**Supporting information for:**

**Identification of a new psychoactive substance in seized material: The synthetic opioid *N*-phenyl-*N*-[1-(2-phenethyl)piperidin-4-yl]prop-2-enamide (Acrylfentanyl)**

**Table of contents**

**Characterisation of acrylfentanyl (seized sample)**

*The numbering of atoms used for NMR assignments does not follow IUPAC recommendations and is only used here for clarity (See Fig. S1 for numbering system).*

**Acrylfentanyl**: *N*-phenyl-*N*-[1-(2-phenethyl)piperidin-4-yl]prop-2-enamide

**$^1$H NMR** (400 MHz, $CD_3OD$): $\delta$ 7.60-7.52 (m, 3H; Ph), 7.39-7.27 (m, 7H, Ph), 6.31 (dd, *J* = 17, 2 Hz, 1H, H-1), 5.91 (dd, *J* = 17, 10.5 Hz, 1H, H-2) 5.59 (dd, *J* = 10.5, 2 Hz, 1H, H-1'), 4.90 (tt, *J* = 12, 4 Hz, 1H, H-5),# 3.78 (m, 2H, H-7 and H-7'),* 3.37-3.33 (m, 2H, H-8),# 3.29-3.22 (m, 2H, H-7 and H-7'),* 3.10-3.05 (m, 2H, H-9), 2.26-2.19 (m, 2H, H-6 and H-6'), 1.86 (qd, *J* = 8, 4 Hz, H-6 and H-6')*. # Overlapping with signal from the NMR solvent; * Overlapping with unidentified impurity.

**HRMS** (MALDI-TOF) *m/z* found: 335.2114 $[M+H]^+$; $C_{22}H_{27}N_2O^+$ requires M, 335.2118.

**Figure S1: Acrylfentanyl (seized sample), $^1$H NMR (400 MHz) in $CD_3OD$**

*Overlapping signals from the NMR solvent and impurity triethylamine hydrochloride are indicated with arrows.*

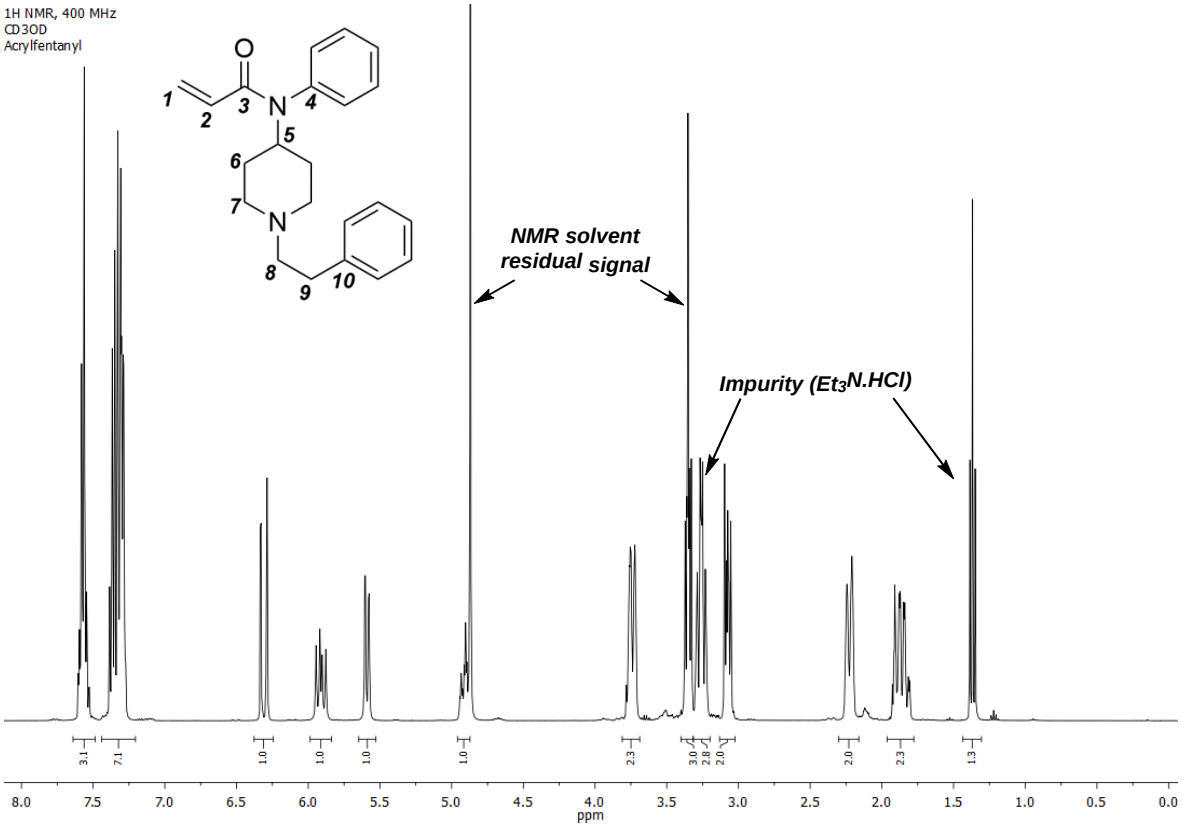


**Figure S2: Acrylfentanyl (seized sample), COSY (400 MHz) in $CD_3OD$**

*Coupling between the $CH_2$- and $CH_3$-groups of triethylamine hydrochloride is observed by COSY*

