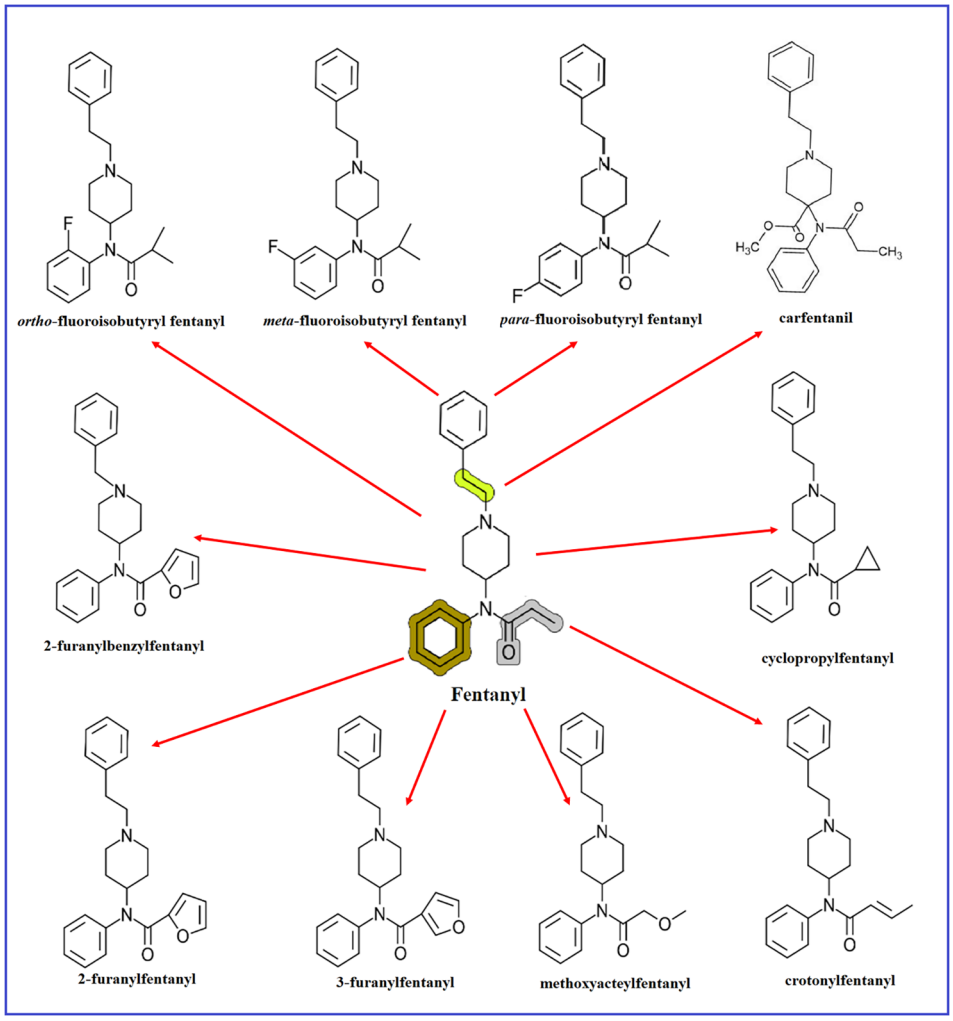

Fig. 1. Chemical structures of the fentanyl substances analyzed in this study.

analysis of fentanyl samples comes with analytical challenges and limitations. These analytical challenges are often due to the nature of samples being submitted to forensic science providers for analysis. Isomeric interferences, low purity, and insufficient amount of samples are among those challenges. Fentanyl and fentanyl-related substances are of high toxicity and present a significant hazard to those ingesting the substance. Thus, most often fentanyl and fentanyl-related substances are mixed with other substances resulting in the fentanyl-related analytes being present in illicit samples at very low concentrations. It is not uncommon to find a sample with numerous fentanyl analogues present or composed of a mixture of substances with very similar chemical structures. In the Maher et al. study on the differentiation of regioisomeric forms of trifluoromethylphenylpiperazines, these researchers describe the challenge of differentiating between identical mass spectra for the three regiosomeric 2-, 3-, and 4-trifluoromethylphenylpiperazines [6]. There have been numerous published studies using GC-IRD for the analysis of controlled substances with isomeric challenges, such as piperazines, phenethylamines, and designer drugs [6–12]. For example, the Clark et al study on the ring and side chain regioisomers of ethoxyphenethylamines demonstrates the advantages of GC-IRD analysis for the identification of MDEA, MDMMA, and MBDB [7].

To overcome limitations associated with GC–MS, scientists have turned to other complementary methods for the analysis of fentanyl analogues, such as liquid chromatography- ion trap mass spectrometry (LC-Ion Trap MS), nuclear magnetic resonance spectroscopy (NMR). In a study comparing analysis by GC–MS and LC-Ion Trap MS for a number of positive cases identified for synthetic opioids in toxicological samples, Shoff et al. demonstrated that while only 9 cases of furanyl fentanyl were detected by GC–MS, 37 cases were detected by LC-Ion Trap MS [13]. More concerning, their study showed only 30 cases of carfentanil detected by GC–MS, compared to 134 cases detected via LC-Ion Trap MS [13]. Recently, Mallette et al. described the characterization and differentiation of cyclopropylfentanyl, crotonylfentanyl, and 3-butenylfentanyl in drug samples using a combination of techniques to include NMR, Fourier transform infrared spectroscopy (FTIR), and GC–MS [14]. Other techniques such as thermal desorption direct analysis in real time (DART) and ion mobility spectrometry (IMS) have also been shown to provide useful characterization of fentanyl analogues [15]. In a study by Lurie et al., incomplete chromatographic resolution between fentanyl-related compounds were subsequently identified using retention times and dual multiple-reaction monitoring [16]. Other studies include the direct-infusion techniques with electrospray-mass spectrometry (ESI-MS), LC-atmospheric pressure ionization-tandem mass spectrometry, coiled microextraction and portable GC–MS, ionic liquid-modified disposable electrochemical sensor strips, and ion mobility spectrometry [17–23].

This paper offers an alternative and complementary technique to the widely used EI-GC–MS method in order to enhance the capability of laboratories when analyzing fentanyl-related substances that prove to be difficult to identify using conventional analytical techniques, such as GC–MS. Following the guidelines developed by the Scientific Working

