Application Note

Methods to Differentiate Base Peak 257 Fentanyls: Methacrylfentanyl, Cyclopropyl Fentanyl, and Crotonyl Fentanyl

Holly Grenke Pierzynski, MS, Jianmei Liu, Michael Miller, MS, Laura J. Neubauer, Roxanne E. Franckowski, MS, and Donna M. Iula, Ph.D.
Cayman Chemical Company

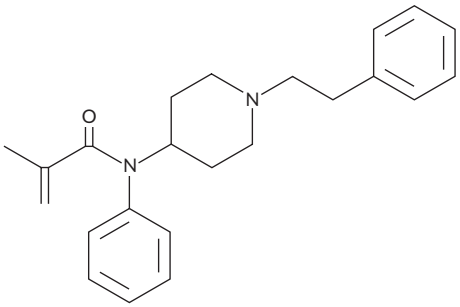
Key Features

- Isobaric fentanyl analogs pose a challenge to identification and differentiation in forensic casework.
- A method has been developed to separate methacrylfentanyl, cyclopropyl fentanyl, and crotonyl fentanyl by gas chromatography–mass spectrometry (GC-MS).
- Close-eluting cyclopropyl fentanyl and crotonyl fentanyl were differentiated by Fourier-transform Infrared Spectroscopy (FTIR).
- A convenient and inexpensive technique to differentiate cyclopropyl fentanyl and crotonyl fentanyl by thin layer chromatography (TLC) was also determined.

Cayman Chemical · 1180 East Ellsworth Road · Ann Arbor, MI, 48108 · (800) 364-9897

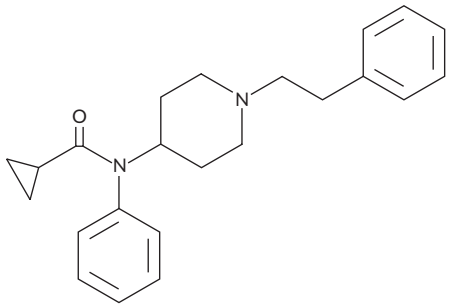
Introduction

Cyclopropyl fentanyl is a new psychoactive substance structurally similar to the potent synthetic opioid pharmaceutical fentanyl. This new fentanyl analog emerged on the designer drug scene in the summer of 2017, but it has never been studied or approved for use in humans. In June of 2017, the Georgia Bureau of Investigation's Crime Lab issued a report that cyclopropyl fentanyl had been positively identified in counterfeit Percocet tablets following an investigation into multiple reported overdoses in the state.[1] Since that initial report, there have been numerous cases of cyclopropyl fentanyl identification across the United States, and the substance was issued a temporary Schedule I controlled substance designation by the Drug Enforcement Administration in January of 2018.[2] This compound has the molecular formula $C_{23}H_{28}N_2O$, exact mass of 348.22, and a prominent GC-MS base peak *m/z* of 257.[3] Isobaric analogs with similar GC-MS fragmentation and close chromatographic retention times have also emerged, which have added to the challenge of identifying and confirming the presence of cyclopropyl fentanyl in forensic casework. Of the three substances, cyclopropyl fentanyl and crotonyl fentanyl are a particular challenge in that they are nearly indistinguishable by routine testing techniques. A recent publication has indicated that there is a difference in the UV maxima, which helps distinguish between the two.[4] Using tools readily available to the forensic community, we have successfully developed methods to unambiguously identify the three isobaric fentanyls shown below.



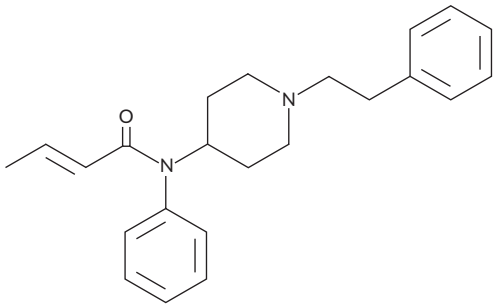
Methacrylfentanyl

Chemical Formula: $C_{23}H_{28}N_2O$
Exact Mass: 348.22
GC-MS base peak: 257



Cyclopropyl fentanyl

Chemical Formula: $C_{23}H_{28}N_2O$
Exact Mass: 348.22
GC-MS base peak: 257



Crotonyl fentanyl

Chemical Formula: $C_{23}H_{28}N_2O$
Exact Mass: 348.22
GC-MS base peak: 257

The Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) has published a set of recommendations. Part IIIB, Methods of Analysis/Drug Identification, recommends a minimum standard of analytical criteria be employed when making an identification.[5] The analytical criteria are separated into three categories (A, B, and C) based on discriminating potential. Category A, considered identification techniques, is the most discriminate (*e.g.*, a spectroscopic technique that provides structural information). Category B includes separation techniques. Category C involves the least discriminate techniques, which are mainly utilized as screening tools (*e.g.*, a presumptive color test).[5] The techniques presented in this application note are classified as Category A and Category B techniques.