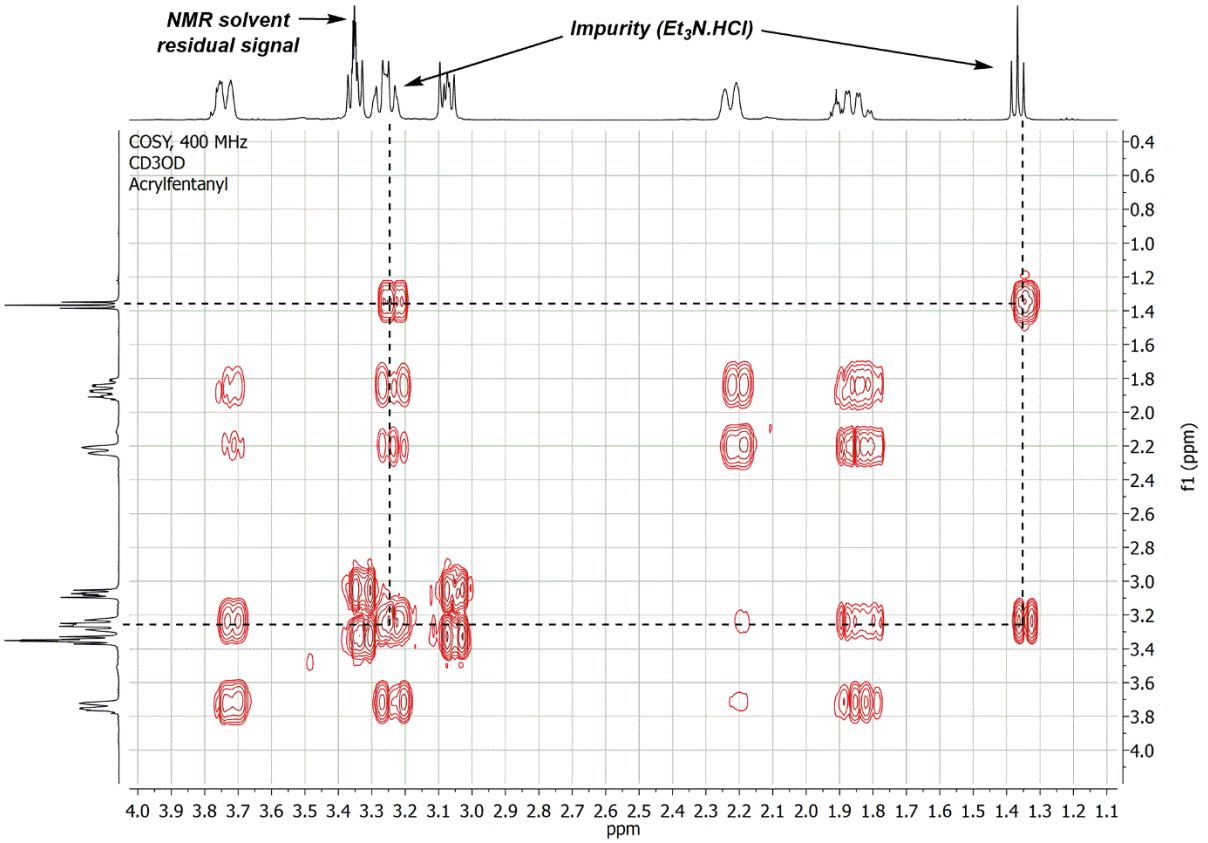
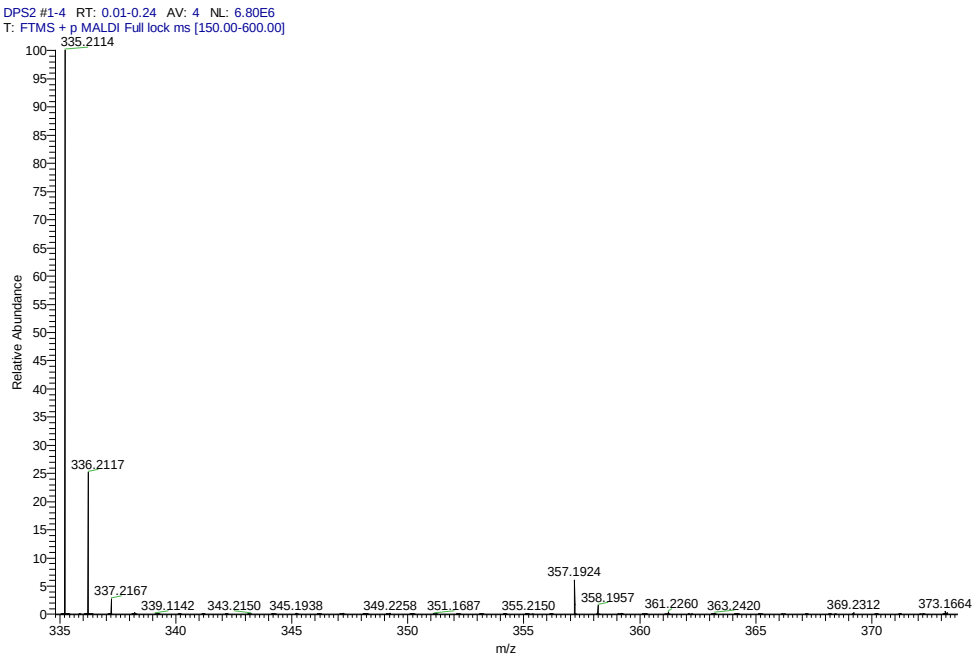



**Figure S3: Acrylfentanyl (seized sample), MALDI-TOF HRMS spectrum**



## Characterisation of fentanyl (standard)

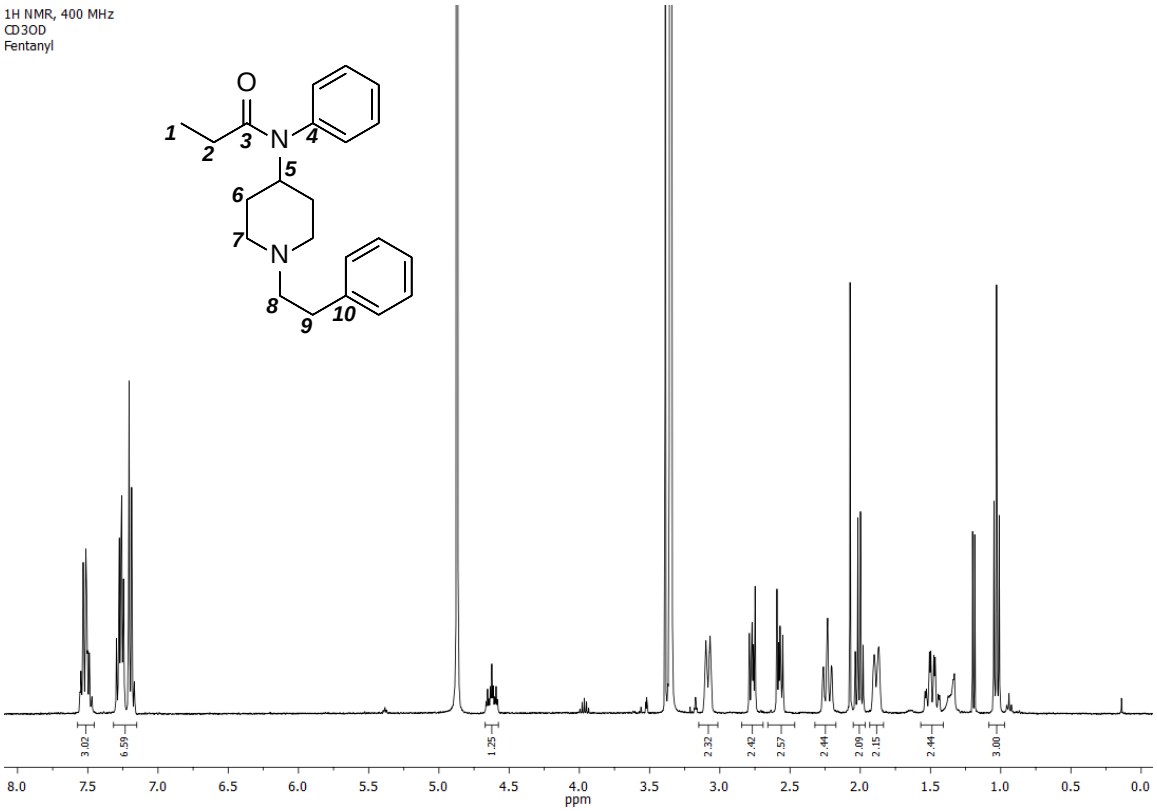
**Fentanyl**: *N*-phenyl-N-[1-(2-phenethyl)piperidin-4-yl]propanamide

*The numbering of atoms used for NMR assignments does not follow IUPAC recommendations and is only used here for clarity (See Fig. S4 for numbering system).*