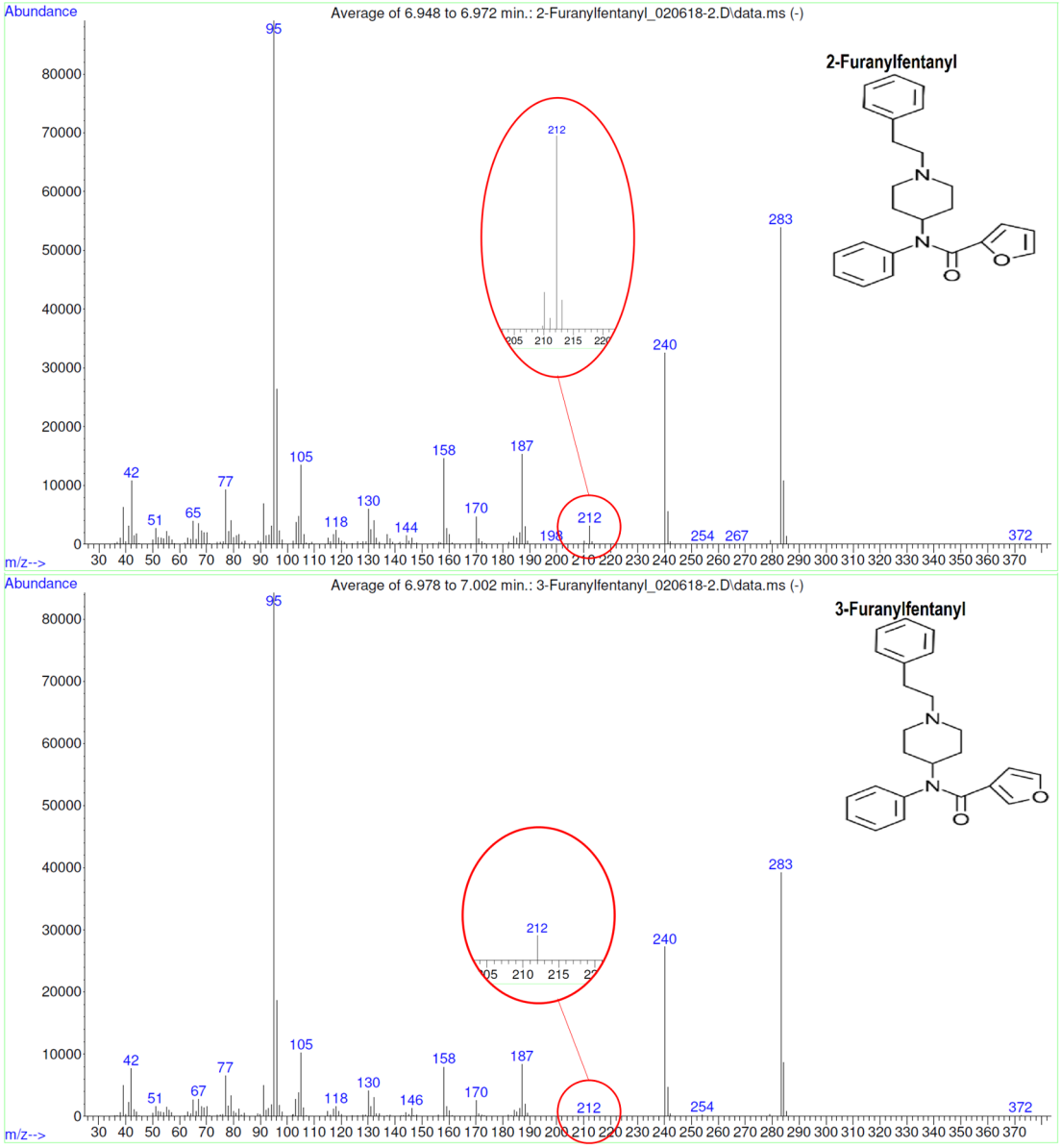

Fig. 2. Mass spectra differences for 2- and 3-furanylfentanyl.

Group for the Analysis of Seized Drugs (SWGDRUG) [24], GC-IRD (a hyphenated category B and A technique) can be utilized to differentiate between fentanyl analogues with the same molecular weight and very similar chemical structures.

To the authors' knowledge, this is the first reporting of a comprehensive optimization study for the analysis of fentanyl analogues by GC-IRD in peer-reviewed literature. There have been brief reports on the GC-IRD analysis of fentanyl-related compounds, for example the comparison of GC-IRD analysis to Q-TOF LC-MS for the detection and identification of 2- and 3-furanylfentanyl isomers [25]. Other studies of fentanyl-related substances have also included GC-IRD as a potential technique for analysis [26–28]. However, GC-IRD optimization and the use of large volume programmable temperature vaporization inlets have not been thoroughly described previously.

GC-IRD utilizes the advantages of infrared spectroscopy (IR) with the separation power of a gas chromatography (GC). There are two modes used for GC-IR, vapor phase and condensed phase. Vibrational frequencies are usually higher in gas-phase IR as compared to condensed-phase IR. This study only considers the vapor phase mode. Samples are prepared in the same manner as GC–MS samples and injected into a GC where analytes are separated as they flow through the GC column. Once separated, analytes enter the IRD through a 120 mm long capillary with a 1 mm internal diameter. This capillary, designated the light pipe, is enclosed in a heating block at the desired temperatures to keep samples in the vapor state while interacting with the IR beam and characteristic absorption spectra are detected [28,29].

One of the limitations of the GC-IRD is its somewhat lower sensitivity, in comparison to GC–MS. Mass loadings of of less than 25 ng on-column (equivalent to a 1 μL injection of a 25 ppm sample) generally produce undefined spectra, making the instrument ineffective for forensic analysis of analytes at low-level concentrations such as might be found in toxicology samples or even some very dilute seized drug samples. While the actual sample amount available for analysis in toxicology cases is often a challenge, seized drug analysts normally have sufficient amounts of sample for analysis and compound identification using routine sample preparation techniques such as simple solvent extraction. However, in the past ten years the landscape of seized drug samples has been changing from single-drug samples to polydrug samples. Often, these mixtures of illicit drug cocktails contain several substances of very similar chemical structures and properties,

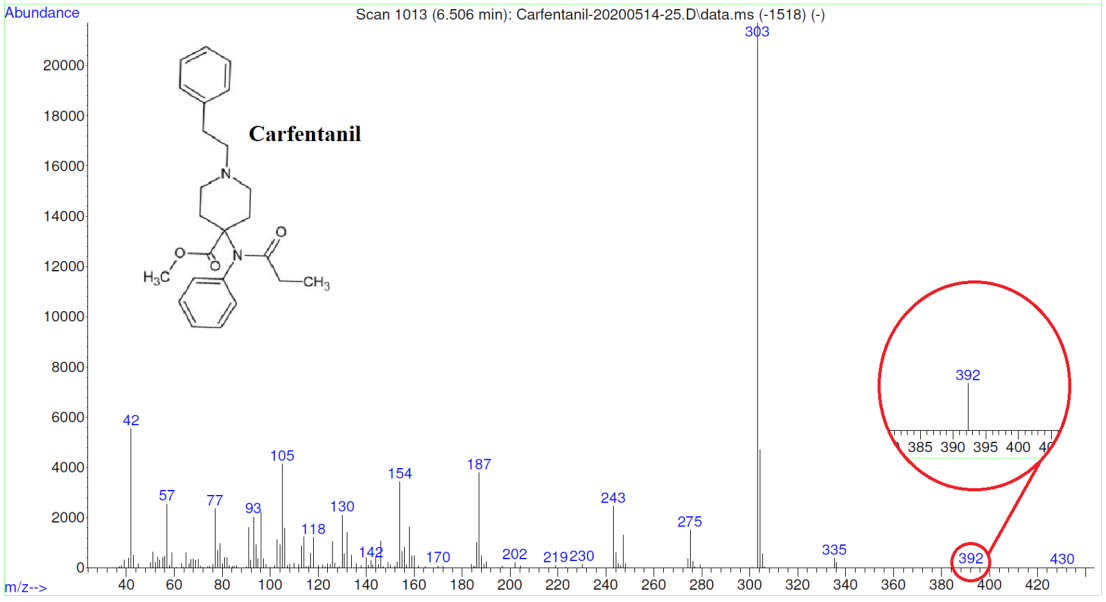

Fig. 3. Mass spectrum of carfentanil demonstrating a lack of the molecular ion.

rendering simple solvent extractions ineffective. Samples submitted to forensic laboratories often consist of complex matrices of multiple analytically challenging substances (i.e. fentanyl analogues at concentrations lower than 5% by volume). Temperature-programmed sample introduction has been around since 1979, demonstrating a valuable option to consider in analytical techniques [28,29]. The temperature of the inlet is set at a lower temperature than the boiling point of the solvent and gradually increased. This causes the solvent to be evaporated and vented through the split line prior to sample introduction on the column [28,29]. New technological scientific advances can accommodate samples with analytes at low concentrations. The inlet functions in solvent vent mode by utilizing a temperature ramp to remove the solvent from the sample injection before being transferred to the column. The vent and solenoid are in the open position and the sample is injected into the inlet at an initial temperature set to the boiling point of the solvent. The analytes remain on the liner wall while solvent vapors are removed via the split vent. The inlet temperature is then ramped to a final set temperature, while the solenoid and vent are closed [28,29]. This process allows for injection volumes of 1 µL to 1000 µL, generating spectra for trace level concentrations [28,29].

