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Characterization and differentiation of cyclopropylfentanyl from *E*-crotonylfentanyl, *Z*-crotonylfentanyl, and 3-butenylfentanyl

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ABSTRACT

Recently, a sample containing cyclopropylfentanyl was analyzed at this laboratory. Cyclopropylfentanyl began to appear in the United States' illicit drug markets in 2017. Unfortunately, cyclopropylfentanyl presents an analytical challenge due to its mass spectrum being almost identical to that of crotonylfentanyl. There are two possible isomers of crotonylfentanyl, *Z*- and *E*- crotonylfentanyl. In order to provide sufficient analytical data to distinguish the two isomers of crotonylfentanyl and cyclopropylfentanyl, crotonylfentanyl was synthesized and fully characterized. Each isomer was analyzed via nuclear magnetic resonance spectroscopy, gas chromatography–mass spectrometry, and Fourier transform infrared spectroscopy. During the synthesis of crotonylfentanyl, an unknown compound was formed. The identification of this compound and the analytical characterization of the two isomers of crotonylfentanyl are presented. Through the comparison of these compounds, it was confirmed that cyclopropylfentanyl can be differentiated from crotonylfentanyl.

1. Introduction

Fentanyl-related compounds (FRCs) have had a long history within the United States and began to resurface again in 2013. FRCs pose serious risks due to their often unknown potencies as well as the relatively easy methods in which they may be synthesized [1–3]. Typically, only one precursor required for traditional fentanyl synthesis needs modification in order to make a different FRC. Often, FRCs produce very similar analytical data due to their structural similarities, which adds to the difficulty in their identification for forensic purposes. There have been several recent publications aiming to assist the forensic community in identifying and differentiating newly encountered FRCs [4–8]. In addition to the difficulty in identification, certified reference materials (CRMs) are often required to confirm the structure of a suspected FRC. This leads to the need to synthesize or purchase potential FRCs before they actually appear on the illicit drug markets.

In 2017, cyclopropylfentanyl (Fig. 1) began to appear in drug exhibits internationally as well as within the U.S. [9–11] Subsequently, it was temporarily designated as a Schedule I controlled substance. Its potency in comparison to fentanyl is unknown. The synthesis, similar to many other FRCs, involves the modification of precursors utilized in fentanyl synthesis. Unfortunately, it was quickly realized by the forensic community that the mass spectrum of cyclopropylfentanyl is almost identical to that of crotonylfentanyl. Therefore, these compounds are often reported collectively as “cylcopropylfentanyl / crotonylfentanyl” [10, 12]. The combined reporting presents challenges for data interpretation. For example, the National Forensic Laboratory Information System's (NFLIS) reporting of cyclopropylfentanyl and crotonylfentanyl exhibits is not clear as to whether exhibits contain only one of these compounds or a mixture of both. As of February 2018, there were 15 exhibits containing cyclopropylfentanyl and/or crotonylfentanyl from reporting laboratories throughout the U.S.

As of March 2018, crotonylfentanyl (Fig. 1) has yet to be identified in any exhibit within the Drug Enforcement Administration's (DEA) laboratory system. However, a possible legal challenge has arisen in terms of its differentiation from cyclopropylfentanyl with traditional analytical methods found in forensic laboratories. In an effort to differentiate crotonylfentanyl from cyclopropylfentanyl, it was necessary to synthesize and characterize crotonylfentanyl. As seen in Fig. 1, there are two isomers of crotonylfentanyl: *Z*- and *E*-crotonylfentanyl, but the *Z*- isomer is the most prominent. During the synthesis of both isomers of crotonylfentanyl, an unknown compound was detected (Fig. 1); herein determined to be 3-butenylfentanyl. The synthesis, isolation, and analysis of the three compounds are discussed as well as their differentiation from cyclopropylfentanyl.

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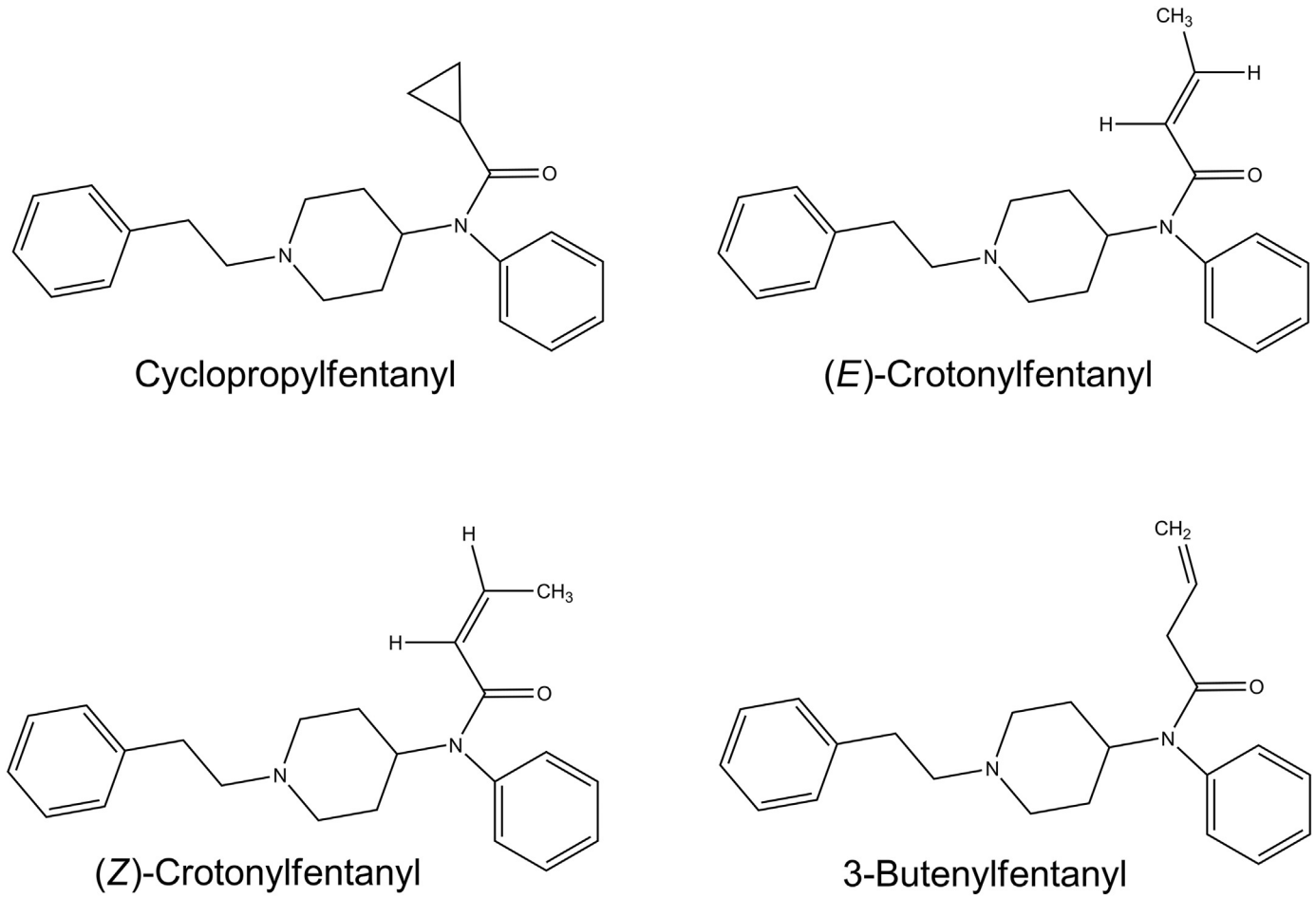