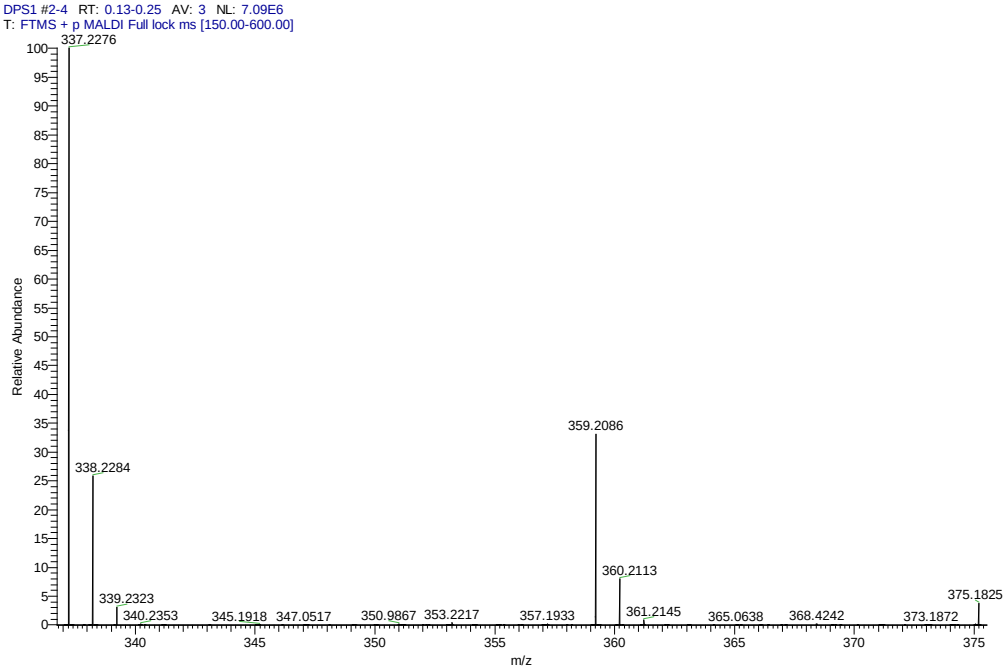
**$^1$H NMR** (400 MHz, $CD_3OD$): $\delta$ 7.57-7.46 (m, 3H, Ph), 7.31-7.23 (m, 4H, Ph), 7.22-7.15 (m, 3H, Ph), 4.62 (tt, *J* = 12, 4 Hz, 1H, H-5), 3.14-3.02 (m, 2H), 2.83-2.71 (m, 2H), 2.62-2.50 (m, 2H), 2.31-2.15 (m, 2H) (H-6, H-6', H7, H-7', H-8 and H-9), 2.01 (q, *J* = 7.5 Hz, 2H, H-2), 1.88 (d, *J* = 12.5 Hz, 2H), 1.49 (qd, *J* = 12.5, 4 Hz, 2H), 1.03 (t, *J* = 7.5 Hz, 3H, H-1).

**HRMS** (MALDI-TOF) m/z found: 335.2114 $[M+H]^+$; $C_{22}H_{27}N_2O^+$ requires M, 335.2118.

**Figure S4: Fentanyl (standard), $^1$H NMR (400 MHz) in $CD_3OD$**



## Figure S5: Fentanyl (standard), MALDI-TOF HRMS spectrum




## Figure S6: $^{1}H$ NMR (600 MHz) in $CDCl_3$

## Top: Acrylfentanyl (standard); Bottom: Acrylfentanyl (seized sample)

*The acrylfentanyl standard conforms to the seized sample, excl. triethylamine hydrochloride*

