The Identification, Derivatization, and Quantitation of Fentanyl Analogs

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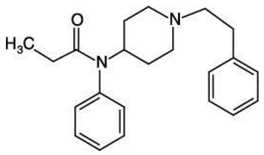
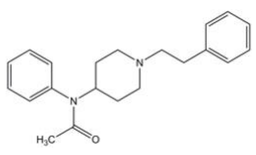
Abstract/Introduction

- Fentanyl is a schedule II synthetic opiate analgesic
- Fentanyl works by binding to opiate receptors in the brain
  - This action causes higher levels of dopamine in the body, resulting in a euphoric, relaxed state
  - Fentanyl is used for treating patients suffering from moderate to severe pain, or chronic pain
- Some Analogs of fentanyl are created clandestinely to supply illicit opiates
  - These analogs have no approved human use
- There are analogs of fentanyl that exist outside of clandestine production, such as sufentanil and alfentanil (used medically in much the same way as fentanyl)
- Drug analysts will need an accurate identification and quantitation method for seized solid-dose drugs
- There is research that has been performed on quantifying these analogs in urine for toxicologists
- The objective of this research was to first identify and potentially derivatize these analogs using GCMS and then validate a quantitation method for acetyl fentanyl



Fentanyl



Acetyl Fentanyl

•Fentanyl analogs used in this study include:
- Acetyl fentanyl
- Acetyl norfentanyl (A metabolite of acetyl fentanyl)
- Butyryl fentanyl
- Para-fluorofentanyl
- Cis 3-methylfentanyl
- Trans 3-methylfentanyl
- Sufentanil
- Alfentanil

- The underivatized Analogs had near-identical retention times but the mass spectra were able to be easily differentiated, save the identical cis and trans isomers
- The derivatization was unsuccessful with the exception of the acetyl norfentanyl
- A quantitation method was developed for acetyl fentanyl
- The calibration models were successful in creating accurate curves that produced results for the controls within 20% error of their experimental values

Materials and Methods

Materials
- Analog standards were obtained from Cayman, Cerilliant, and Lipomed as either 1 mg/mL methanolic standards or powders that were then made into 1 mg/mL methanolic standards
- Methanol was purchased from Fisher-Scientific
- Ethyl Acetate, Heptafluorobutyric Anhydride (HFBA), Pentafluoropropionic Acid (PFPA), and Procaine were obtained from Sigma-Aldrich
- Deuterated Fentanyl, Cocaine, Heroin, Methamphetamine, Oxycodone, & Alprazolam standards purchased from Cerilliant
- Reagents/solvents were GCMS, LCMS, or HPLC grade

Sample Preparation for Identification/Derivatization
- A method was adapted from Sabina Strano-Rossi *et al*.
- 50µL of 1.0mg/mL analog standard was placed in a test tube and the methanol was evaporated off
- The residue was reconstituted with 100µL of a derivatization agent (either heptafluorobutyric anhydride or pentafluoropropionic acid) and then heated for 20 min. at 55°C
- Evaporated solvent off & reconstituted w/ 300µL ethyl acetate
- 5µL of 30 mg/mL procaine was added as the internal standard.
- For underivatized samples, the same procedure was followed, save for the addition of the derivatization agent

Sample Preparation for Calibration Model
- Calibrators were prepared at 1.0, 0.75, 0.5, 0.1, and 0.01mg/mL by diluting1.0mg/mL standard with methanol.
- The controls were prepared the same, with concentrations of 0.9, 0.4, and 0.05mg/mL
- 40µL of 100 µg/mL deuterated fentanyl added as the internal standard

GC-FID / GCMS
- Agilent Technologies - 6890N gas chromatograph with flame ionization detector and 5973 mass spectrometer detector (dual column)
- The same temperature programming was used for GC-FID and GCMS
- Initial temperature was 85°C, held for 0.75min, then increased at a rate of 15.0°C/min to the final temperature of 305°C, held for 0.75min.
- Pressure initially 5.0 psi, then ramped at 150 psi/min to 15.0 psi, held for 6 minutes, then ramped at 150 psi/min to 40.0 psi
- For the quantitation phase of the study, the same parameters were used, save the pressure, which held constant at 20.0 psi