In this study, the authors focused on the development and optimization of GC-IR methodology to address the detection and subsequent identification of fentanyl analogues, as well as addressing the analytical challenges inherent in fentanyl samples (e.g. polydrug samples and low concentration substances of interest).

2. Experimental

2.1. Instrumentation

GC-IRD studies were conducted using an Agilent Technologies 7890 B Gas Chromatograph coupled with an IRD 3 detector obtained from Analytical Solutions and Providers (ASAP) equipped with a TITAN XL Large Volume Programmable Temperature Vaporization (PTV) inlet. The GC was operated in splitless mode and solvent vent mode with a carrier gas (helium) flow rate of 2 mL/min and a column head pressure of 10 psi. The column used was a 30 m × 0.32 mm i.d. coated with 0.25 µm (HP-5) purchased from Agilent Technologies. Temperature programs utilizing the PTV in splitless mode consisted of an initial temperature of 55 °C for 1 min, ramped up to 295 °C at a rate of 30 °C per minute, followed by a hold at 295 °C for 9 min. Sample injection volume was 2 µL with a flow rate of 3.0 mL/min. Samples analyzed in solvent vent mode consisted of 5 µL and 25 µL injections at an initial temperature of 55 °C for 0.1 min and ramped at 305 °C/min to 290 °C with no hold and a 3.0 mL/min flow rate. Temperature programming for the oven started at 70 °C for 1 min, and increased at a rate of 25 °C/min to 295 °C and held for 8 min. Samples were dissolved and diluted using methanol and introduced individually and in mixtures using an Agilent Technologies 7693 Autosampler.

GC–MS analysis was performed using a GC Agilent Technologies 7890B Gas Chromatograph, equipped with a DB-1, 15 m × 0.25 mm id × 0.25 um film column. The mass spectrometry was an Agilent Technologies 5979B MSD instrument. The GC injector temperature was set to 270 °C with an injector volume of 1 µL. The MS source temperature was set at 280 °C, while the quadrupole temperature was set to 150 °C. The MS was used in full scan mode with EI at 70.0 eV.

2.2. References and reagents

Reference materials were obtained from the DEA Special Testing and Research Laboratory, who procured the reference materials from Cayman Chemical (Ann Arbor, MI), before subjecting them to an authentication process. Reference materials were dissolved in methanol to an approximate 1000 ppm concentration and then diluted to specific concentrations for analysis.

3. Results and discussion

3.1. Gas chromatography mass spectrometry (GC–MS)

In this study, ten substances were selected based on their structural similarities. Five of the ten substances (cyclopropylfentanyl, crotonylfentanyl, methoxyacetylfentanyl, 2- and 3-furanylfentanyl) involved replacement of the n-propionyl group in the core fentanyl structure with other acyl substituents (Fig. 1). Cyclopropylfentanyl, crotonylfentanyl, and methoxyacetylfentanyl co-elute in a routine gas chromatograph method due to their similar structures of 348.48, 348.8, and 352.47, respectively. While the mass spectra for cyclopropylfentanyl and crotonyl are almost identical, the mass spectra for methoxyacetylfentanyl is significantly different from the other two compounds. The MS spectra for these positional isomers offers little

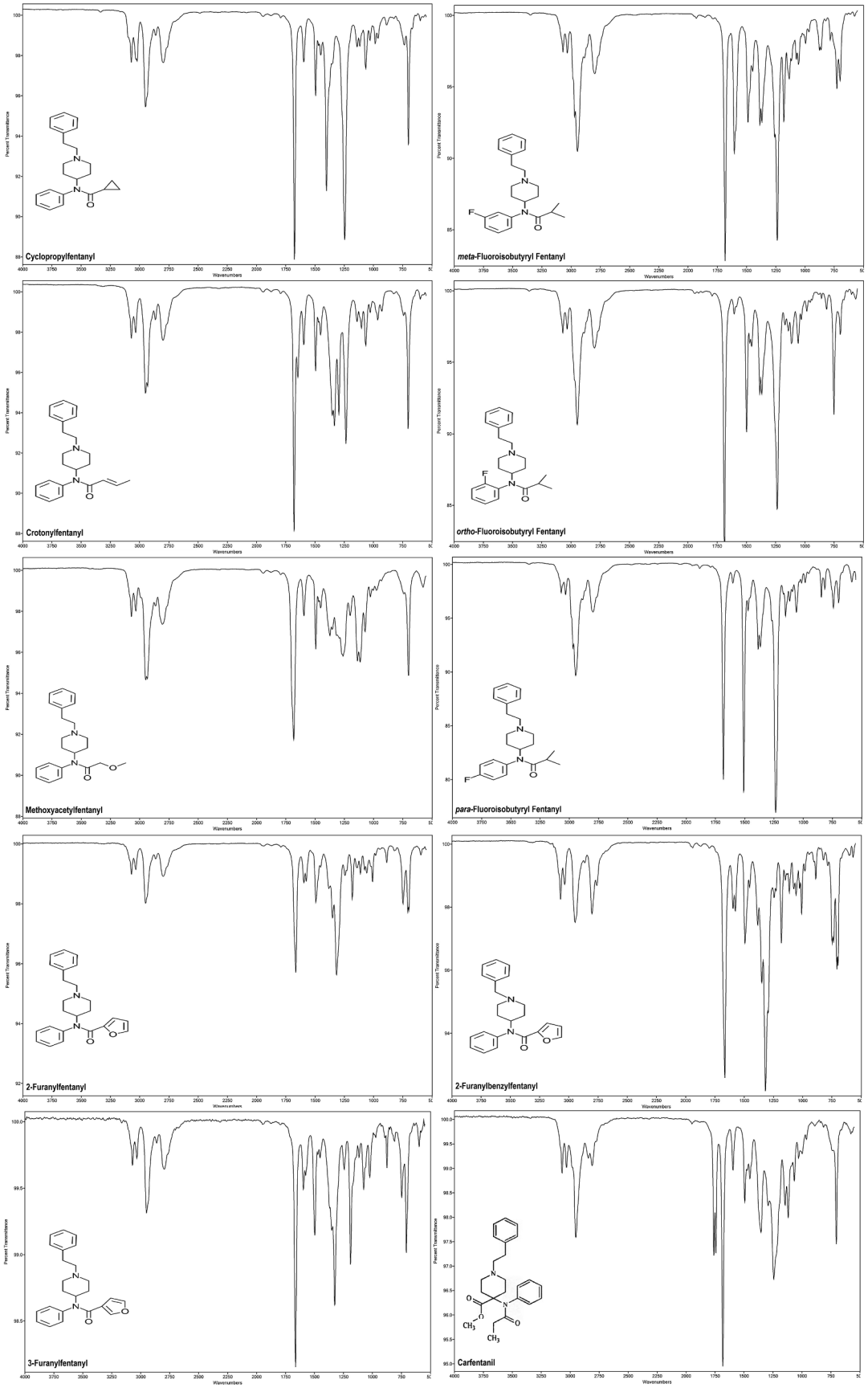

Fig. 4. Gas-phase infrared (IR) spectra for fentanyl-related substances analyzed with a 250 °C flow cell (light pipe) temperature at 8 cm^{-1} resolution.