Fig. 1. Structures of cyclopropylfentanyl, *E*-crotonylfentanyl, *Z*-crotonylfentanyl, and 3-butenylfentanyl.

2. Material and methods

2.1. Gas chromatography/mass spectrometry (GC/MS)

GC/MS analyses were performed using an Agilent Model 5975C quadrupole mass-selective detector (MSD) interfaced with an Agilent 7890A gas chromatograph (GC) (Agilent, Santa Clara, CA). The MSD was operated in the electron ionization mode with an ionization potential of 70 eV, a scan range of 34–700 mass units, and at 1.34 scans/s. The GC system was fitted with a 30 m × 0.25 mm ID fused-silica capillary column coated with DB-1 (0.25 μm) in constant flow mode, at 36.5 cm/s of Helium. The GC oven was temperature programmed as follows: Initial temperature, 100 °C; initial hold, 0.0 min; temperature program rate, 6 °C/min to 300 °C; final hold, 5.6 min. The injector was operated in the split mode (22:1) and at a temperature of 280 °C. The auxiliary transfer line to the MSD was operated at 280 °C. Samples (ca. 1 mg/mL) were injected at a volume of 2 μL.

2.2. Fourier transform infrared spectroscopy (FTIR)

Infrared spectra were obtained on a Thermo-Nicolet Nexus 670 FTIR equipped with a SensIR Dura-Scope single bounce attenuated total reflectance (ATR) accessory. Instrument parameters were: Resolution = 4 cm^{-1}; gain = 1; optical velocity = 0.4747; aperture = 150; and scans/sample = 32.

2.3. Nuclear magnetic resonance spectroscopy (NMR)

NMR spectra were obtained using an Agilent 600MR-DD2 600 MHz NMR with a 5 mm OneNMR pulse field gradient probe (Palo Alto, CA). The sample temperature was maintained at 25 °C. Standard Agilent pulse sequences were used to obtain proton, carbon (proton decoupled), HSQC (C13 and N15), HMBC (C13 and N15), COSY, H2BC, NOESY, and HSQC-TOCSY. Samples were dissolved in 1 mL deuterated chloroform ($CDCl_3$) containing 0.03% *v*/v tetramethylsilane (TMS, 0 ppm reference) (Cambridge Isotopes, Tewksbury, MA). Data processing and structure elucidation were performed using Structure Elucidator software from Applied Chemistry Development (ACD/Labs, Toronto, Canada).

2.4. Synthesis

Due to the nature and sensitivity of fentanyl-related syntheses, exact synthetic details are not given, but are outlined in Fig. 2 and briefly