GC-MS

Among the three fentanyl analogs, methacrylfentanyl is the most easily identified and differentiated by its retention time and mass spectrum. It was difficult, however, to distinguish cyclopropyl fentanyl and crotonyl fentanyl with GC-MS techniques routinely used by forensic labs and during quality control testing as they share similar mass spectra, and chromatographic baseline resolution was not achievable. Using the chromatographic conditions we have developed and have listed in the GC-MS method below (**Table 1**), a well-defined separation for methacrylfentanyl, cyclopropyl fentanyl, and crotonyl fentanyl was achieved. Retention times for methacrylfentanyl, cyclopropyl fentanyl, and crotonyl fentanyl were 11.63, 13.23, and 13.47 minutes, respectively. The peaks were confirmed by mass spectra (**Figure 1**). In the present study, we found that oven temperature is key to achieve successful separation. Slowing the rate of the oven temperature program led to complete chromatographic separation and achieving a better than baseline resolution equal to 2.01.

A mixture containing methacrylfentanyl, cyclopropyl fentanyl (HCl), and crotonyl fentanyl was prepared by dissolving 1 mg of each material in 1 ml of HPLC-grade methanol. The final concentration of each of the three components in the solution was 1 mg/ml. A 1 µl solution was injected into the GC-MS and analyzed using the conditions in **Table 1**. A Standard Spectra Autotune (S-Tune) using Perfluorotributylamine (PFTBA) was performed on the mass spectrometer prior to sample analysis.

Table 1. GC-MS conditions as well as sample preparation to achieve data shown in Figure 1.

Instrument	Agilent 6890 Gas Chromatograph equipped with an Agilent 5973 Mass Selective Detector	
Column	Restek, Rtx-5MS, 30 m × 0.32 mm I.D., 0.5 µm film thickness (Phase composition: Crossbond 5% diphenyl / 95% dimethyl polysiloxane, similar columns: DB-5MS)	
Injector Temperature	300°C	
Oven Temperature	240°C for 1 minute, 2.5°C/minute to 275°C, 30°C/minute to 300°C, 4.2 minute hold Total run time: 20 min	
Carrier Gas	Helium at 2.0 ml/minute, split ratio = 15:1	
MS Settings	Transfer line temperature:	300°C
	MS Source:	230°C
	MS Quad:	150°C
	Scan Range:	40-600 *m/z*
	Electron Ionization:	70 eV
S-Tune Parameters	Target Tune Masses:	69, 219, 502
	69 Abundance Target, Counts:	800,000
	Mass 219 Target %:	55
	Mass 502 Target %:	2.5