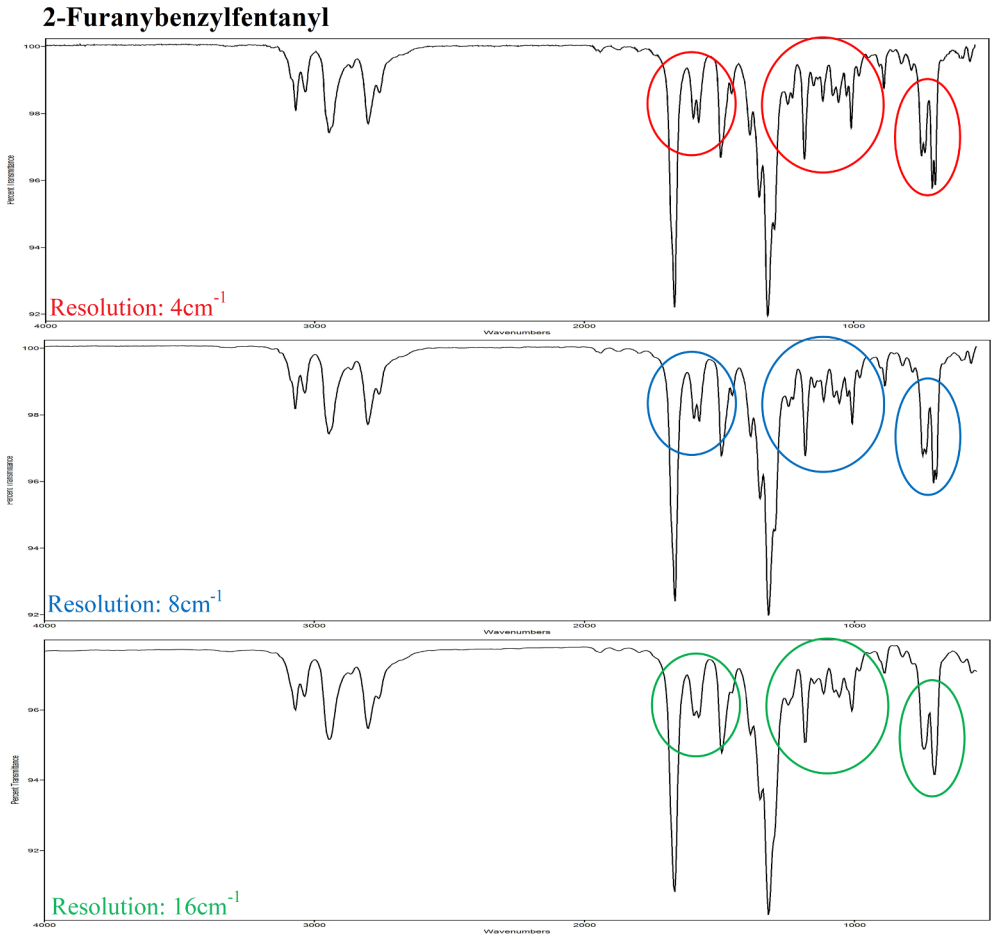

Fig. 5. Gas-phase infrared spectra of 2-Furanylbenzylfentanyl at different resolutions (4 cm^{-1} - top, 8 cm^{-1} – middle, and 16 cm^{-1} - bottom) demonstrating an increase in peak definition and noise with increased resolution.

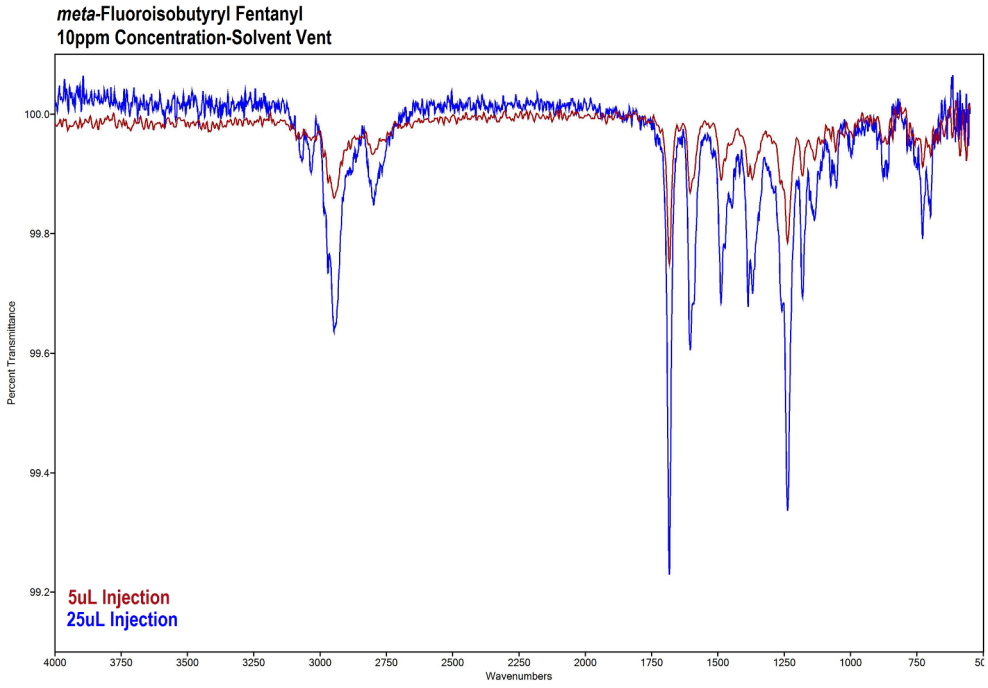

Fig. 6. Gas-phase infrared spectra of of *meta*-fluoroisobutyryl fentanyl at a 10 ppm concentration solution using solvent vent mode at 5 μL (red) and 25 μL (blue) injections illustrating the differences in quality spectra at the 10 ng on-column mass loadings for the optimized conditions. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

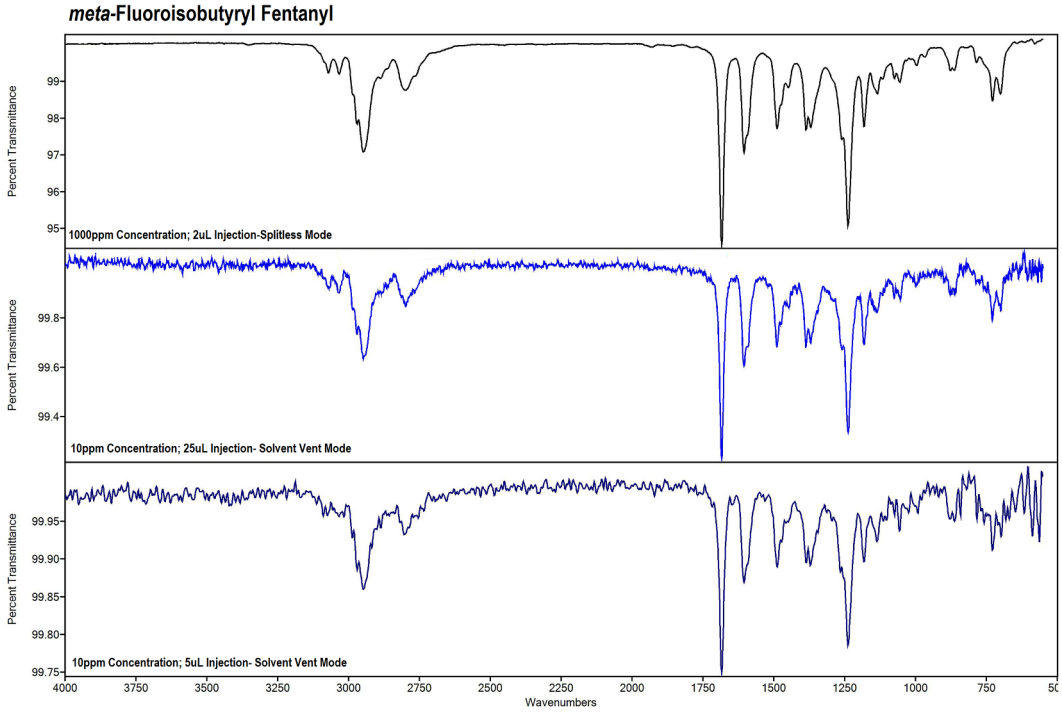

Fig. 7. Gas-phase infrared spectra of *meta*-fluoroisobutyryl fentanyl at a 10 ppm concentration solution using solvent vent mode at 5 μL and 25 μL injections illustrating the differences in quality spectra, as compared to 1000 ppm concentrations at 2 μL injections for the optimized conditions.

differences. For example, the only significant difference between the MS spectra of 2-furanylfentanyl and 3-furanylfentanyl is the *m/z* 212 fragment (Fig. 2). According to the National Forensic Laboratory Information System special fentanyl report released in January of 2019, crime laboratories in all but seven states reported in 2017 seeing cases of furanylfentanyl, while 35 states reported cases of cyclopropylfentanyl and 22 states reported seeing (*para*-) 4-fluoroisobutyryl fentanyl.