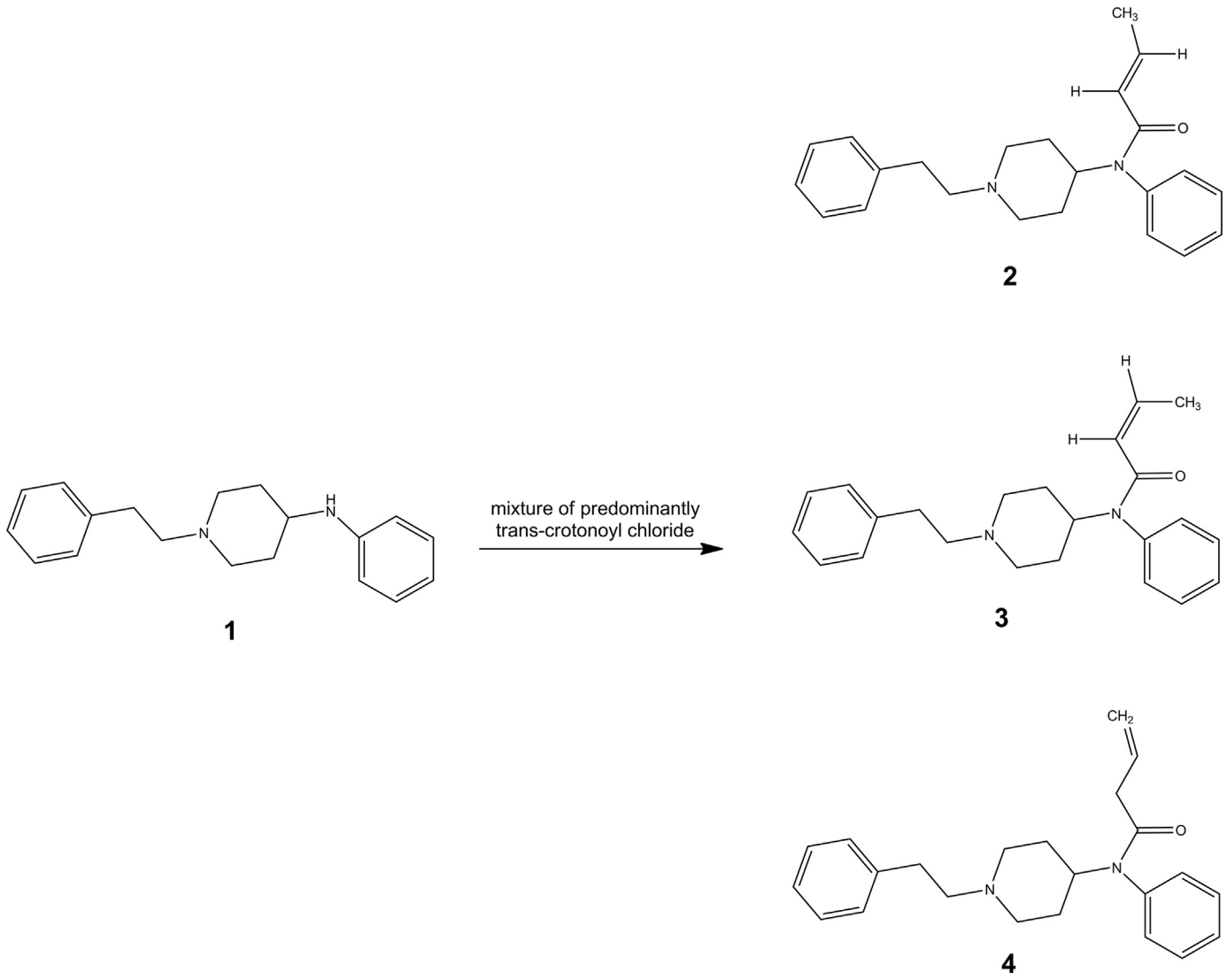

Fig. 2. Synthetic scheme to *E*-crotonylfentanyl **2**, *Z*-crotonylfentanyl **3**, and 3-butenylfentanyl **4**.

described below (yields not optimized). All reagents were products of Sigma-Aldrich Chemical Company (Milwaukee, WI), and were used without further purification. 4-Anilino-N-phenethylpiperidine (4-ANPP) was obtained from the authentic reference collection of this laboratory. 4- ANPP **1** was reacted with two equivalents of crotonyl chloride (predominantly *trans*-isomer) to provide a mixture of *E*-crotonylfentanyl **2**, *Z*-crotonylfentanyl **3**, and 3-butenylfentanyl **4** at ratios of approximately 91:5:4. Compounds **2**, **3**, and **4** were isolated via alumina column chromatography in yields of 44%, 1.3%, and 0.2%, respectively.

2.5. (2E)-Crotonylfentanyl base

^{1}H NMR (600 MHz, CHLOROFORM-d) δ ppm 1.46 (br q, J = 11.7 Hz, 2H), 1.68 (d, J = 7.1 Hz, 3H), 1.83 (br d, J = 11.7 Hz, 2H), 2.18 (br t, J = 11.7 Hz, 2H), 2.54 (m, 2H), 2.73 (m, 2H), 3.01 (br d, J = 10.6 Hz, 2H), 4.73 (br t, J = 11.8 Hz, 1H), 5.47 (br d, J = 14.8 Hz, 1H), 6.90 (dq, J = 14.8, 7.1Hz, 1 H), 7.08 (d, J = 7.7 Hz, 2H), 7.15 (m, 2H), 7.18 (m, 1H), 7.25 (m, 2H), 7.38 (m, 1H), 7.39 (m, 2H).

^{13}C NMR (151 MHz, CHLOROFORM-d) δ ppm 17.95, 30.58 (2C), 33.86, 52.33, 53.12 (2C), 60.52, 123.51, 126.02, 128.23, 128.38 (2C), 128.63 (2C), 129.18 (2C), 130.73 (2C), 138.45, 140.28, 141.09, 165.69.

2.6. (2Z)-Crotonylfentanyl base

^{1}H NMR (600 MHz, CHLOROFORM-d) δ ppm 1.47 (br q, J = 11.3 Hz, 2H), 1.84 (br d, J = 12.0 Hz, 2H), 2.08 (d, J = 7.0 Hz, 3H), 2.18 (br t, J = 11.3 Hz, 2H), 2.55 (m, 2H), 2.74 (m, 2H), 3.02 (br d, J = 11.3 Hz, 2H), 4.71 (br t, J = 12.0 Hz, 1H), 5.42 (br d, J = 11.3 Hz, 1H), 5.86 (dq, J = 11.3, 7.3 Hz, 1H), 7.07 (d, J = 7.7 Hz, 2H), 7.16 (m, 2H), 7.17 (m, 1H), 7.26 (m, 2H), 7.35 (m, 1H), 7.37 (m, 2H).