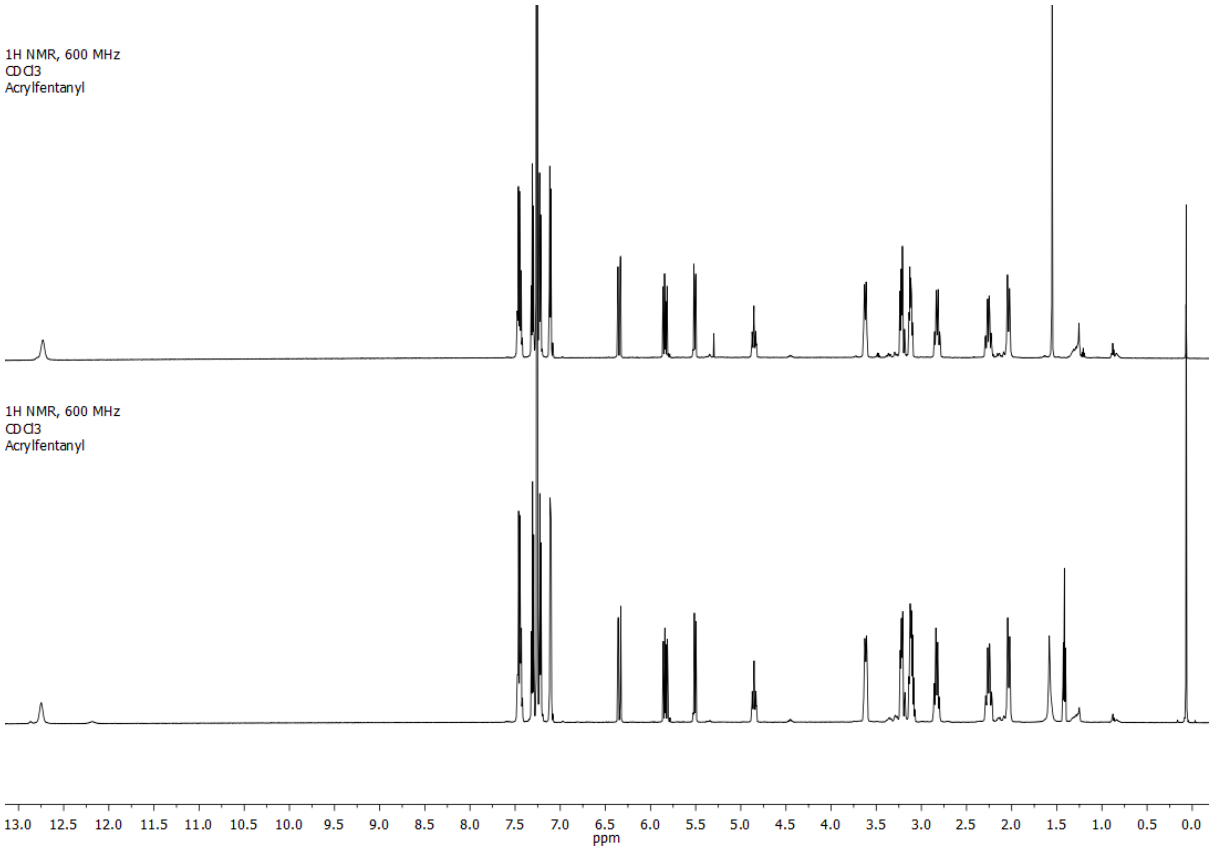


**Figure S7: $^{1}H$ NMR (600 MHz) in $CDCl_3$ : Enlargement (4.7–7.7 ppm)**

**Top: Acrylfentanyl (standard); Bottom: Acrylfentanyl (seized sample)**

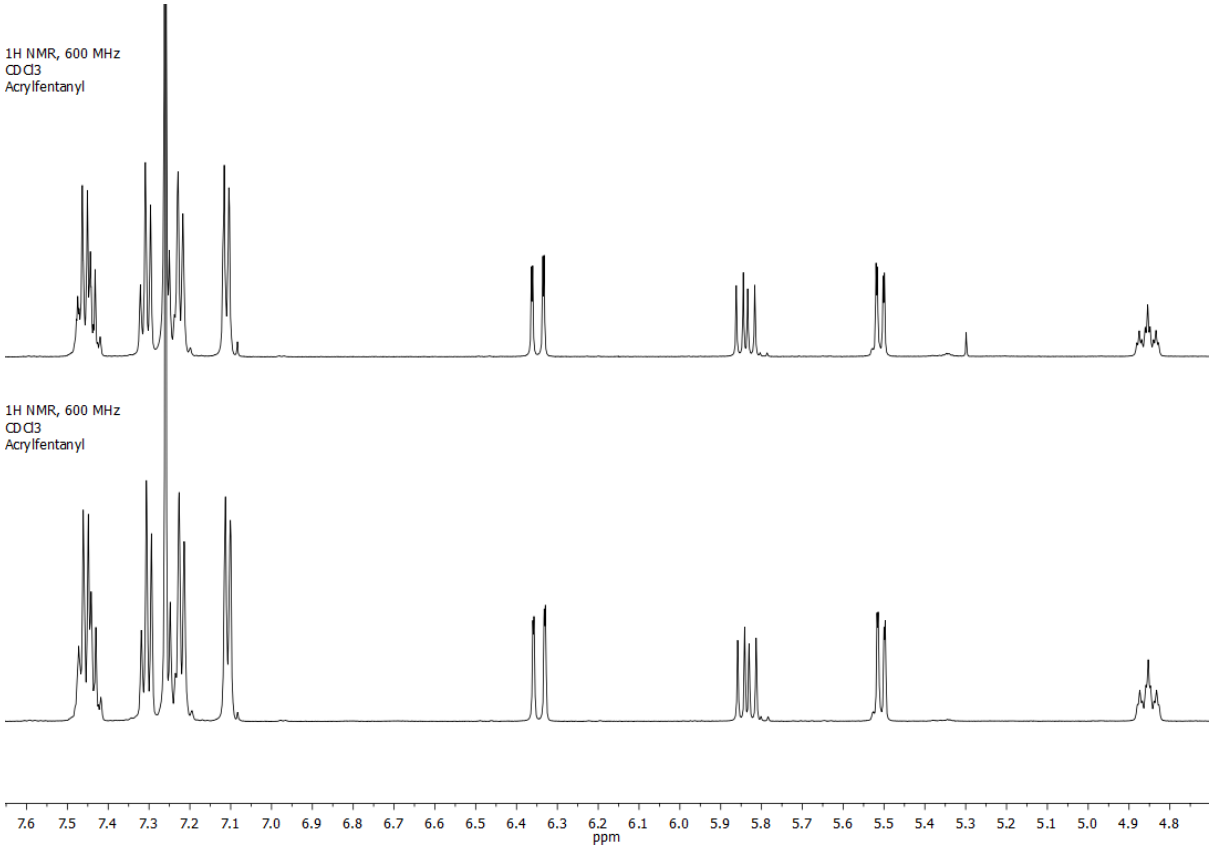



**Figure S8: $^{1}H$ NMR (600 MHz) in $CDCl_3$ : Enlargement (1.1–3.8 ppm)**

**Top: Acrylfentanyl (standard); Bottom: Acrylfentanyl (seized sample)**

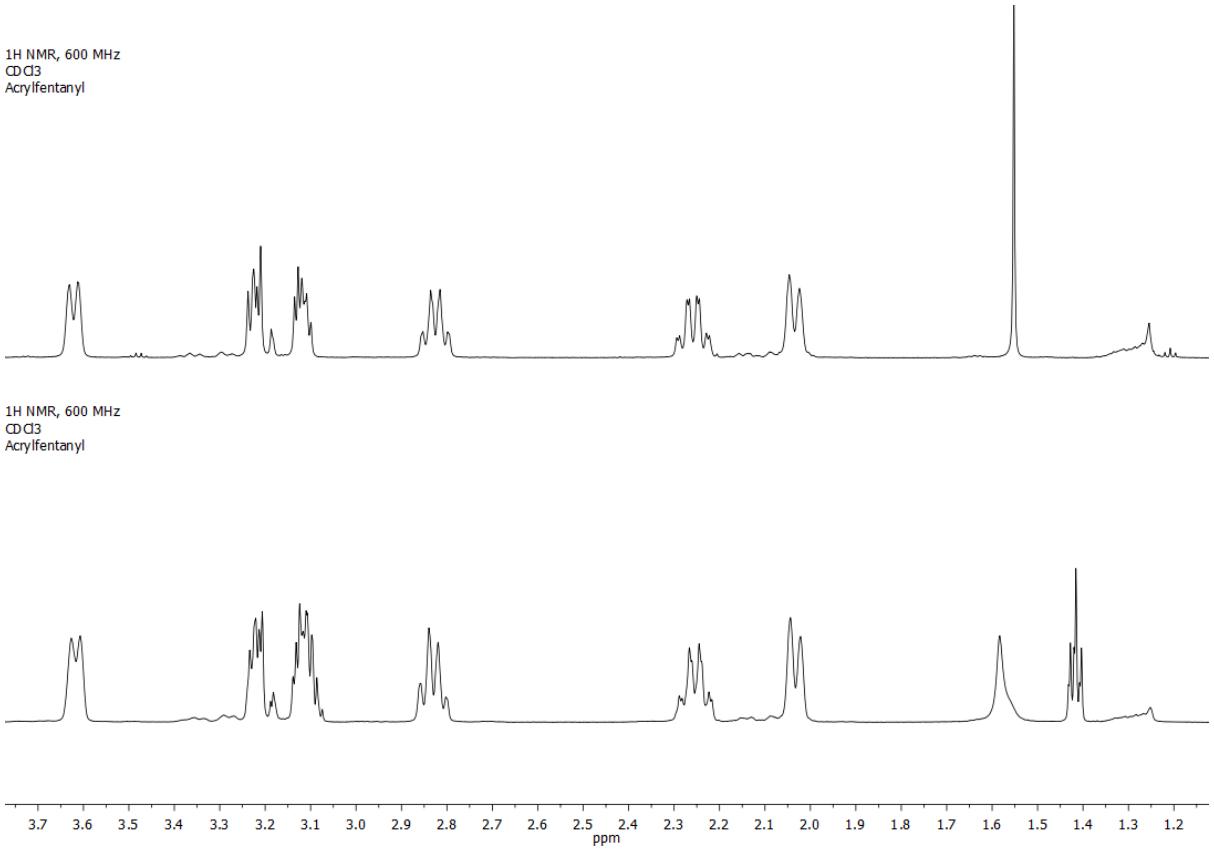


**Figure S9: DEPT (600 MHz) in $CDCl_3$**

**Top: Acrylfentanyl (standard); Bottom: Acrylfentanyl (seized sample)**

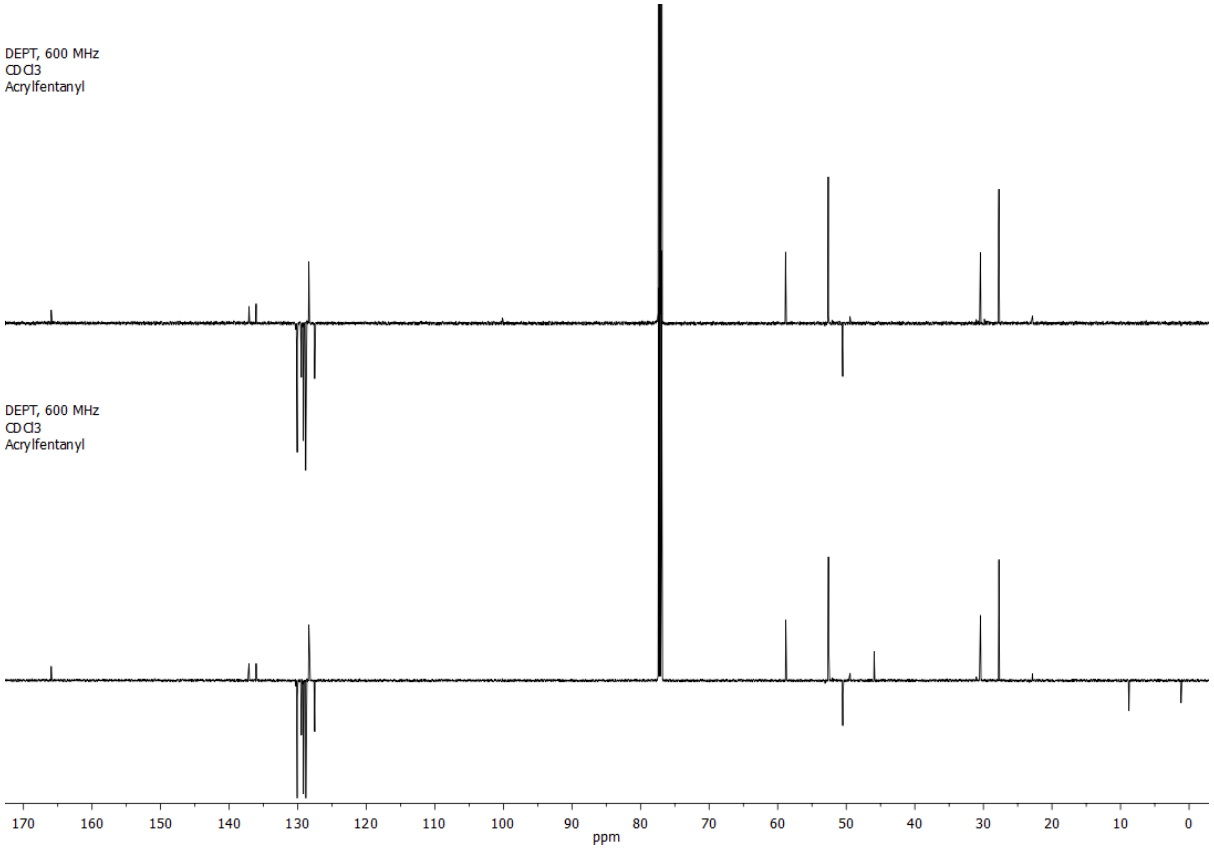