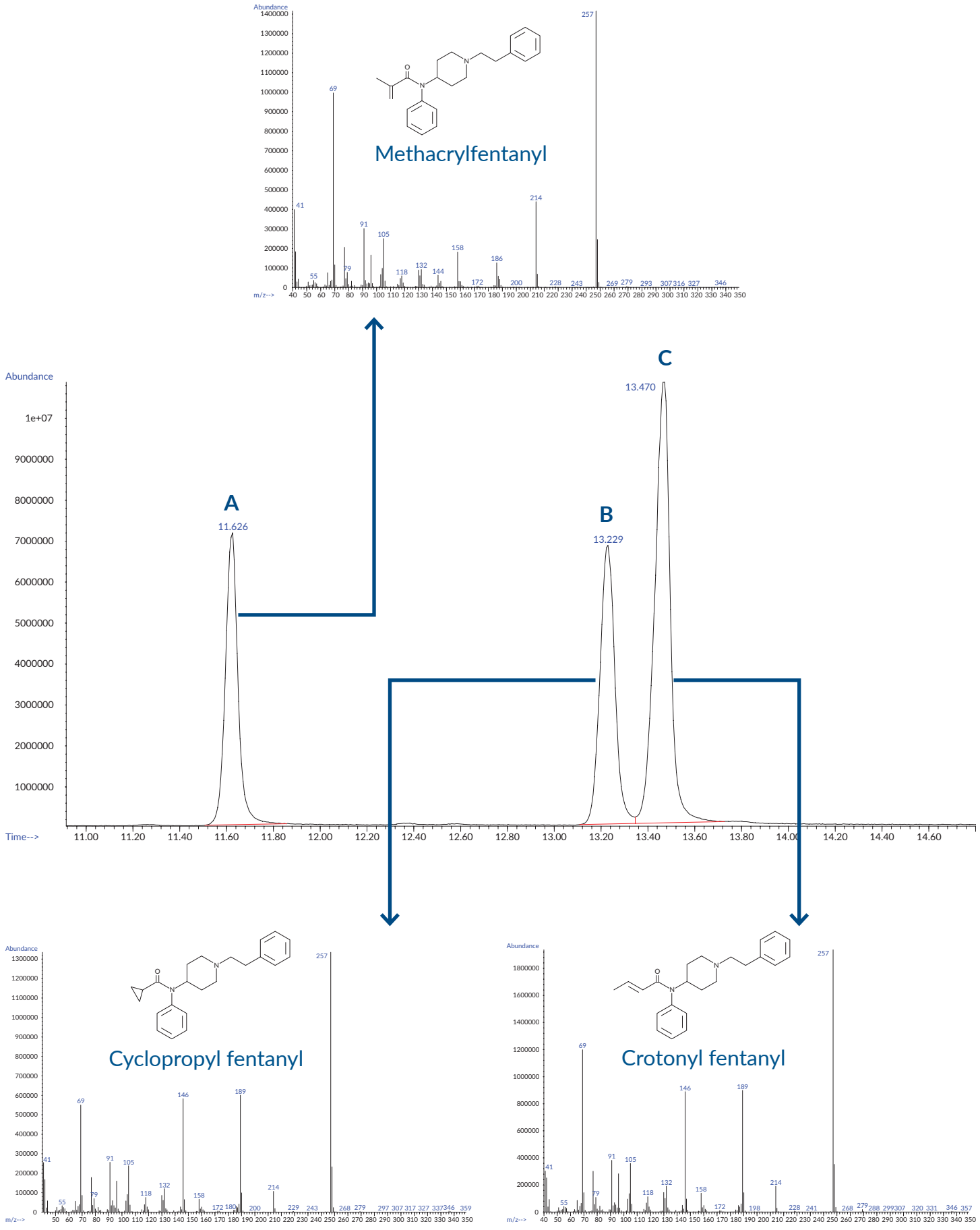


Figure 1. GC-MS chromatograms of (**A**) methacrylfentanyl, (**B**) cyclopropyl fentanyl, and (**C**) crotonyl fentanyl.

ATR-FTIR

Since methacrylfentanyl can be easily differentiated by GC-MS, we focused our attention on the use of FTIR and TLC to differentiate the other two isomers, cyclopropyl fentanyl and crotonyl fentanyl. Attenuated Total Reflection (ATR)-FTIR is a commonly utilized technique that allows the compound to be analyzed in a solid or liquid state without further preparation, while identifying functional groups based on the absorption of IR radiation. There are several noteworthy differences in the spectra of the two molecules that are due to their specific structural features (**Figures 2** and **3**). For example, the amide carbonyl stretch in cyclopropyl fentanyl is at 1640.50 cm^{-1} whereas it is at 1620.79 cm^{-1} in crotonyl fentanyl. This is due to the fact that the carbonyl in crotonyl fentanyl is conjugated to the double bond, which typically results in a lower wavenumber for the C=O vibrational frequency.[6] Cyclopropyl fentanyl's unique feature is its strained cyclopropane ring system. The stretch is prominent and easily identifiable in the fingerprint region at 1415.12 cm^{-1}. This stretch is characteristic of cycloalkane C-H bond vibrations.[7] There are additional differences that are apparent in the expanded overlays given in **Figure 3**.

The free base forms of each molecule were used in this study to avoid any IR differences due to salt form. 5 mg of each material was applied directly to the ATR stage and analyzed using the conditions listed in **Table 2**. A traceable polystyrene standard was analyzed prior to the samples to verify proper instrument performance.

Table 2. ATR-FTIR conditions to achieve data shown in **Figures 2** and **3**.