^{13}C NMR (151 MHz, CHLOROFORM-d) δ ppm 15.27, 30.64 (2C),

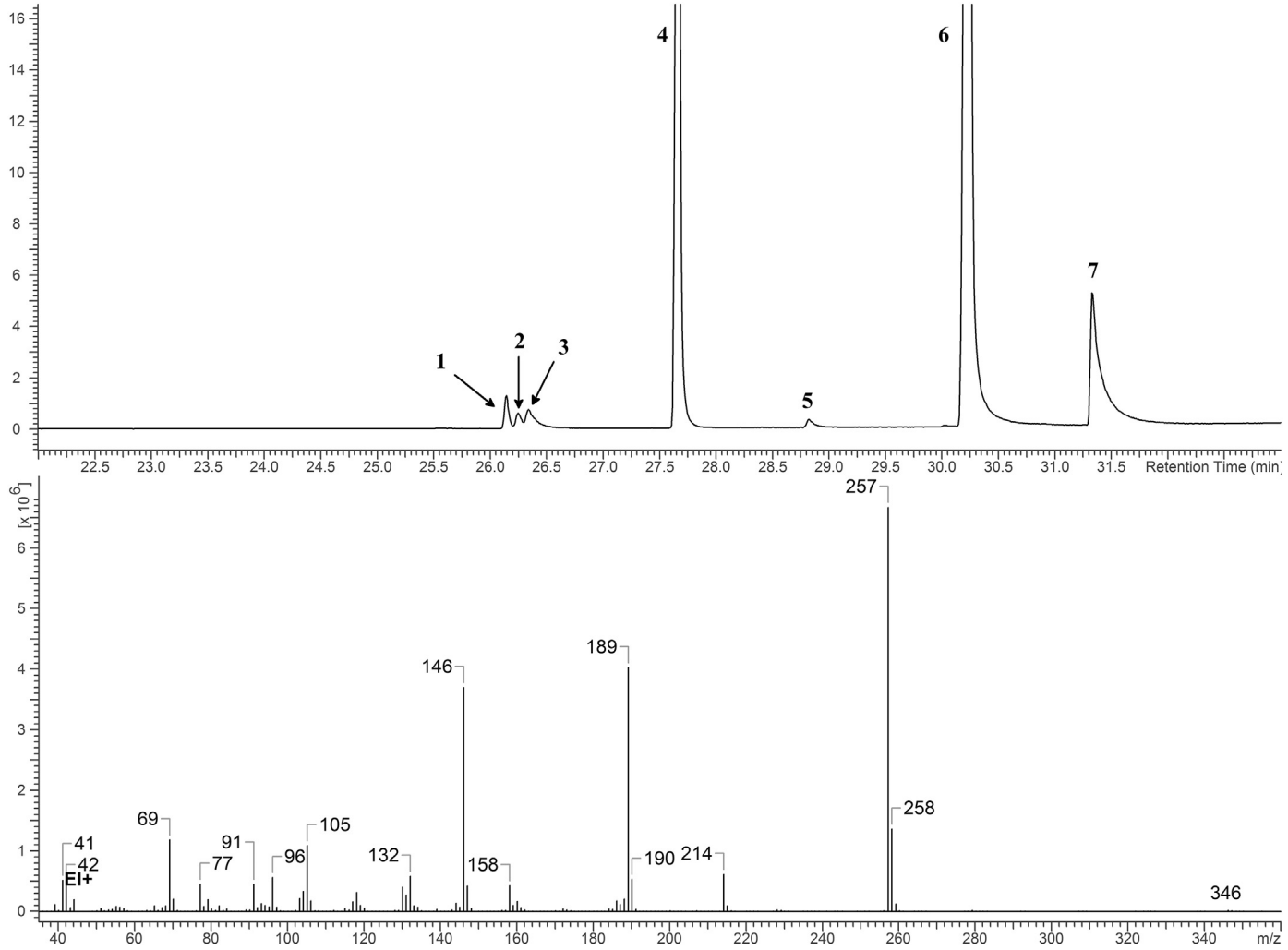

Fig. 3. Reconstructed total ion chromatogram of an illicit sample containing acetylcodeine (1), U-48800 (2), O6-monoacetylmorphine (3), heroin (4), fentanyl (5), cyclopropylfentanyl (6), and alprazolam (7) with the electron mass spectrum mass spectra of cyclopropylfentanyl below.

33.86, 51.87, 53.16 (2C), 60.51, 122.79, 126.03, 128.14, 128.38 (2C), 128.63 (2C), 129.15 (2C), 130.50 (2C), 138.78, 139.37, 140.28, 166.53.

2.7. 3-Butenylfentanyl (crotonylfentanyl impurity)

^{1}H NMR (600 MHz, CHLOROFORM-d) δ ppm 1.44 (m, 2H), 1.81 (br d, J = 11.7 Hz, 2H), 2.16 (br t, J = 11.7 Hz, 2H), 2.54 (m, 2H), 2.72 (m, 2H), 2.74 (m, 2H), 3.00 (br d, J = 11.7 Hz, 2H), 4.68 (tt, J = 12.1, 3.7 Hz, 1H), 4.89 (d, J = 17.1Hz, 1H), 5.04 (d, J = 10.3 Hz, 1H), 5.88 (ddt, J = 17.1, 10.3, 6.7 Hz, 1 H), 7.09 (d, J = 7.2Hz, 2H), 7.15 (m, 2 H), 7.17 (m, 1H), 7.25 (m, 2H), 7.38 (m, 1H), 7.39 (m, 2H).

^{13}C NMR (151 MHz, CHLOROFORM-d) δ 30.5 (2C), 33.8, 40.1, 52.3, 53.1 (2C), 60.5, 117.5, 126.0, 128.4 (2C), 128.5, 128.6 (2C), 129.3 (2C), 130.5 (2C), 132.1, 138.5, 140.2, 170.56.