Additional modifications to the fentanyl core structure create other fentanyl related substances, of which many have associated isomers. Positional isomers *meta*-fluoroisobutyryl fentanyl, *para*-fluoroisobutyryl fentanyl, and *ortho*-fluoroisobutyryl are generated by the insertion of a fluorine atom to create a halogen substituted aniline ring and the addition of a second methyl group to the n-propionyl group (Fig. 1). Modification to the phenethyl group of the fentanyl structure and the replacement of the ethyl chain of the propionamide group with an aromatic heterocyclic furan moiety generates the furanylbenzylfentanyl compound, which was also evaluated in this study (Fig. 1). All ten compounds have the same fentanyl core structure, increasing the potential for co-elution or isomeric interferences with traditional GC–MS methods. In addition, these substances are often observed in low concentrations causing the respective GC–MS spectrum to lack the molecular ion. This is often the case with carfentanil samples. Due to its high and lethal potency, carfentanil is often detected in very low concentrations and often found in the presence of other substances. It is not uncommon for GC–MS data of carfentanil in illicit samples to lack its molecular ion *m/z* 393 (Fig. 3). The lack of molecular ions in GC–MS data may often lead forensic scientists to conduct experiments with other techniques that can confirm the presence of the respective substance.

3.2. Gas chromatography infrared detector (GC-IRD) results

GC-IRD is a good alternative for analyzing fentanyl derivatives in forensic samples. The vapor-phase infrared spectra were obtained in the range of 4000–550 cm^{-1}, at a scan rate of 1.5 scans per second and a resolution of 8 cm^{-1}. The effects of light pipe temperature, resolution, and sample concentration were evaluated. The IRD light pipe temperature was optimized for maximum signal to noise.

The same prominent peaks attributed to the fentanyl core structure were observed in the IR spectra for the ten different fentanyl analogues analyzed in this study. The most prominent peak in all ten spectra (at 1680–1666 cm^{-1}) is attributed to the C═O absorption of the tertiary amide of the fentanyl core structure of the substances (Fig. 4). Substances with electron withdrawing groups attached to the nitrogen will have a higher frequency of absorption, since they are competing with the carbonyl oxygen for the electrons of the nitrogen [9]. The C—H bond stretching vibrations associated with the phenyl ring of the phenethyl group in each substance can be seen around 2948 cm^{-1} and with a strong peak around 700 cm^{-1} attributed to the ring C═C bend. The absorbances observed at 1080–1066 cm^{-1} and 1265–1235 cm^{-1} are likely results from the C—N stretch from the piperidine ring that is expected with all fentanyl derivatives. As expected with cyclization, there is a decrease in frequency of the CH_2 scissoring vibration of the piperidine ring with absorptions peaks at 1400 cm^{-1} and 1390 cm^{-1}, respectively. In cyclopropylfentanyl, the CH_2 scissoring vibration of the cyclopropyl group shows an absorption around 1440 cm^{-1} attributed to the strain of the cyclopropyl ring. While for crotonylfentanyl, the medium peaks at 1600 cm^{-1} and 1400 cm^{-1} are probably attributed to the alkene moiety of crotonylfentanyl.

The fingerprint region (1300–500 cm^{-1}) of the spectrum is complex resulting from interacting vibrational bands and can be used to differentiate between similar structural fentanyl derivatives. For example, methoxyacetylfentanyl has a strong, sharp band at around 1150 cm^{-1} associated with the stretching vibration of the aliphatic C—O—C ether part of the molecule, whereas the symmetrical C—O—C stretching vibration of the ether group in the 5-member heterocyclic furan ring of 2 or 3-furanylfentanyl and 2-furanylbenzylfentanyl is at a higher wavenumber. There are also differences resulting from the aryl halides in the fluoroisobutyryl fentanyl compounds. Absorptions at around 1250 cm^{-1} and 1100 cm^{-1} are probably attributed to the fluorobenzene groups.

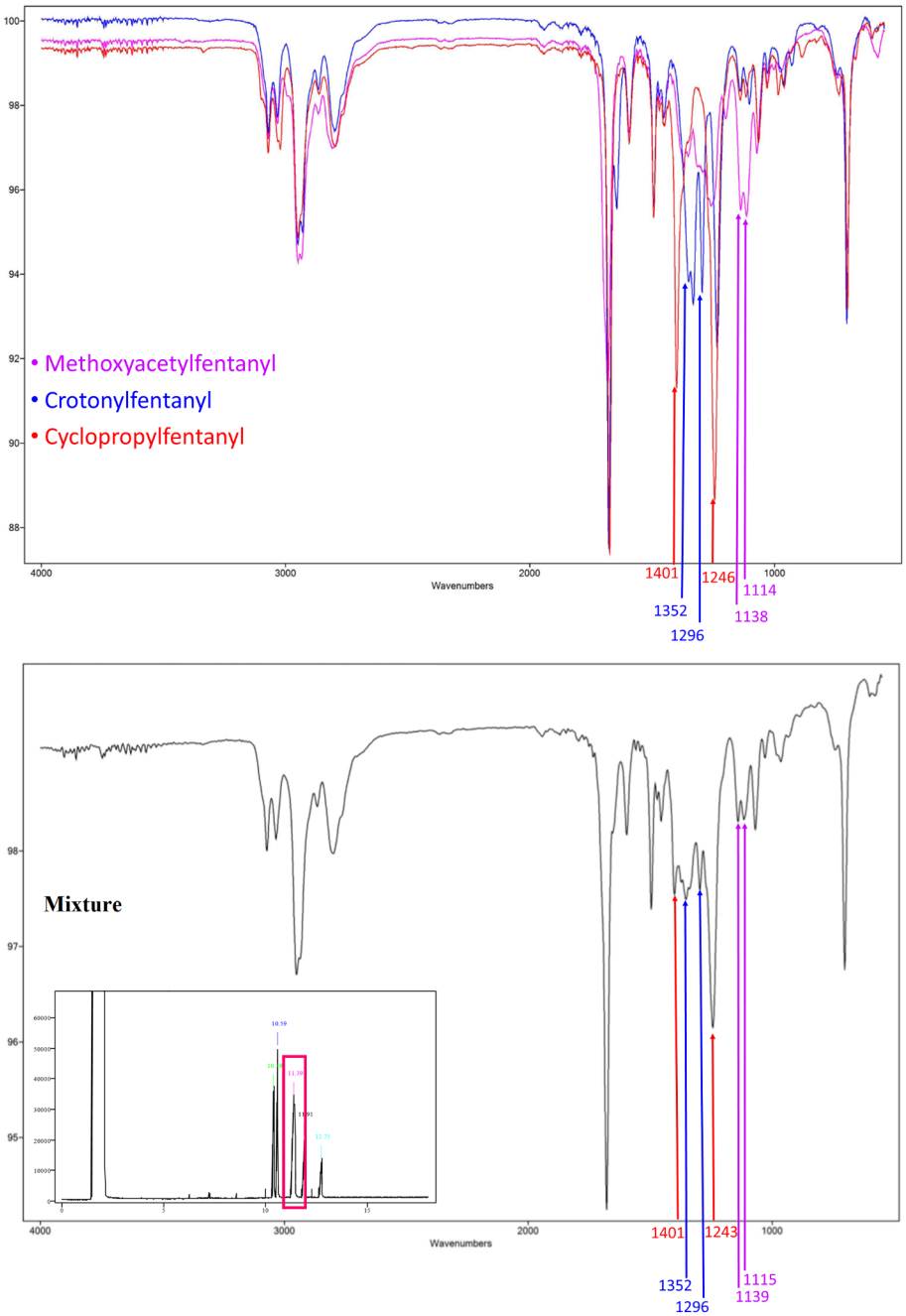

Fig. 8. Sample mixture of 7 fentanyl analogues, including Cyclopropylfentanyl, Crotonylfentanyl, and Methyoxyacetylfentanyl, which have similar retention times and illustrates the ability to identify specific peaks between overlay (top) vs the actual sample mixture (below).