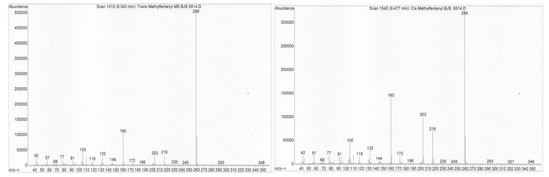
Selectivity
- Established by spiking a 1.0mg/mL sample of acetyl fentanyl with 50µL each of 1 mg/mL heroin, cocaine, methamphetamine, oxycodone, and alprazolam standards
- The sample was run using the same GCMS method as the quantitation model

LOD/LOQ
- Determined by locating the approximate point of elution of acetyl fentanyl on a gas chromatograph of a blank sample
- The integration of the noise was forced and then multiplied by three to determine LOD and by ten to determine LOQ

Ion Ratios
- Established by running standards of the middle three calibrators and determining the average primary and secondary ion ratios by dividing the peak area of the quantitation (largest) ion by the first qualifier (second largest) ion for the primary ion ratio and by the second qualifier (third largest) ion for the secondary ion ratio

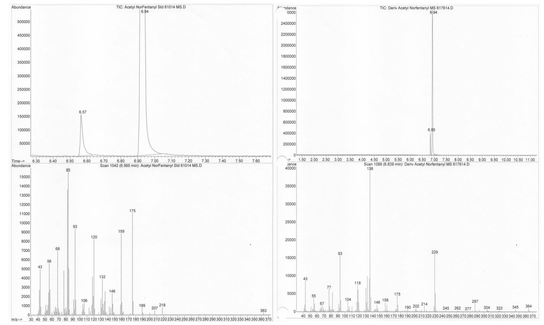
Results and Discussion

Identification
- Mass Spectra of the analogs were differentiated save the 3-methylfentanyl cis and trans isomers



Trans **Cis**

Derivatization
- Derivatization was unsuccessful for all analogs with no changes occurring to their GC-FID or GCMS spectra.
- The exception was the metabolite acetyl norfentanyl



Underivatized **Derivatized**

- Literature showed that derivatization for fentanyl and its analogs has only been accomplished in the past for increasing the sensitivity of metabolites to GC-FID/GCMS methods

Quantitation
- Calibration Model produced excellent quantitation curves with near perfect linearity and high consistency
- Accuracy and Precision both demonstrated
- All controls within ± 20% of the expected values
- The model could be implemented in casework with little issue for the GC-FID method

Control	Run 1	Run 2	Run 3	Run 4	Run 5	Percent Error	RSD	%CV
0.9 mg/mL	0.898	0.890	0.890	0.880	0.883	1.42%	0.00768	0.768%
0.4 mg/mL	0.399	0.395	0.396	0.391	0.394	1.27%	0.00743	0.743%
0.05 mg/mL	0.0444	0.0446	0.0424	0.0441	0.0454	13.2%	0.0250	2.50%

- GCMS method had tailing problems - causing acetyl fentanyl and deuterated fentanyl peaks to be unresolved
  - Attempts to solve this issue were unsuccessful
- Ion ratios were within ± 20% of the standard ion ratios

Run	Average Primary Ion Ratio	Average Secondary Ion Ratio
Standards	*2.08*	*3.31*
Calibrator Run 1	2.00	3.33
Calibrator Run 2	2.15	3.42
Calibrator Run 3	1.96	3.50
Calibrator Run 4	2.02	3.04
Calibrator Run 5	2.14	3.35

- Selectivity study showed heroin & acetyl fentanyl co-elute
  - A potential problem as the drugs are often seen together
  - Method adjustments did not resolve this
- LOD was determined to be ~0.003mg/mL
- LOQ was determined to be ~0.007mg/mL

Conclusions

- Most of the analogs were able to be sufficiently differentiated from each other without derivatization
  - Similar retention times but unique mass spectra
  - Cis and Trans 3-methylfentanyl spectra were identical
  - The Acetyl norfentanyl metabolite was not detected by the method as well as the other non-metabolite analogs
- The derivatization method was successful for the acetyl norfentanyl (metabolite) but unsuccessful for the cis/trans isomers or other fentanyl analogs
- The GC-FID method for the calibration model was successful with data showing little variation & high accuracy
- The GCMS data was not as successful in regards to resolution and co-elution issues
- Tailing was a problem, it caused the deuterated fentanyl and heroin peaks to be unresolved
- Another problem was acetyl fentanyl and heroin co-eluting, the mass spectra were still able to be isolated though by subtracting out one spectra from the other and vice-versa.
- Future studies should address resolving the derivatization, tailing, and co-elution issues.
  - Finding a derivatization agent that works with fentanyl analogs would be a key step in future experiments

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