3. Results and discussion

This laboratory has received eight separate cyclopropylfentanyl seizures within the past year. All were seized between June and July 2017 and contained cyclopropylfentanyl with concentrations ranging from 1.6–91.4%. Six of the seizures were in powder form, while two were tablets. Half of those exhibits also contained another FRC. An example GC/MS reconstructed total ion chromatogram of a seizure containing cyclopropylfentanyl (7% purity by NMR), heroin, U-48800, fentanyl, and alprazolam is illustrated in Fig. 3. The mass spectrum of cyclopropylfentanyl is shown within the same figure. In order to differentiate cyclopropylfentanyl from its closely related FRCs, *E*- and *Z*-crotonylfentanyl, as well as 3-butenylfentanyl were synthesized to obtain their analytical profiles for comparison. The structures of each compound and purities were confirmed with NMR.

The two isomers of crotonylfentanyl, 3-butenylfentanyl, and cyclopropylfentanyl were compared via FTIR and GC/MS (Figs. 4-8). The infrared spectra of *E*-crotonylfentanyl base (most prominent isomer) and cyclopropylfentanyl base are shown in Fig. 4. Both spectra have the characteristic absorbances for aliphatic and aromatic carbons between 3100 cm^{-1} and 2700 cm^{-1}. Differences are observed in the region around 1600 cm^{-1}. In the cyclopropylfentanyl spectrum, there are only two major absorbances with the most prominent being at 1639 cm^{-1}. *E*-crotonylfentanyl IR spectra will have three absorbances in this region

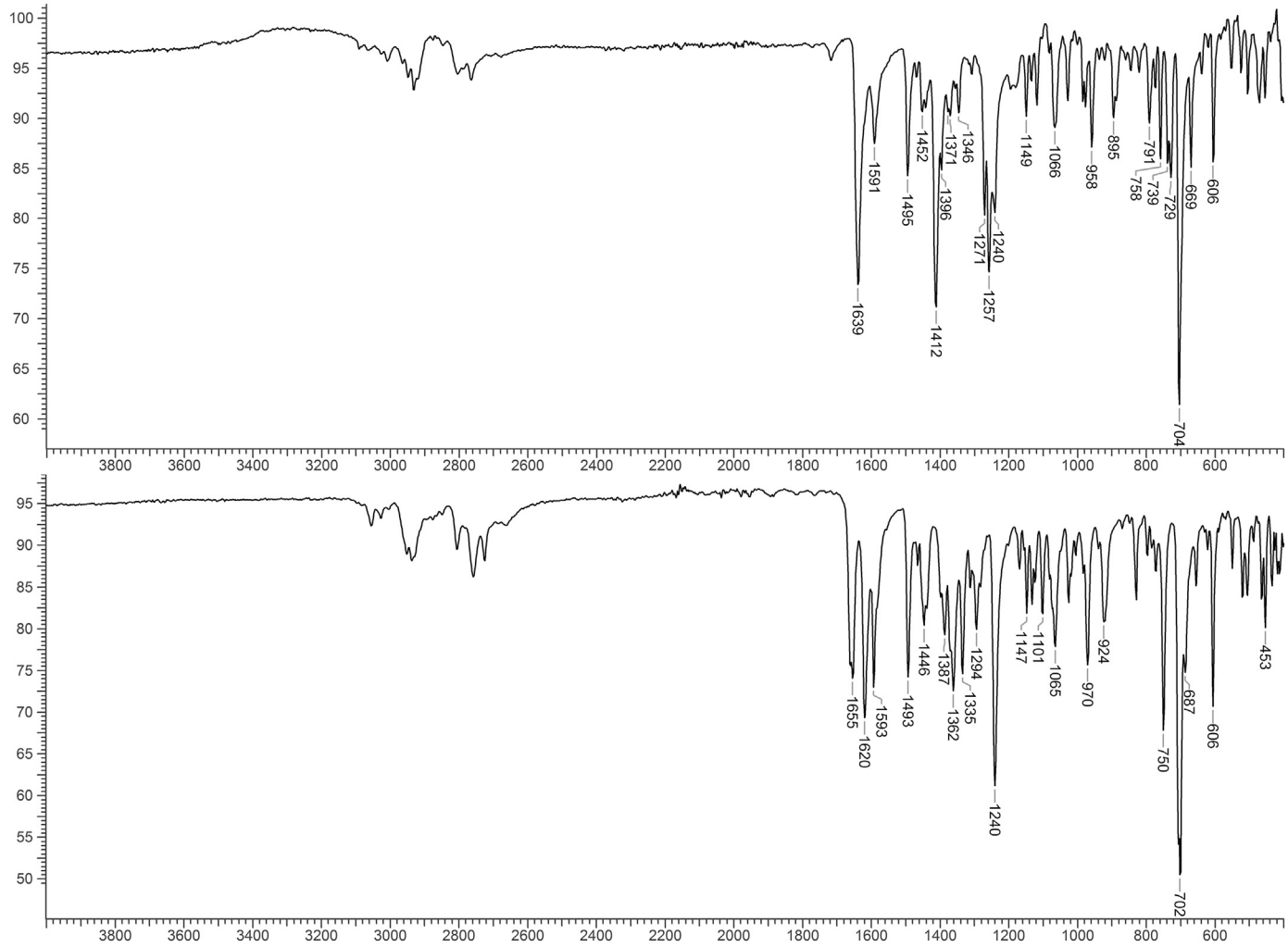

Fig. 4. Infrared spectra (FTIR-ATR) of (upper) cyclopropylfentanyl and (lower) *E*-crotonylfentanyl.

of relatively equal proportion. Cyclopropylfentanyl also has a large absorbance at 1412 that is absent in the spectrum of *E*-crotonylfentanyl, while the spectrum of *E*-crotonylfentanyl has a large absorbance at 750 cm^{-1} that is absent in the cyclopropylfentanyl spectrum. The infrared spectra of *Z*-crotonylfentanyl base (least prominent isomer) and 3-butenylfentanyl base are shown in Fig. 5. As expected, the IR spectrum of *Z*-crotonylfentanyl is almost identical to the *E*-isomer IR spectrum. There are significant differences between the spectra of 3-butenylfentanyl and cyclopropylfentanyl. The most notable is the large absorbance present at 1647 cm^{-1} in the 3-butenylfentanyl spectrum. This could be due to the overlap in the amide and terminal alkene absorbances typically observed in this region. Additionally, an absorbance at 918 cm^{-1} is observed in the 3-butenylfentanyl spectrum, which is absent from the other three presented spectra. Finally, there is splitting observed at 702 cm^{-1} that is absent in all other spectra. Despite the structural similarities in the presented FRCs, there are notable differences between the IR spectra of cyclopropylfentanyl and crotonylfentanyl that could be used to differentiate them.