3.3. Flow cell (light pipe) temperature

The purpose of the light pipe is to keep the sample in the vapor phase while the sample interacts with the IR beam, maximizing the path length for increased sensitivity. Thus, optimizing the temperature of the heating block involves evaluating a range of temperatures suitable for the analysts of interest. The temperature must be high enough to prevent substances with high molecular weights from condensing inside the light pipe and low enough to prevent excess noise from the increased vibration. Forensic applications commonly use a 280 °C flow cell temperature [8]. In this study, the effect of different light pipe temperatures was evaluated using two substances. Early eluter, *meta*-fluoroisobutyryl fentanyl, and late eluter, 2-furanylbenzylfentanyl, were selected for this evaluation to consider a large range of temperatures. A solution containing the two fentanyl derivatives was evaluated at flow cell temperatures of 240 °C, 250 °C, 260 °C, 270 °C, 280 °C, and 290 °C. As expected, the peaks were sharper as the temperature was decreased. The most prominent differences were found at the 240 °C and 270 °C. After a series of experiments, it was determined that 250 °C produced the sharpest peaks, with little broadening effect to the corresponding chromatographic peak shape.

3.4. Resolution

Resolution indicates the minimum spectral peak interval that can be distinguished. The effect of various resolution values was also evaluated. The compound 2-furanylbenzylfentanyl was analyzed at 4, 8, and 16 cm^{-1} resolutions. While it was expected that sharper peaks

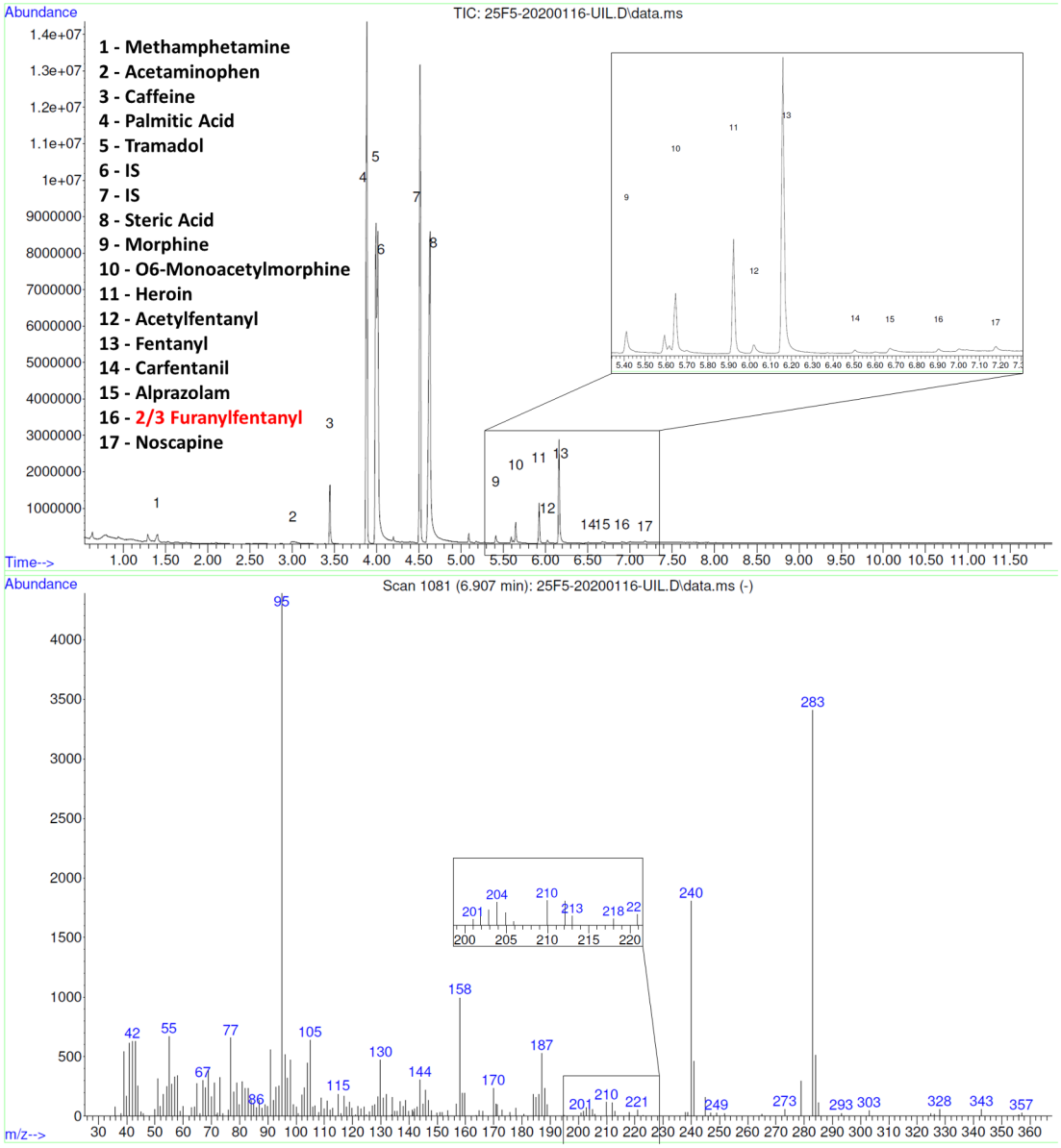

Fig. 9. Gas chromatogram of a real case sample containing illicit tablets that was submitted to a forensic laboratory for analysis. The instrumental conditions of the GC–MS are DB-1MS 15 m × 250 μm × 0.25um film column with an initial oven temperature of 100 °C and a ramp of 30 °C/min. until 310 °C. The inset shows 2- or 3-furanylfentanyl compound that cannot be differentiated.

would be generated at higher resolutions, the relative signal-to-noise was also expected to decrease given that, as the resolution is increased, the intensity of the signal entering the detector is reduced. Fig. 5 shows the differences in resolution for the collection of IR spectra under the same conditions for 2-furanylbenzylfentanyl. Peak broadening at 1600 cm^{-1} and 1100 cm^{-1} can be observed at a resolution of 16 cm^{-1} but no significant difference was observed between resolutions 4 and 8 cm^{-1} for this example and for others investigated and therefore 8 cm^{-1} was selected for collection of spectra.

3.5. Splitless mode vs solvent vent mode

In this study, the inlet temperature was set at 55 °C, which is below the 64.7 °C boiling point of methanol (sample solvent). Using the PVT inlet of the GC-IRD, large volume injections were conducted to allow for sample concentration and overcome the sensitivity limitations associated with GC-IRD analysis. *Meta*-fluoroisobutyryl fentanyl was evaluated at 10 ppm, 50 ppm, 100 ppm, 500 ppm, and 1000 ppm using splitless mode (2 μL injection volume) and solvent vent mode (5 μL and 25 μL injection volume) (Fig. 6). The light pipe temperature was maintained at 250 °C with an internal resolution of 8 cm^{-1} and a scan rate of 1.5 scans per second. A 2 μL injection volume at 10 ppm (20 ng on-column mass loading) resulted in poor IR spectra using both splitless mode and in solvent vent mode. In Fig. 6, the 25 μL injection showed more noise, but sharper peaks than the 5 μL injection at the 10 ppm level. Fig. 7 illustrates the advantages to increased injection volumes for these very low concentration samples.