Instrument	Thermo Scientific™ Nicolet™ iS™10 with Thermo Scientific™ Smart iTR™ accessory for ATR analysis	
Temperature	Ambient	
Processing Software	Omnic	
Instrument Method Details	Number of Scans:	32
	Resolution:	4
	Final Format:	Log(1/R)
	Detector:	DTGS KBr
	Source:	IR
	Accessory:	Smart iTR™
	Max Range:	4,000 cm^{-1}
	Min Range:	650 cm^{-1}
	Gain:	2.0
	Optical Velocity:	0.4747
	Aperture:	Medium Resolution

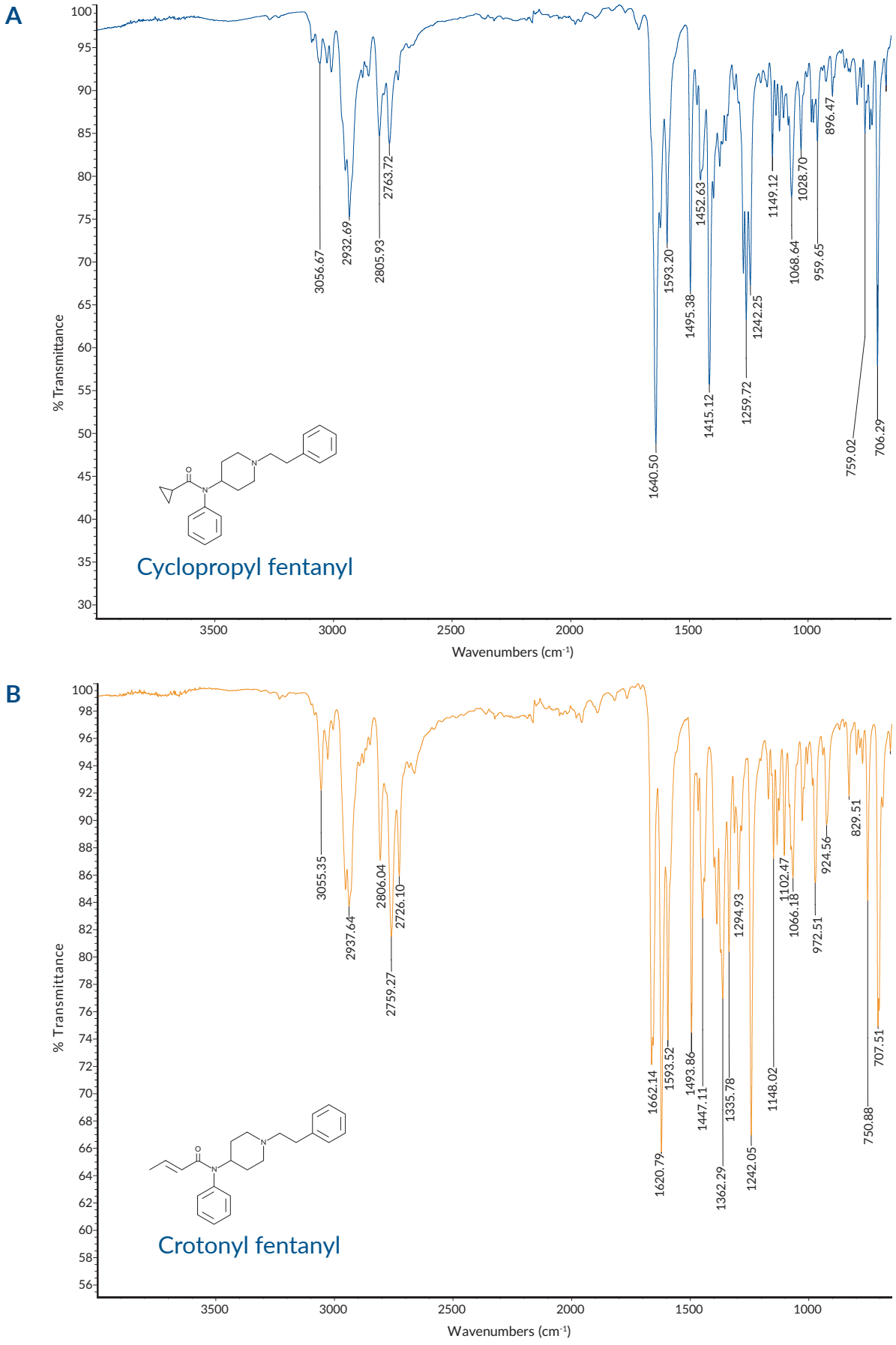


Figure 2. ATR-FTIR spectrums of cyclopropyl fentanyl (**A**) and crotonyl fentanyl (**B**).