Since most all forensic identifications of FRCs are made via GC/MS due to the low FRC concentrations typically encountered, this will be the major emphasis of the discussion. The GC retention times for each compound and fentanyl are listed in Table 1. As shown, all compounds were fully resolved utilizing the described method. The electron ionization (EI) mass spectrum of cyclopropylfentanyl (Fig. 3) is remarkably quite similar to both the EI mass spectrum of *E*- and *Z*-crotonylfentanyl (Fig. 7). The only apparent minor differences are in the *m/z* 69 ion in which the ratio of *m/z* 69 to *m/z* /105 for cyclopropylfentanyl is approximately 1:1, while these same ions give a slightly enriched *m/z* 69 in the crotonylfentanyl isomers; yielding a ratio to *m/z* 105 of approximately 1.5 to 1. It should be noted that these ratios can be influenced by differing on-column concentrations and/or tuning parameters on another instrument. For this reason, we recommend that another technique be utilized if possible (FTIR or NMR). Fortunately, the isomers of crotonylfentanyl and cyclopropylfentanyl were all separated from each other by retention time, so despite the similarities in mass spectral data between these compounds, retention times can be used to differentiate them with the presented method (Fig. 6). The EI mass spectrum of 3-butenylfentanyl is shown in Fig. 8. It is more readily differentiated from cyclopropylfentanyl by its abundances of major ions at *m/z* 146 and *m/z* 189. 3-Butenylfentanyl produces a ratio of *m/z* 146 to *m/z* /189 at approximately 2:1, while cyclopropylfentanyl yields a ratio of these ions at approximately 0.9 to 1. Additionally, *m/z* 69 is much less abundant relative to *m/z* 105 giving a ratio of approximately 1:2.

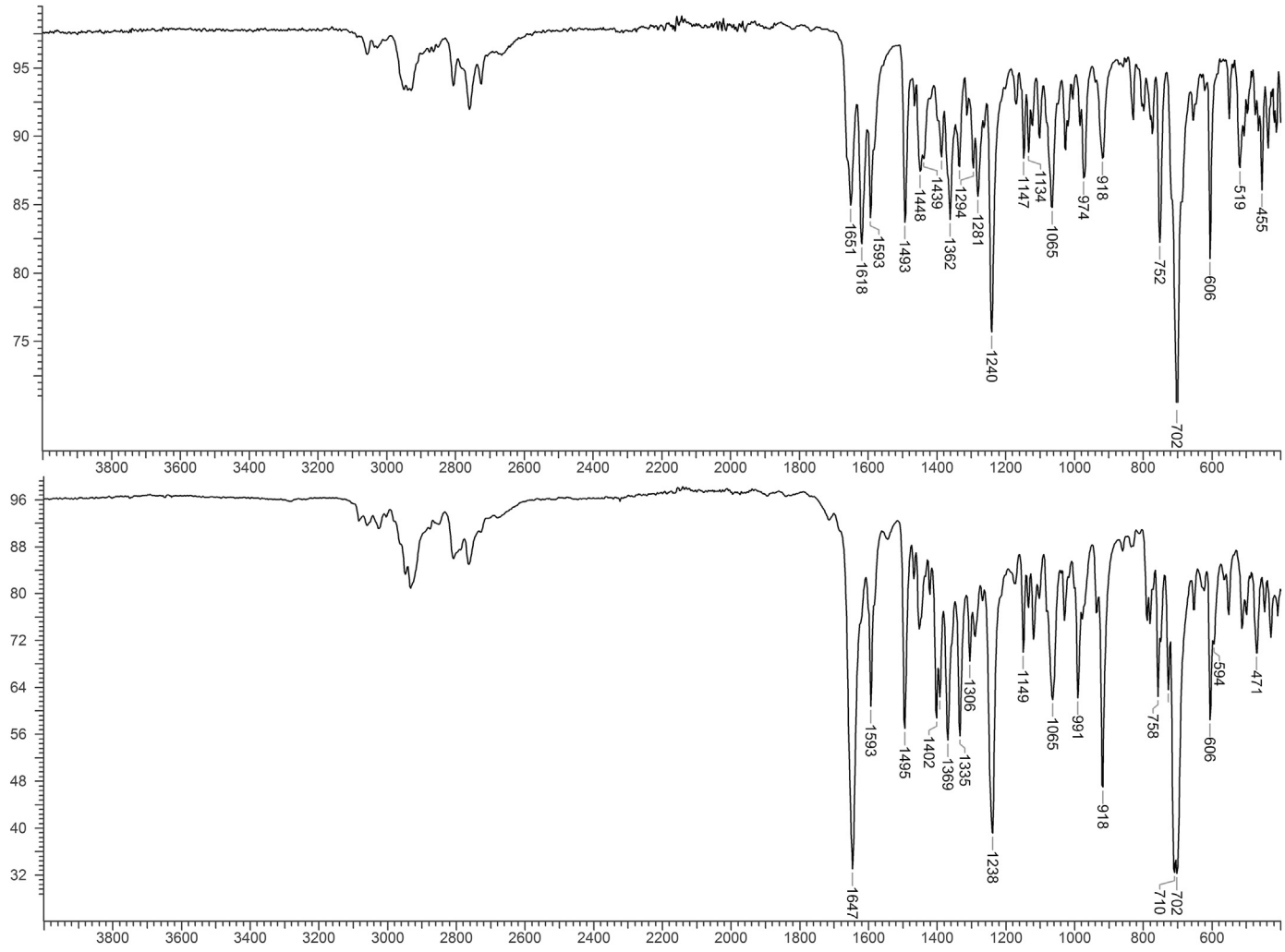

Fig. 5. Infrared spectra (FTIR-ATR) of (upper) *Z*-crotonylfentanyl and (lower) 3-butenylfentanyl.