3.6. Repeatability

Meta-fluoroisobutyryl fentanyl and 2-furanylfentanyl were analyzed 10 times at a light pipe temperature of 250 °C, resolution of 8 cm^{-1} in splitless mode. The spectra of the 10 injections presented no significant differences between them demonstrating the high repeatability advantages of using GC-IRD.

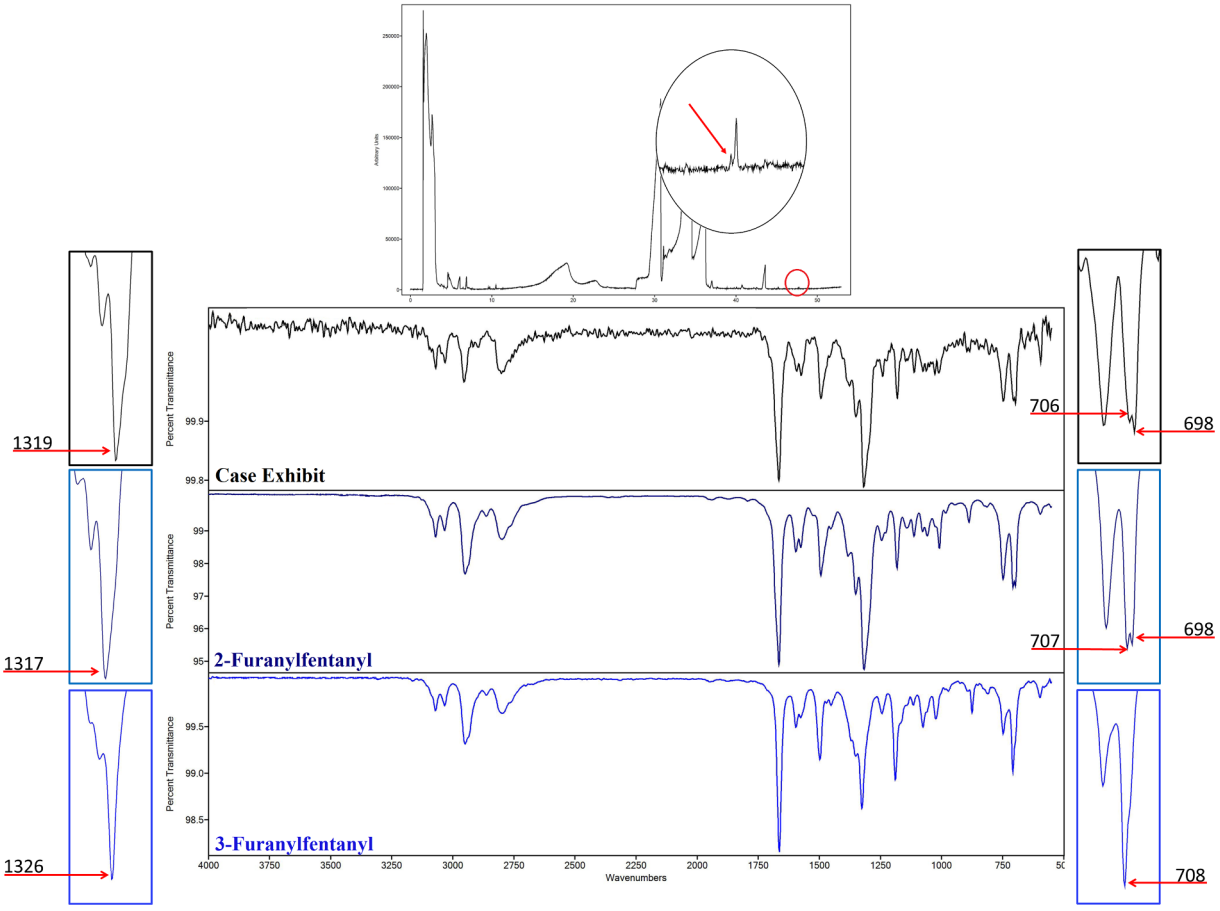

Fig. 10. Gas-phase infrared spectra of a real case sample of illicit tablets that was submitted to a forensic laboratory for analysis. Case Sample via GC-IR showing discernable differences between 2- and 3-furanylfentanyl even at estimated levels of less than 0.5%.

3.7. Complex mixtures

While the compounds evaluated in this study have similar structural configurations, they were able to be differentiated and distinguished using GC-IRD. A mixture of cyclopropylfentanyl, crotonylfentanyl, methoxyacetylfentanyl, 2-furanylfentanyl, *meta*-fluoroisobutyryl fentanyl, *ortho*-fluoroisobutyryl fentanyl, and 2-furanylbenzyl fentanyl was prepared at approximately 0.718 mg/mL. The three substances, cyclopropylfentanyl, crotonylfentanyl, and methoxyacetylfentanyl co-eluted and generated a mixed IR spectrum. However, the data shows discernable differences in their IR spectra that can be used to identify cyclopropylfentanyl, crotonylfenantyl, and methoxyacetylfentanyl, even in a complex mixture of similar structural substances, such as the one used in this study (Fig. 8).

3.8. Case example

In the past ten years, the number of polydrug samples submitted to forensic laboratories for analysis has significantly increased [30]. Scientists have transitioned from identifying 1–3 substances in a sample to currently trying to separate and identify 3–8 substances or more in a routine sample. These substances are usually similar in structure and some are found in very low purity, making extraction techniques and other sample preparation methodologies less effective in isolating analytes of interest. In Fig. 9, an example of a real case of illicit tablets containing 13 target analyte substances is shown. This sample contains four different types of fentanyl related substances (fentanyl, acetyfentanyl, carfentanil, and either 2- or 3-furanylfentanyl). Conventional instrumentation such as GC–MS may not necessarily be the best technique to identify the low purity fentanyl related substances, such as carfentanil (no molecular ion present) or to differentiate between 2- or 3-furanylfentanyl. The optimized GC-IRD method was used to determine whether this technique could differentiate between 2- and 3-furanylfentanyl and generate IR data at very low levels to detect carfentanil. The concentration levels of furanylfentanyl and carfentanil in this real-world case sample were estimated to be less than 0.5% and less than 0.1%, respectively. Several injection volumes (mass loadings) were analyzed (using the PTV mode to achieve good results. While the PTV optimized conditions shown above suggest using 5 µL injections, injections as high as 25 µL total volume were necessary for this particular (very low concentration) sample. The very small size of the carfentanil peak in Fig. 9 (RT of ~6.5 min.) is indicative of the extremely low relative concentrations expected in actual casework samples. The low concentrations, even when using the PTV and large volume injections, produce an analytical challenge for both GC–MS and GC-IRD analyses especially in the presence of other substances of interest at high concentrations. In this particular example, while the presence of carfentanil could not be detected via GC-IRD, the presence of 2-furanylfentanyl was able to be differentiated from 3-furanylfentanyl (Fig. 10).