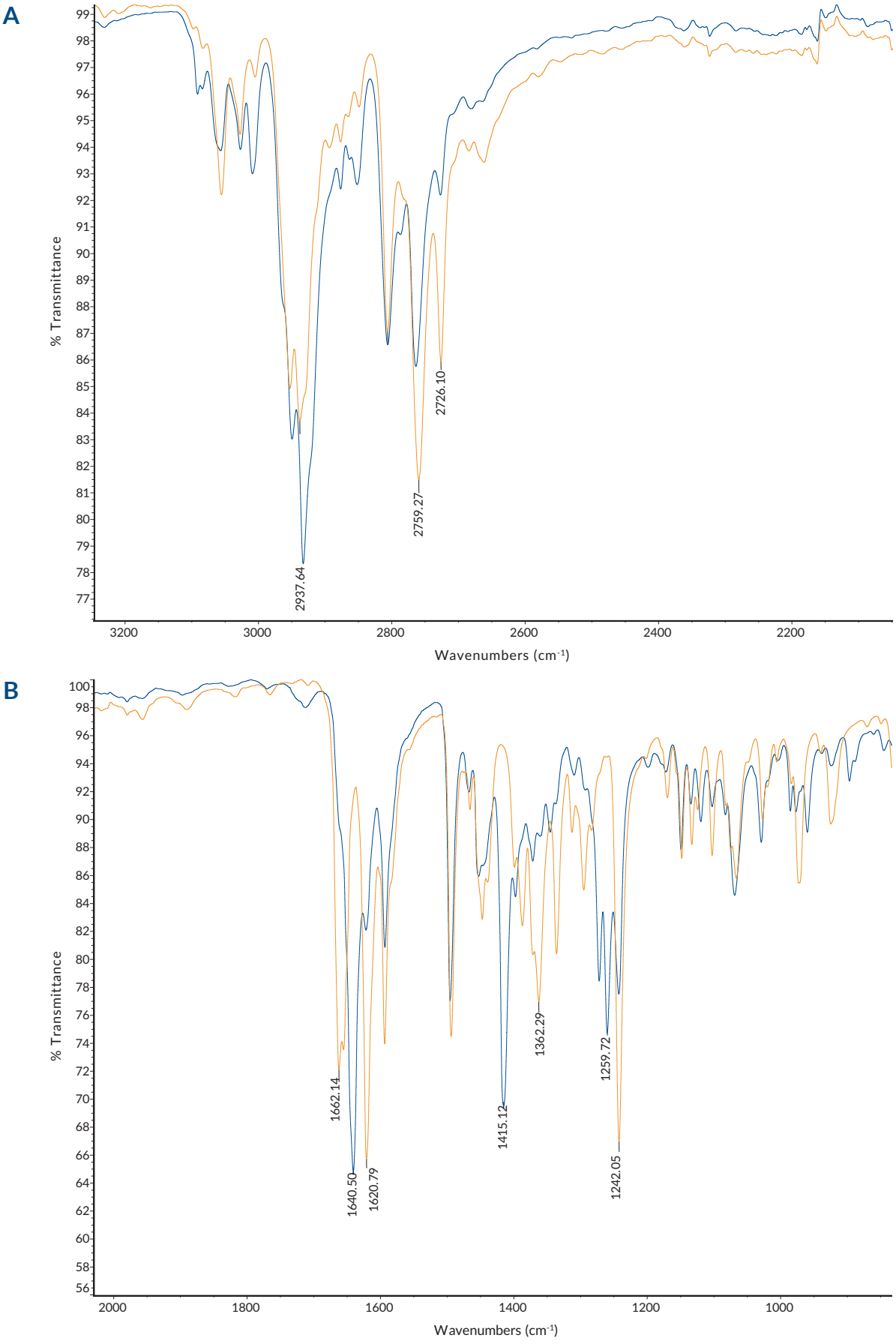


Figure 3. The following expanded regions show a difference in crotonyl fentanyl (orange) and cyclopropyl fentanyl (blue). (**A**) is an overlay of the region 3300 cm^{-1} to 2000 cm^{-1} and (**B**) is an overlay of the region 2000 cm^{-1} to 800 cm^{-1}.