**Figure S10: Identification of the impurity as triethylamine hydrochloride by $^1$H NMR (600 MHz) in DMSO-$d_6$. Acrylfentanyl (seized sample),**

*Peak separation was achieved in DMSO-$d_6$ allowing identification of the impurity.*

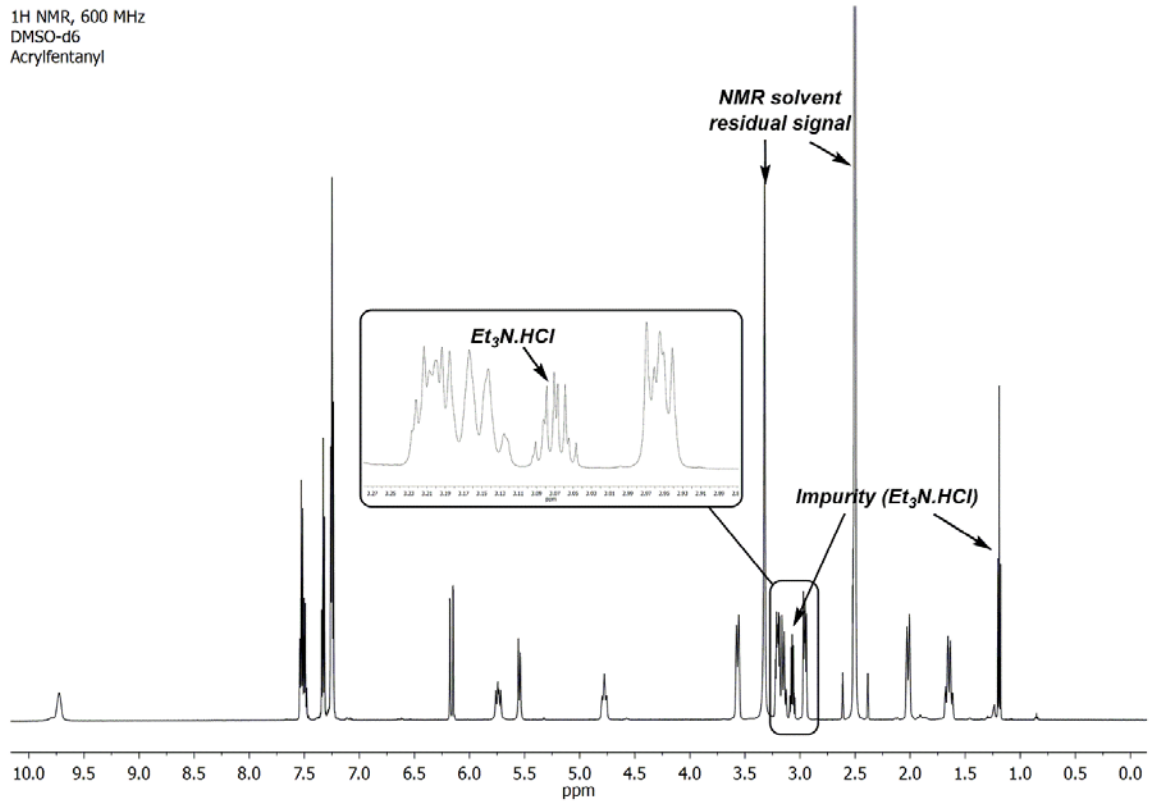


**Figure S11: Identification of the triethylamine hydrochloride impurity by $^{13}$C NMR (600 MHz) in DMSO-*d*$_6$**

**Top: Acrylfentanyl (seized sample); Bottom: Triethylamine hydrochloride (standard).**

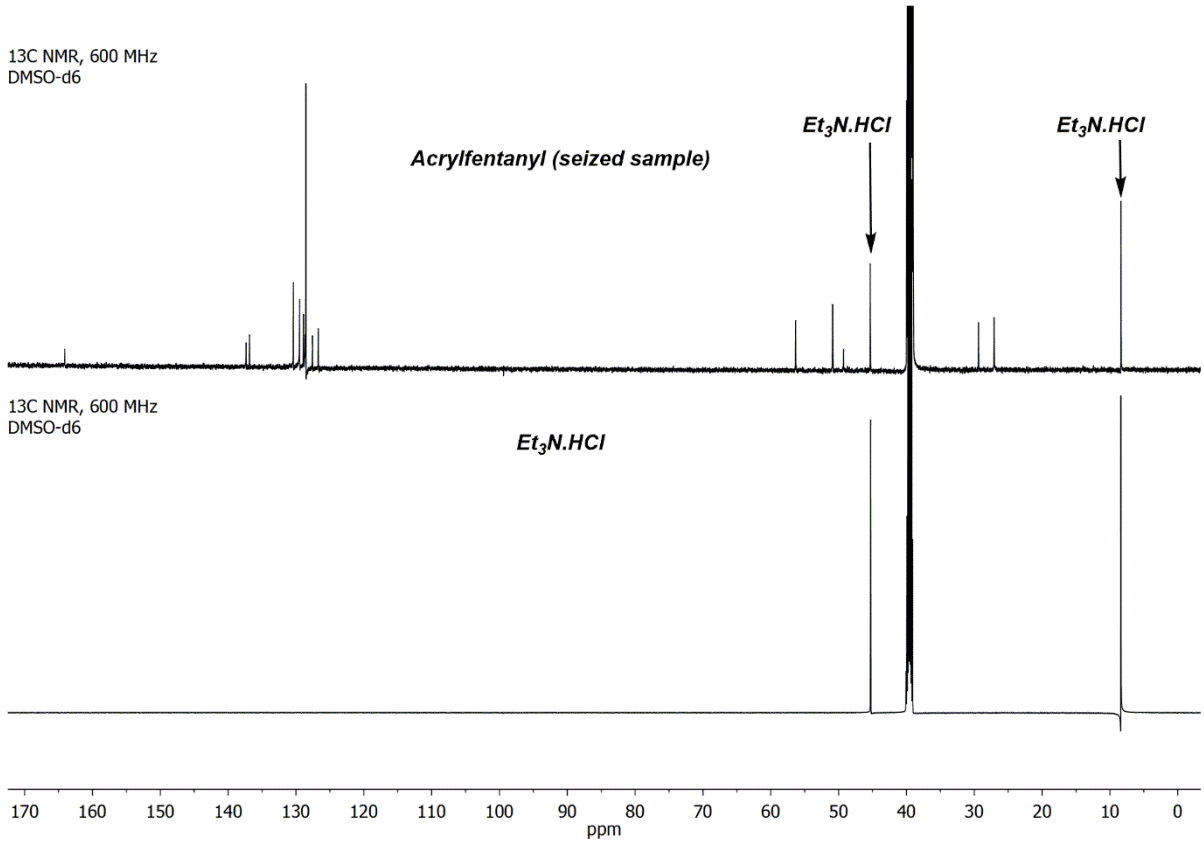



**Figure S12: Identification of the impurity as triethylamine hydrochloride. Enlargement of aliphatic region for A) Acrylfentanyl (seized sample); and B) Acrylfentanyl (seized sample) spiked with triethylamine hydrochloride, $^{1}$H NMR (600 MHz) in DMSO-*d*$_6$**

