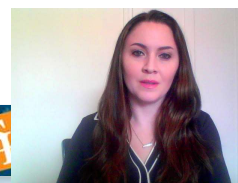


Long-Term Stability Assessment of Fentanyl Analogues in Blood using Liquid Chromatography/Quadrupole Time-of- Flight/Mass Spectrometry (LC-QTOF-MS)

Kaitlyn B. Palmquist, BS, Madeleine J. Swortwood, PhD

*Department of Forensic Science
Sam Houston State University
Huntsville, TX, USA*

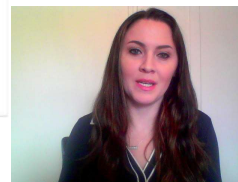


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FSF Emerging Forensic Scientist Award Competition



FSF Emerging Forensic Scientist Award
Paper Presentation



2

Disclosures

None



3



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Fentanyl

Synthetic opioid analgesic

- μ -receptor agonist
 - Decrease transmission of painful stimuli
- 50-100x more potent than morphine
- Schedule II
- Prescription/medical use:
 - Intravenous
 - Transdermal
 - Oral, Transmucosal
- Side effects: respiratory depression, bradycardia, nausea, constipation, etc.



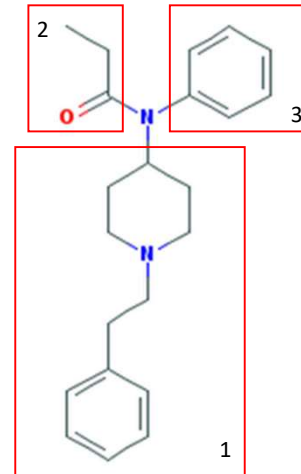
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Fentanyl Analogs

- Originally developed for medicinal and veterinary use → increase in opioid related deaths
- Introduction into illicit drug market as cutting agents for heroin
- Up to 10,000x potency of morphine
- Structural alterations to the fentanyl backbone
- Temporarily placed under Schedule I (updated: from Feb 2020 to May 2021)



5



5

Prevalence (2019)

Narcotic Analgesic Reports	Number	Percent
Fentanyl	98,954	49.74%
Oxycodone	22,470	11.30%
Buprenorphine	20,552	10.33%
Hydrocodone	12,747	6.41%
Acetyl fentanyl	12,190	6.13%
Tramadol	8,196	4.12%
ANPP ²	5,798	2.91%
Carfentanil	3,288	1.65%
Morphine	3,003	1.51%
Codeine	2,210	1.11%
Valeryl fentanyl	2,042	1.03%
Methadone	1,839	0.92%
Hydromorphone	1,582	0.80%
Oxymorphone	565	0.28%
Fluoroisobutyl fentanyl	436	0.22%
Other narcotic analgesics	3,056	1.54%
<i>Total Narcotic Analgesic Reports³</i>	198,929	100.00%
<i>Total Drug Reports</i>	1,521,360	



6

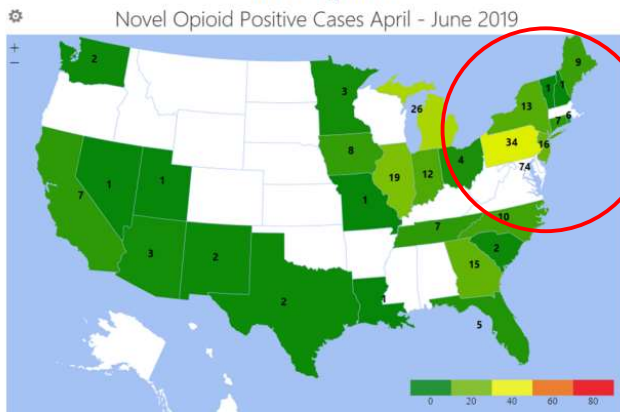

<https://www.nflis.deadiversion.usdoj.gov/>

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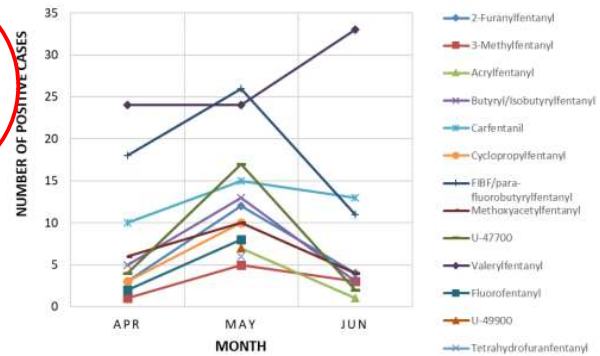
Opioid Trend Report- Q2:2019