TLC

TLC is a standard technique used by forensic chemists in the presumptive identification of drugs of abuse. This inexpensive technique can be quite successful when used in tandem with more sophisticated analytical methods. After investigating several TLC solvent systems and visualization techniques, we have discovered that it is possible to unambiguously differentiate cyclopropyl fentanyl from crotonyl fentanyl. As shown in **Figure 4**, there is a clear R_f value difference between the two analogs (0.57 for cyclopropyl fentanyl and 0.54 for crotonyl fentanyl) when using our recommended TLC eluent system (70:30:1 hexane:acetone: triethylamine). One point to note is that the concentration of cyclopropyl fentanyl is preferred at 5 mg/ml, whereas the concentration of crotonyl fentanyl could be less (1 mg/ml), due to its higher UV activity. The stronger UV activity is presumably due to the presence of the conjugated crotonamide. As noted below, a small amount of triethylamine was used in the eluent to allow for the ability to skip any base-catalyzed neutralization of the sample prior to spotting. For example, the R_f will be the same under these conditions regardless of whether you have cyclopropyl fentanyl HCl or the cyclopropyl fentanyl free base.

Cyclopropyl fentanyl HCl (5 mg) was dissolved in 1 ml of methanol for a 5 mg/ml solution. Crotonyl fentanyl (1 mg) was dissolved in 1 ml of methanol for a 1 mg/ml solution. Using the conditions in **Table 3**, cyclopropyl fentanyl HCl was spotted on the left in lane A, a co-spot in the middle, lane B, and crotonyl fentanyl was spotted on the right, lane C (**Figure 4**).

Table 3. TLC conditions to achieve results in **Figure 4.**

TLC plate	250 µm Analtech Uniplate silica gel GF
Spotting	The standards were spotted with a 2 µl Drummond Microcaps® micropipette, spotting once using the full 2 µl, and allowed to dry.
Plate eluting procedure and solvent system	The plate was eluted in a TLC chamber/tank using a 70:30:1 hexane:acetone:triethylamine solution. The origin and solvent front were noted then air dried prior to visualization.
Visualization technique	UV lamp (245 nm)

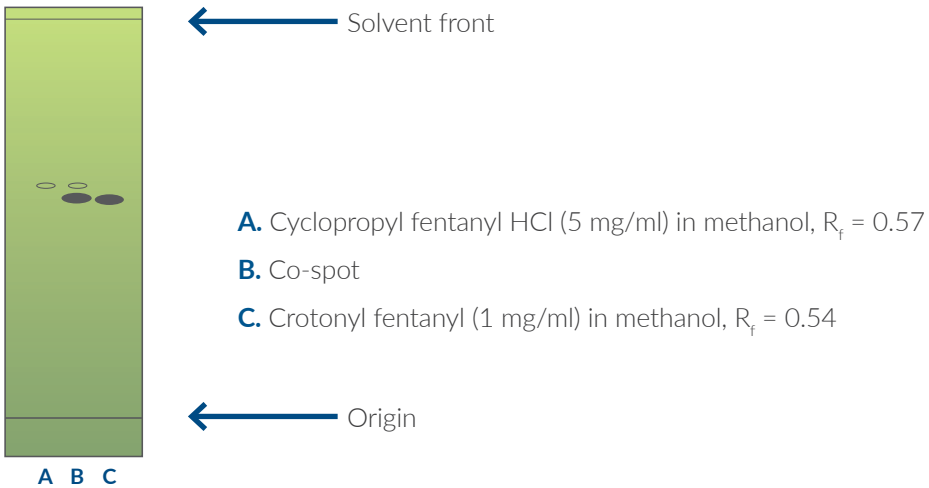


Figure 4. TLC of cyclopropyl fentanyl HCl *vs.* crotonyl fentanyl.

Conclusion

Forensic scientists should be aware that isobaric compounds exist and take extra care in scrutinizing the data before making an identification. The SWGDRUG recommendations require the use of uncorrelated techniques for the identification of an unknown substance.[5] When a Category A technique, such as mass spectrometry or infrared spectroscopy, is utilized in the analytical scheme, a Category B technique may be used to corroborate the identification of an unknown. In the analytical schemes provided, mass spectrometry and infrared spectroscopy are the Category A techniques. The GC method could be the corroborating Category B technique; however, due to procedural or economic reasons, laboratories may not be able to recreate the above GC or IR conditions. Thus, TLC conditions provided herein offer an alternative Category B chromatographic technique to confirm the presence of either cyclopropyl fentanyl and/or crotonyl fentanyl. The use of these methods will aid in proper identification, but reference material standards should be used to confirm the GC retention times, MS spectra, IR spectra, and TLC R_f values.

Cayman products used in this application

Item No.	Product Name
22801	Crotonyl fentanyl
21739	Cyclopropyl fentanyl (hydrochloride)
22884	Methacrylfentanyl

References

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