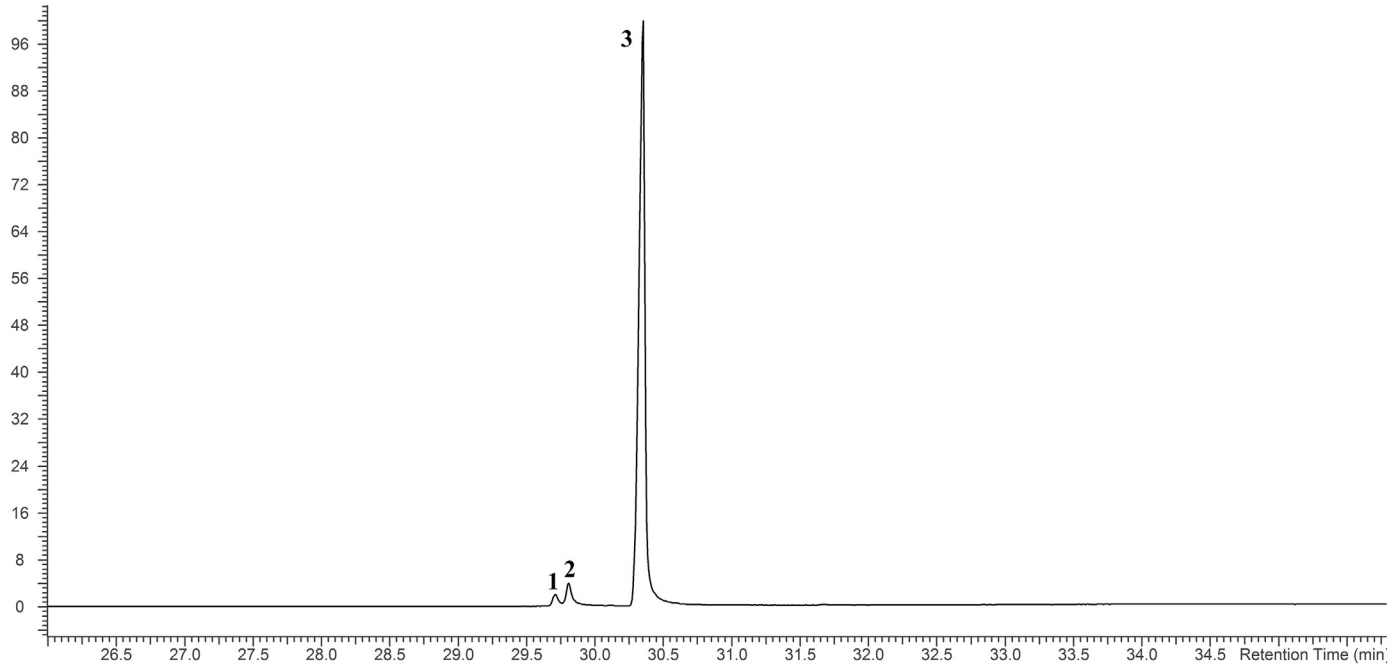

Fig. 6. Reconstructed total ion chromatogram of 3-butenylfentanyl (1), *Z*-crotonylfentanyl (2), and *E*-crotonylfentanyl (3).