Novel Opioids

Novel Opioid Positive Cases April - June 2019



NOVEL OPIOID POSITIVITY APRIL - JUNE 2019

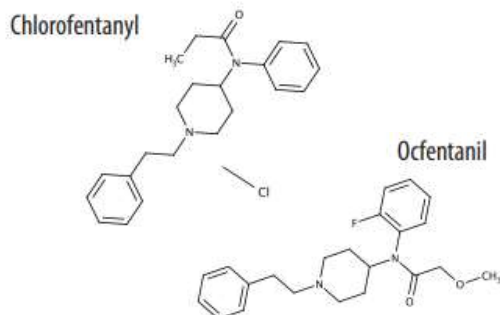


7 <https://www.nmslabs.com>

7

Prevalence (NFLIS 2020 Snapshot)

Drugs reported to NFLIS between Jul 1- Sept 30, 2020



Fentanyl and Fentanyl-Related Compounds	
Fentanyl	20,838
ANPP	2,127
Acetyl fentanyl	916
Carfentanyl	178
Valeryl fentanyl	150



8 <https://www.nflis.deadiversion.usdoj.gov/>

8

Previous Research

Author	Analogs	Instrument	Matrix	Stability Conditions	Stable?
Fogarty et al. (2018)	18	LC-MS/MS	Blood	Frozen: -80°C -20°C (30 days)	All except norfentanyl
Kahl et al. (2018)	6	UHPLC-MS/MS	Postmortem	Refrigerated: 4°C (9 mon) Processed: 15°C (6-72 h)	Yes
Moody et al. (2018)	14	LC-MS/MS	Blood Serum Urine	Room temp (30 days) Refrigeration (30 days) Frozen (30 days)	Blood- 2 weeks, except acrylfentanyl (RT: 1 day; R: 1 week; F: 30 days) Serum- 2 weeks, except acrylfentanyl & MT-45
Nan et al. (2019)	28	UHPLC-MS/MS	Blood	Room temp (24 h) Frozen: -20°C (1month) Processed: 4°C (24 h) 3 freeze/thaw cycles	Yes
Jung et al. (2020)	14	LC-MSMS	Blood Urine	Refrigerated: 4°C Frozen: -20°C (30 days)	Urine- yes, except remifentanyl Blood- some instability, recommend freezer

9

Objectives

- Develop a targeted, quantification method for 13 fentanyl analogs in blood using LC-QTOF-MS
- Validate the method according to ANSI/ASB Standard 036: Standard Practices for Method Validation in Forensic Toxicology
- Determine the long-term stability of fentanyl analogs in blood under various temperature conditions
 - Optimal storage conditions
 - Prioritization of casework

10

LC-QTOF-MS Conditions

Agilent Technologies 1290 Infinity LC	
Column	Poroshell 120 EC-C18 (2.1 x 100mm x 2.7 μ m)
Mobile Phase	A: 0.1% Formic Acid in water B: 0.1% Formic Acid in ACN
Flow Rate	0.400 mL/min
Agilent Technologies 6530 Accurate Mass Time-of-Flight MS	
Ionization Type	Electrospray ionization (ESI) positive
Injection Volume	5 μ L
Data Acquisition	Targeted Acquisition