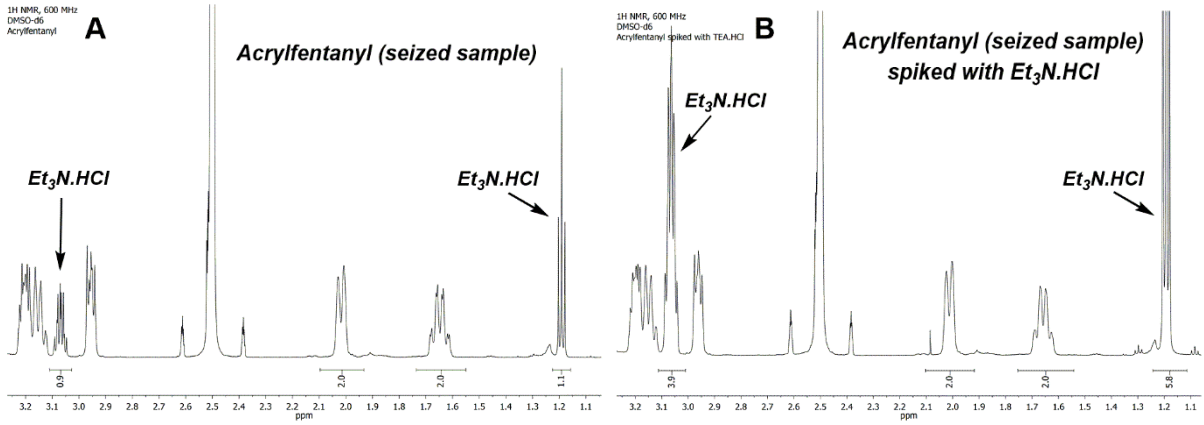


**Figure S13: IR spectrum of acrylfentanyl (seized sample)**

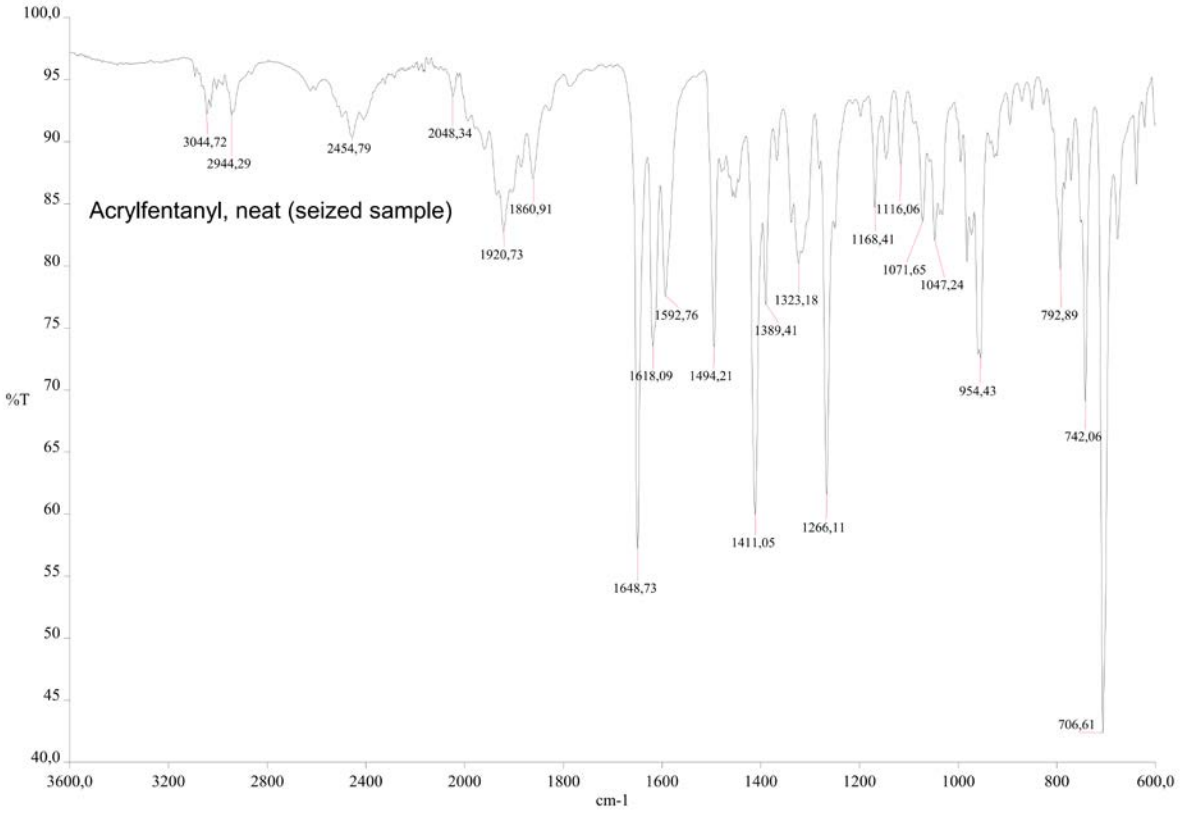
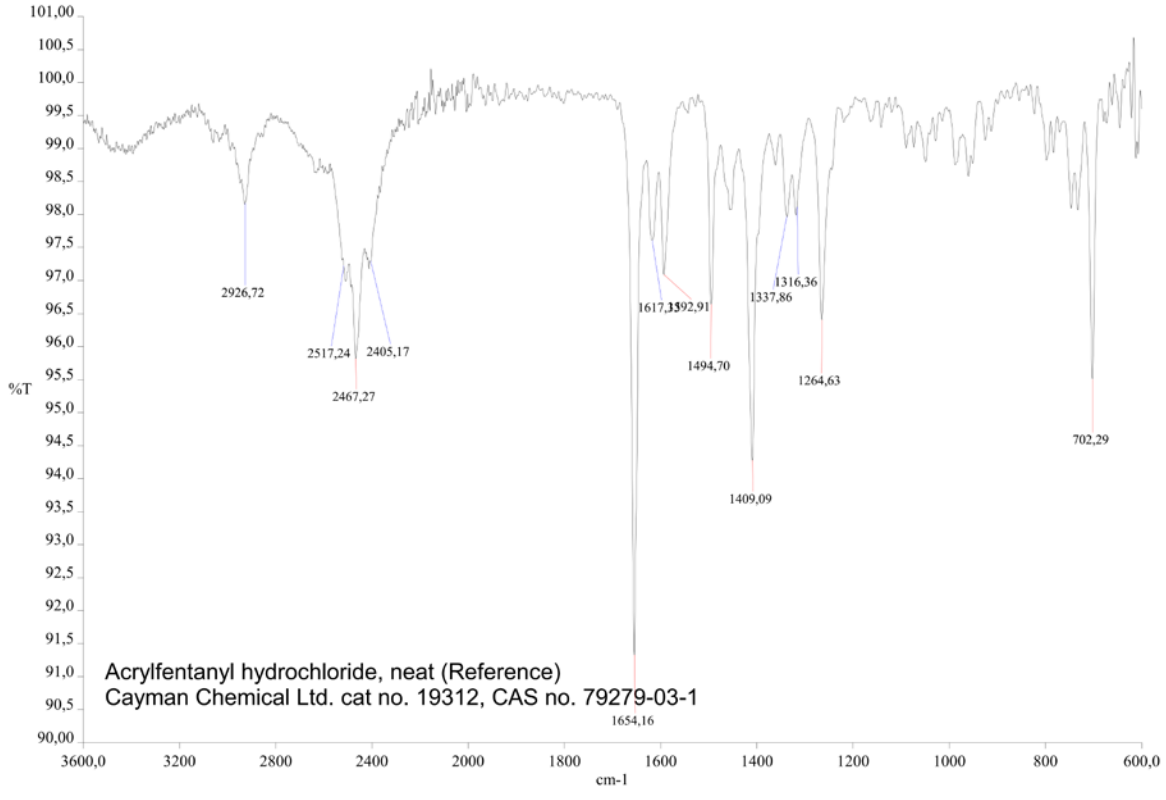