4. Conclusion

The application of gas-phase IR detection following GC separation is a useful alternative or complementary technique for the analysis of drugs which routinely use analytical techniques such as a GC–MS that yield limited spectral information. In this study, the GC-IRD data generated were highly repeatable and offered complementary information

to the GC–MS spectra presenting significant differences between structurally similar fentanyl analogues and enabling the rapid and unambiguous identification of these substances. The PVT inlet options of the GC-IRD allow for large-volume injections, overcoming some of the sensitivity limitations traditionally associated with GC-IRD analysis. A real-world case example illustrates the challenges now more frequently encountered in casework and the utility of GC-IRD to overcome some of the challenges in fentanyl differentiation and identification is presented here, for the first time.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] National Center for Health Statistics, Vital statistics rapid release. Provisional drug overdose death counts, Atlanta, GA: Centers for Disease Control and Prevention, 2018. https://www.cdc.gov/nchs/nvss/vsrr/drug-overdose-data.htm.
[2] J.K. O'Donnell, J. Halpin, C.I. Mattson, B.A. Goldberger, R.M. Gladden. Deaths involving fentanyl, fentanyl analogs, and u-47700-10 states, July-December 2016, Morbidity and Mortality Weekly Report (MMWR), 2017, 66, 1197-1202. DOI:10.15585/mmwr.mm6643e1.
[3] Drug Enforcement Administration, NFLIS. https://www.nflis.deadiversion.usdoj.gov, 2019.
[4] Drug Enforcement Administration. 2018 Synth-Opioids Report. Drug Enforcement Administration, 2018, Southeast Laboratory.
[5] J.B. Morrow, J.D. Ropero-Miller, M.L. Catlin, A.D. Winokur, A.B. Cadwallader, J.L. Staymates, et al., The opioid epidemic: moving toward an integrated, holistic analytical response, J. Anal. Toxicol. (2018) 1–9, https://doi.org/10.1093/jat/bky049.
[6] H.M. Maher, T. Awad, C.R. Clark. M. Differentiation of the regioisomeric 2-, 3-, and 4- trifluoromethylphenylpiperazines (TFMPP) by GC-IRD and GC-MS. Forensic Sci. Int. 2009, 188, 31. DOI:10.1016/j.forsciint.2009.03.009.
[7] R.C. Clark, A.M. Al-Hossaini, T. Awad, J. DeRuiter, GC-MS and GC/IRD analysis of ring and side chain regioisomers of ethoxyphenethylamines related to the controlled substances MDEA, MDMMA, and MBDB, Forensic Sci. Int. 200 (2010) 73–86, https://doi.org/10.1016/j.forsciint.2010.03.033.
[8] R.C. Clark, K.M. Abdel-Hay, J. DeRuiter, Differentiation of the 1-(methylenedioxyphenyl)-2-piperzinopropanes and 1-(methoxyphenyl)-2-piperazinopropanones by GC-IRD and GC-MS, Forensic Sci. Int. 235 (2014) 40–51, https://doi.org/10.1016/j.forsciint.2013.11.015.
[9] K.M. Abdel-Hay, T. Awad, J. DeRuiter, C.R. Clark, Differentiation of methylenedioxybenzylpiperazines (MDBPs) and methoxymethylbenzylpiperazines (MMBPs) by GC-IRD and GC-MS, Forensic Sci. Int. 210 (2011) 122–128, https://doi.org/10.1016/j.forsciint.2011.02.018.
[10] Y. Abiedalla, C. Charles, J. DeRuiter, K. Abdelhay. GC-MS and GC-IRD studies on S cathinones: Bath salt-type aminoketone designer drugs related to MDPV, in: Abstracts of Papers, 252nd ACS National Meeting and Exposition, American Chemical Society, 2016, medi-390 In English CODEN: 69UGR2.
[11] Maher, Hadir, M., Awad, Tamer, DeRuiter, Jack, Clark, C. Randall, GC-MS and GC-IRD studies on brominated dimethoxyamphetamines: Regioisomers related to 4-BR-2,5-DMA (DOB), Drug Testing Analysis, 2012, 4(7-8), 591-600. DOI:10.1002/dta.409.
[12] S. Davis, K. Blakey, K. Rands-Trevor, GC-MS and GC-IRD Analysis of 2-, 3- and 4-methylmethamphetamine and 2-, 3- and 4-methylamphetamine, Forensic Sci. Int. 220 (2012) 67–73, https://doi.org/10.1016/j.forsciint.2012.01.028.
[13] E.N. Shoff, M.E. Zaney, J.H. Kahl, G.W. Hime, D.M. Boland, Qualitative identification of fentanyl analogs and other opioids in postmortem cases by UHPLC-Ion trap MS, J. Anal. Toxicol. (2017) 1–9.
[14] J. Mallette, J.F. Casale, P. Hays, Characterization and differentiation of cyclopropylfentanyl from E-crotonylfentanyl, Z-crotonylfentanyl, and 3-butenylfentanyl, Sci. Justice (2019) 67–74.
[15] E. Sisco, J. Verkouteren, J. Staymates, J. 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 Chem. (2017) 108–115.
[16] I.S. Lurie, R. Lio, Use of multiple-reaction monitoring ratios for identifying incompletely resolved fentanyl homologs and analogs via ultra-high-pressure liquid chromatography-tandem mass spectrometry, J. Chromatogr. A (2009) 1515–1519.
[17] E.M. McBride, R.E. Keller, G.F. Verbeck, Direct-infusion electrospray ionization-mass spectrometry profiling of fentanyl and acetylfentanyl reaction mixtures, Int. J. Mass Spectrom. (2018) 55–61.
[18] L. Wang, J.T. Bernert, Analysis of 13 fentanils, including sufentanil and carfentanil, in human urine by liquid chromatography-atmospheric-pressure ionization-tandem mass spectrometry, J. Anal. Toxicol. (2006) 335–341.
[19] E. McBride, G. Verbeck, Z. Lawton. Rapid In-Field Screening for Fentanyl Analogs Using Coiled Microextraction and Portable GC/MS. PerkinElmer. Application Note. 2018.
[20] S. A. Goodchild, L. J. Hubble, R. K. Mishra, Z. Li. Ionic Liquid-Modified Disposable Electrochemical Sensor Strip for Analysis of Fentanyl. Analytical Chemistry. 2019. 3747-3753.
[21] H. Zaknoun, M. Binette, M. Tam, Analyzing fentanyl and fentanyl analogues by ion mobility spectrometry, Int. J. Ion Mobility Spectrom. (2019) 1–10.
[22] A.M. Armas, K. Song. The Analytical Profile of Fluorobutyryl fentanyl isomers. DEA Laboratory Note. January 2019.
[23] United Nations Office of Drugs and Crime. Recommended methods for the Identification and Analysis of Fentanyl and its Analogues in Biological Specimens. United Nations, 2017.
[24] Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG). Welcome to the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) Website. http://www.swgdrug.org (May 3, 2018).
[25] S. Ho. Using Q-TOF LC-MS to distinguish 2- and 3- furanyl fentanyl and other fentanyl related compounds. DEA Laboratory Notes. 2017. LN-17-06-01.
[26] M. Skov-Skov Bergh, I.L. Bogen, A. Wohlfarth, S. R. Wilson, A. M. Leere Oiestad. Distinguishing between cyclopropylfentanyl and crotonylfentanyl by methods commonly available in the forensic laboratory. Therapeutic Drug Monitoring. Pending Publication.
[27] R.S. Vardanyan, V.J. Hruby, Fentanyl-related compounds and derivatives: current status and future prospects for pharmaceutical applications, Future Med. Chem. 6 (4) (2014) 385–412, https://doi.org/10.4155/fmc.13.215.
[28] Shipman, T. Conti, T. Tighe, E. Buel, Research and Development in the Area of Controlled Substances Forensic Drug Identification by Gas Chromatography-Infrared Spectroscopy. U.S. Department of Justice, Award Number: 2008-DN-BX-K161.
[29] Titan XL Large Volume PTV Inlet, Analytical Solutions and Providers, Revision 2.1 – November 2013. R.
[30] National Forensic Science Technology Center, Florida International University, December 2019. https://www.youtube.com/watch?v=YFTPHDnj0q8.