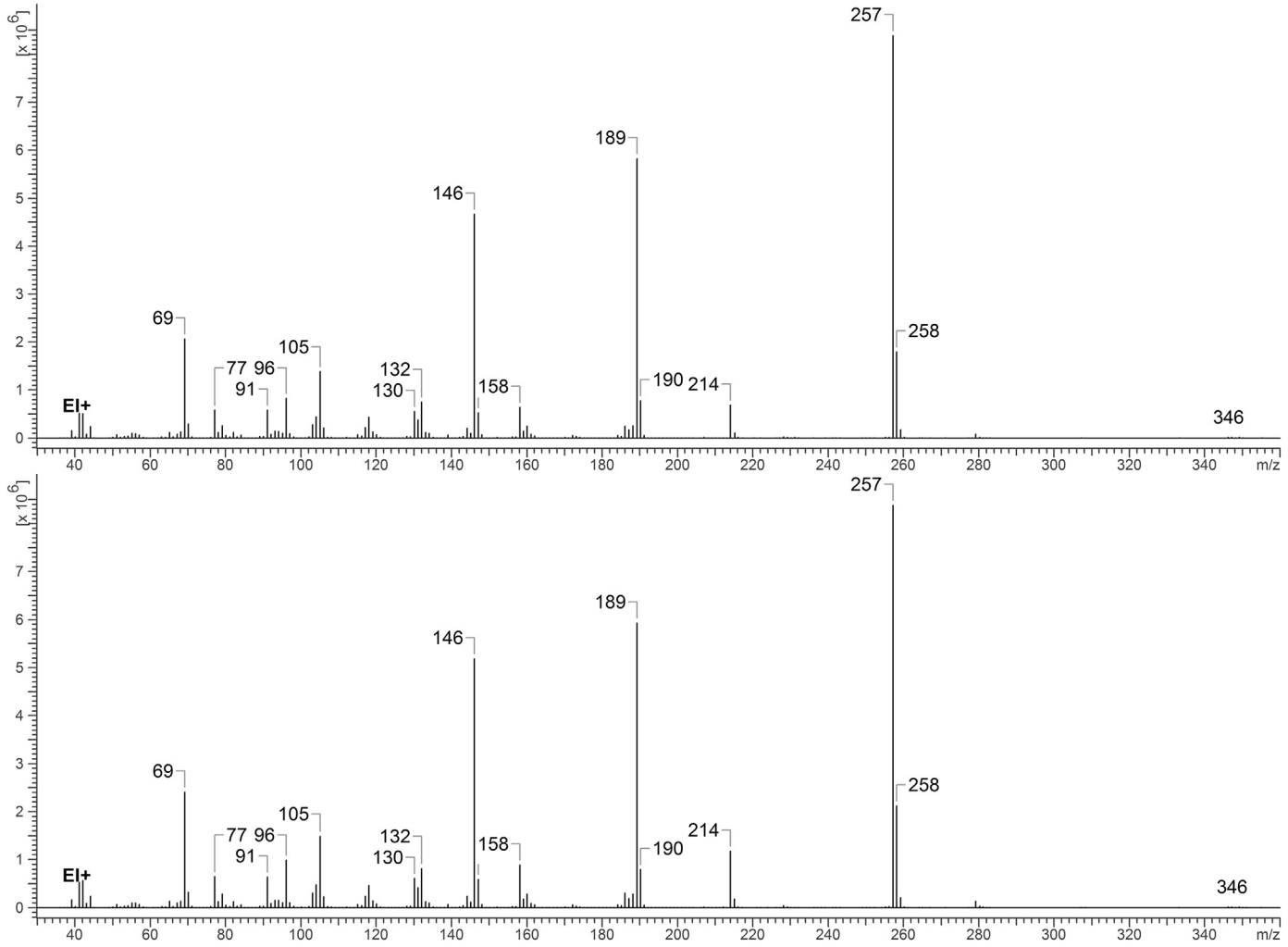

Fig. 7. Electron ionization mass spectra of (upper) *Z*-crotonylfentanyl and (lower) *E*-crotonylfentanyl.

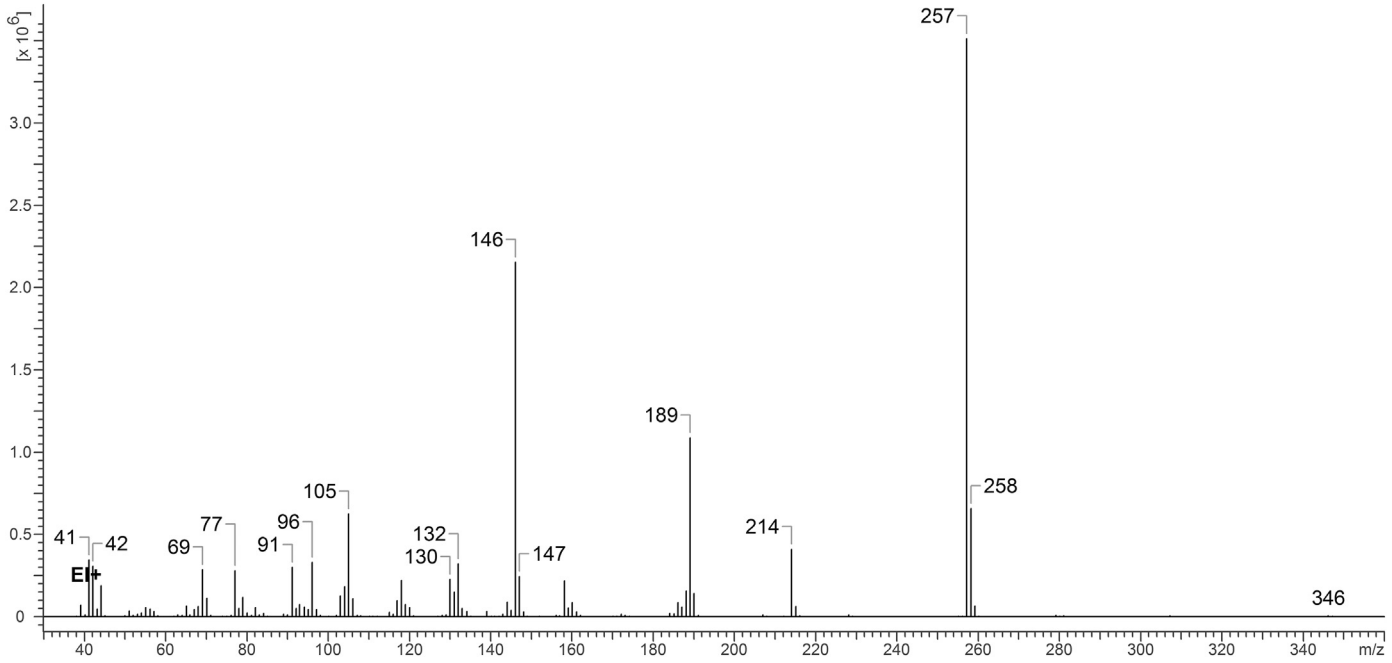

Fig. 8. Electron ionization mass spectra of 3-butenylfentanyl.

Table 1
GC/MS retention times of fentanyl-related compounds in order of elution. See Experimental for GC/MS conditions.

Compound	Retention time (min)
Fentanyl	28.81
3-butenylfentanyl	29.71
Z-crotonylfentanyl	29.81
Cyclopropylfentanyl	30.21
E-crotonylfentanyl	30.35

4. Conclusions

The two isomers of crotonylfentanyl and 3-butenylfentanyl were synthesized and can be separated from cyclopropylfentanyl through traditional analytical methods typically utilized by forensic laboratories. Despite their mass spectral similarity to cyclopropylfentanyl, each of these compounds can be differentiated from another through slight mass spectral and retention time differences. In samples where the concentration allows for isolation of these FRCs, FTIR can be used as an additional method to distinguish the two compounds.

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