**Figure S14: IR spectrum of acrylfentanyl (standard)**



**Instrumentation (LC-MS/MS) for quantification of acrylfentanyl**

The LC system modules were all from Agilent Technologies (Palo Alto, CA, USA) including a 1200 binary pump, 1200 SL autosampler and 1200 column department unit. The damper and mixer were bypassed in order to optimize the pumping system to low dead volume as described in the Agilent User Manual. Autosampler injection volume was 2 µL. The analytical column was an Kinetex Biphenyl (Phenomenex, Torrace, CA, USA), 100 × 3 mm, i.d., packed with 2.6 µm particles. The flow rate was 550 µL/min and the column temperature was 40 $^{o}$C. Mobile phase A was 0.1 % formic acid. Mobile phase B was 0.1% formic acid in methanol. The binary pump gradient started at 2% phase B for 0.5 min and then went up to 95% phase B in 5 min. It was maintained at 95 % phase B for 3 min and then returned to the initial conditions for equilibration. The total run time was 9 min.

The MS system consisted of an Agilent 6460 triple quadrupole mass spectrometer (Palo Alto, CA, USA) equipped with a jet stream electrospray ion source operated in positive mode. The capillary voltage was 3500 V, the nebuliser pressure 25 p.s.i., the gas temperature 350 $^{o}$C, the gas flow 8 L/min, the sheath gas temperature 375 $^{o}$C ion, the sheath gas flow 8.5 L/min, the nozzle voltage 400 V and the declustering potential 130 V. Data were acquired in dynamic multiple reaction monitoring mode (*d*MRM). All calculations were performed used the MassHunter software (Agilent Technologies).

**Table S1: Multiple Reaction Monitoring (MRM) parameters**

| **Compound** | **Transition type** | **MRM transistion** | **Collision energy** |
|---|---|---|---|
| Fentanyl | Target ion | 337 → 188 | 20 |
| | Qualifying ion | 337 → 105 | 40 |
| | Qualifying ion | 337 → 79 | 50 |
| Acrylfentanyl | Target ion | 335 → 188 | 20 |
| | Qualifying ion | 335 → 105 | 40 |
| | Qualifying ion | 335 → 79 | 50 |
| Fentanyl- *d*5 | Target ion | 343 → 105 | 40 |

The calibrators of acrylfentanyl were prepared from a 250 µg/mL aqueous working solution of acrylfentanyl at six concentration levels: 15.625, 31.25, 62.5, 156.25, 312.5 and 625 ng/mL (free base). The concentration of acrylfentanyl in the seized sample was calculated from a 6-point calibration curve based on peak area using a linear regression curve fit, not forced through zero, with no weighting.

A working solution of the seized sample (powder) at concentration 250 ng/mL was used in the following sample preparation procedure (for sample(s) and calibrators):
100 µL sample was added to 25 µL internal standard solution (fentanyl-*d*5, 1 µg/mL) and 125 µL water.

The determined acrylfentanyl free base concentration from LC-MS/MS was used to calculate the mass-% of acrylfentanyl hydrochloride in the seized sample.

**Figure S15: LC-MS/MS calibration curve**

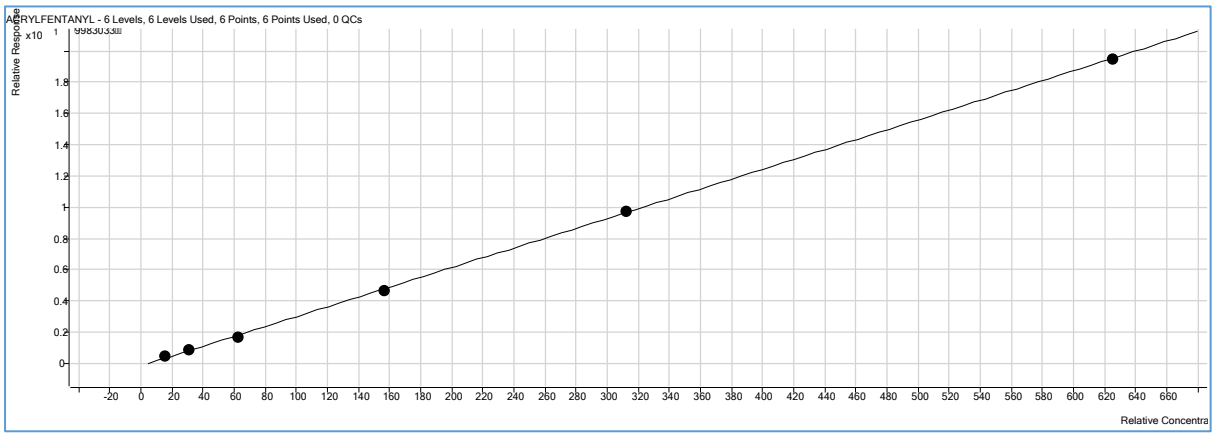



**Figure S16: MRM signal for *m/z* 335 → *m/z* 188 (acrylfentanyl in seized sample)**

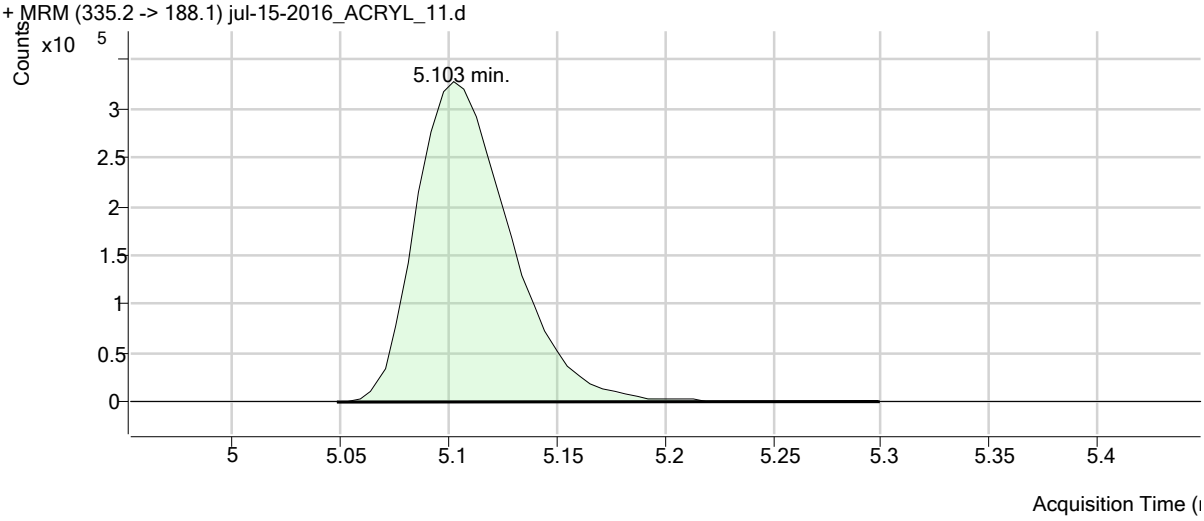


**Figure S17: Qualifying ion transitions (overlaid) for acrylfentanyl in seized sample**

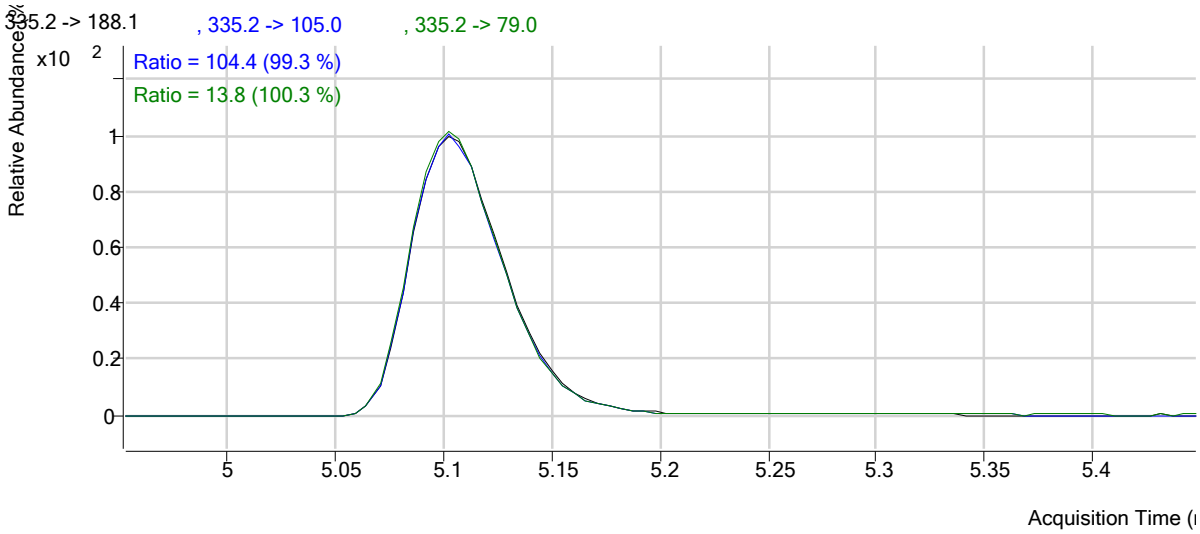



**Figure S18: MRM signal for *m/z* 343 → *m/z* 105 (internal standard, fentanyl-*d*5) spiked to seized sample during sample preparation**

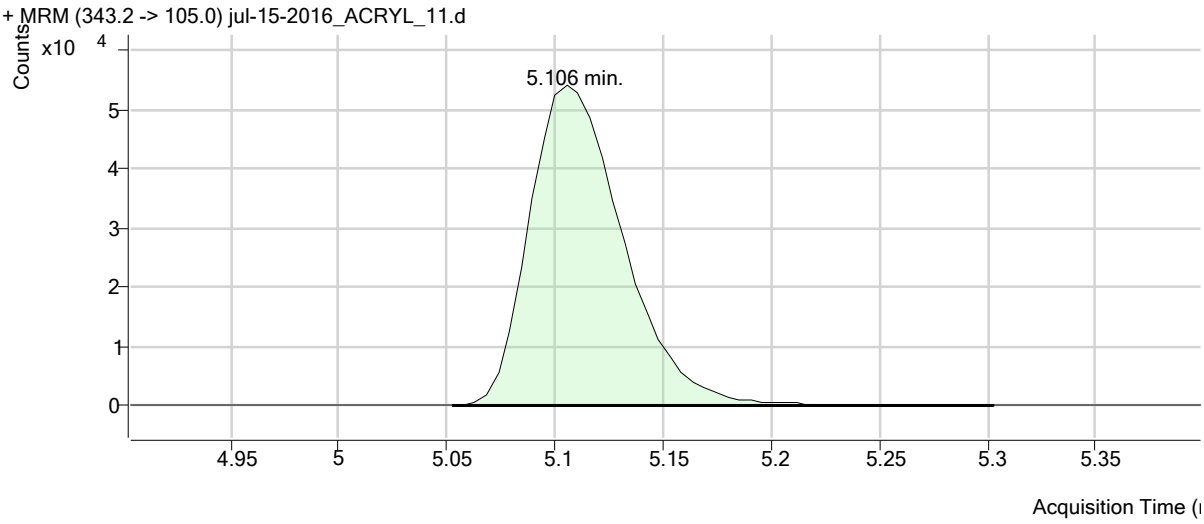


**Figure S19: MRM signal for *m/z* 335 → *m/z* 188**

**Acrylfentanyl calibrator, 156.25 ng/mL (free base)**

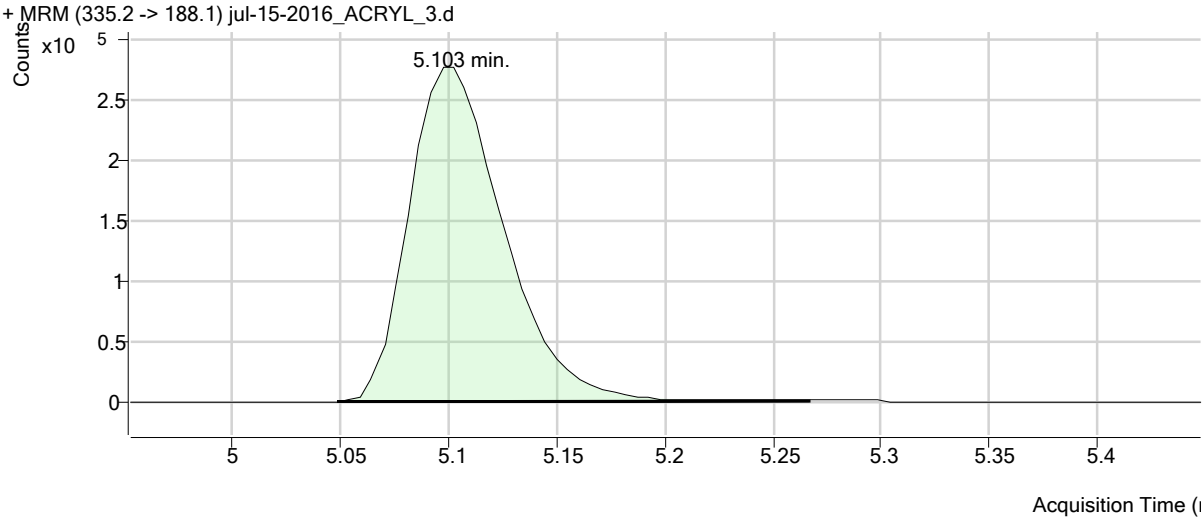



**Figure S20: Qualifying ion transitions (overlaid)**

**Acrylfentanyl calibrator, 156.25 ng/mL (free base)**