11

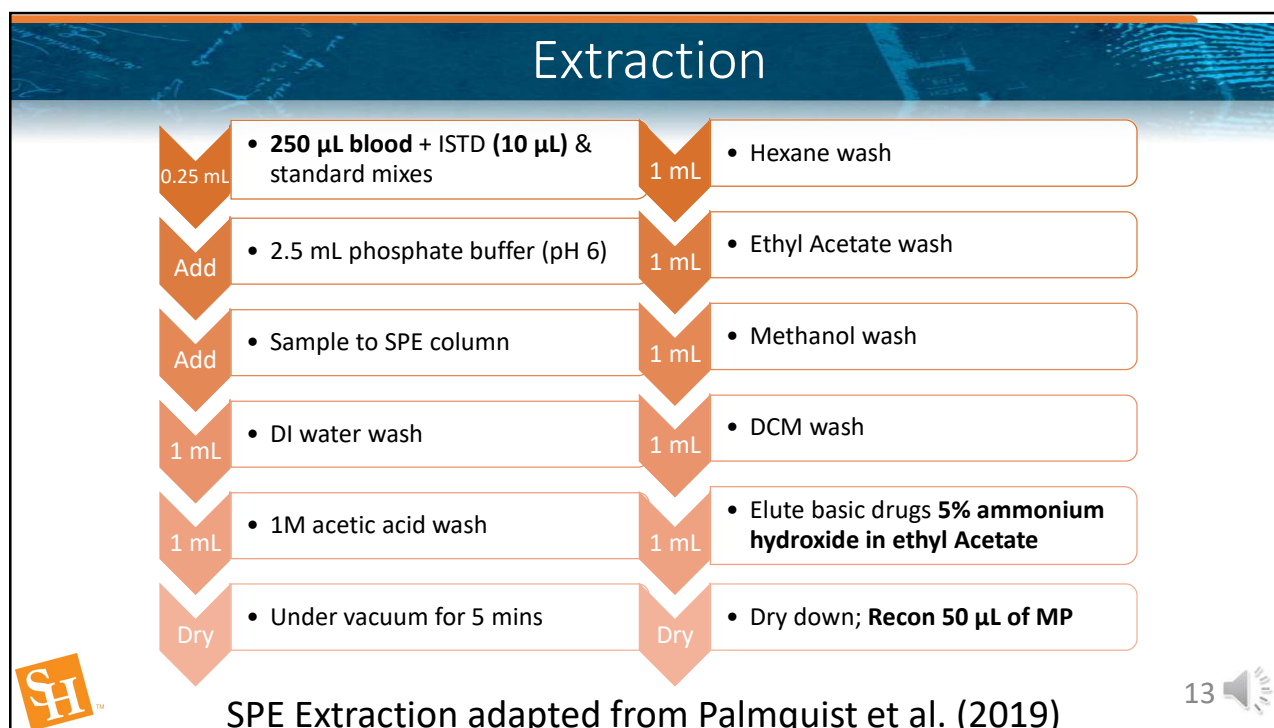


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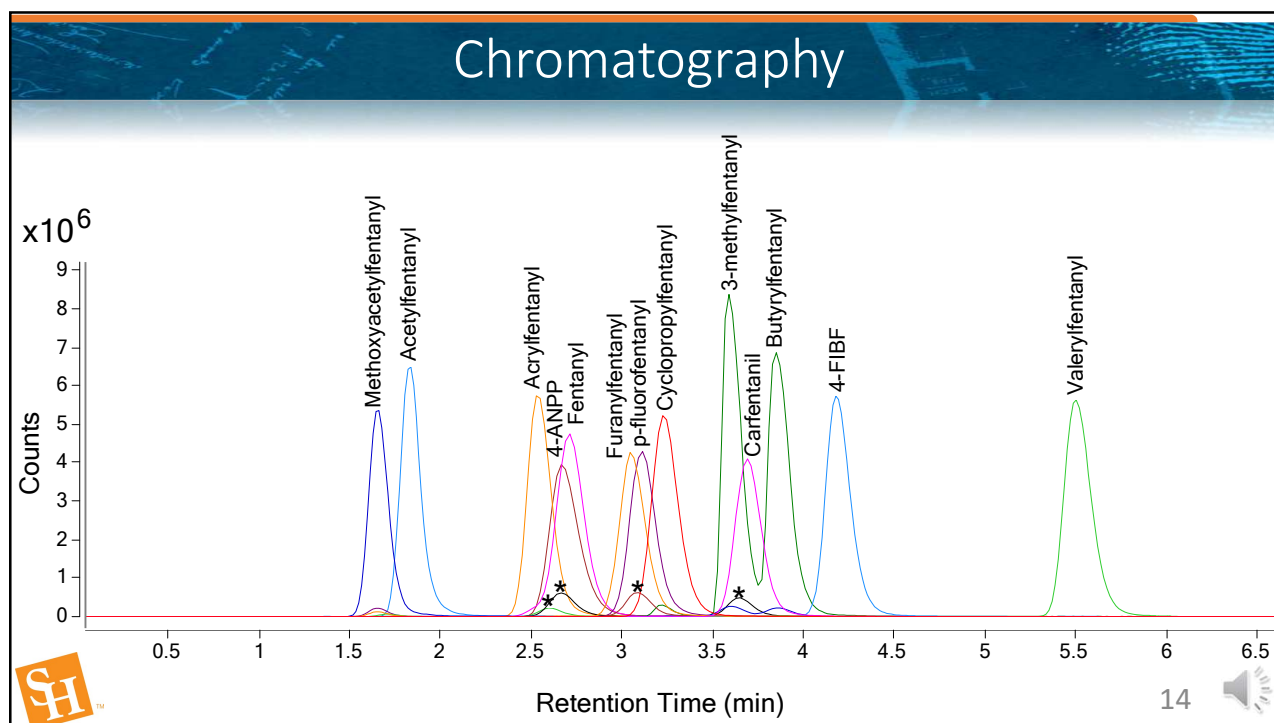
Targeted Acquisition

Analyte	Paired ISTD	Retention Time (min)	Precursor Ion	Collision Energy (eV)	Product Ion (Quant)	Product Ion (Qual)
Methoxyacetylfentanyl	Fentanyl-D5	1.60	353.2224	20	188.1400	105.0702
Acetylfentanyl	Fentanyl-D5	1.80	323.2118	25	188.1400	105.0700
Acrylfentanyl	Fentanyl-D5	2.50	335.2118	20	188.1400	105.0708
4-ANPP	4-ANPP-D5	2.60	281.2012	20	188.1400	105.0705
Fentanyl	Fentanyl-D5	2.66	337.2274	25	188.1400	105.0704
Furanylfentanyl	Fentanyl-D5	3.01	375.2067	20	188.1400	105.0701
p-fluorofentanyl	p-fluorofentanyl-D3	3.06	355.2180	25	188.1400	105.0698
Cyclopropylfentanyl	Fentanyl-D5	3.17	349.2274	25	188.1400	105.0694
3-methylfentanyl	Carfentanil-D5	3.55	351.2431	25	202.1500	105.0705
Carfentanil	Carfentanil-D5	3.60	395.2329	15	335.2100	246.1483
Butyrylfentanyl	Fentanyl-D5	3.79	351.2431	25	188.1400	105.0709
4-FIBF	p-fluorofentanyl-D3	4.10	369.2337	25	188.1400	105.0700
Valerylfentanyl	Fentanyl-D5	5.40	365.2587	25	188.1400	105.0697

12



13



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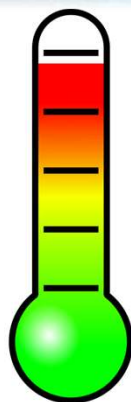
Method Validation

Method Validation Parameter	Results
Calibration Model	Linear, $1/x^2$ weighting
Calibration Range	1-100 ng/mL
R ² values	0.995- 0.999
Limit of Detection (ng/mL)	0.5
Lower Limit of Quantification (ng/mL)	1
Within- and Between-run precision (%CV)	Within ± 7.2
Bias (%)	Within ± 15.2
Matrix Effects (%)	Acceptable Ion Enhancement, <i>except carfentanil & 4-ANPP in LQC (>25% enhancement)</i>
Carryover	None
Interferences	None
Short-term Stability: Processed (48 h) Room temperature (24 h) Refrigerated (72 h)	All analytes stable, <i>except acrylfentanyl at room temperature (>20% bias)</i>

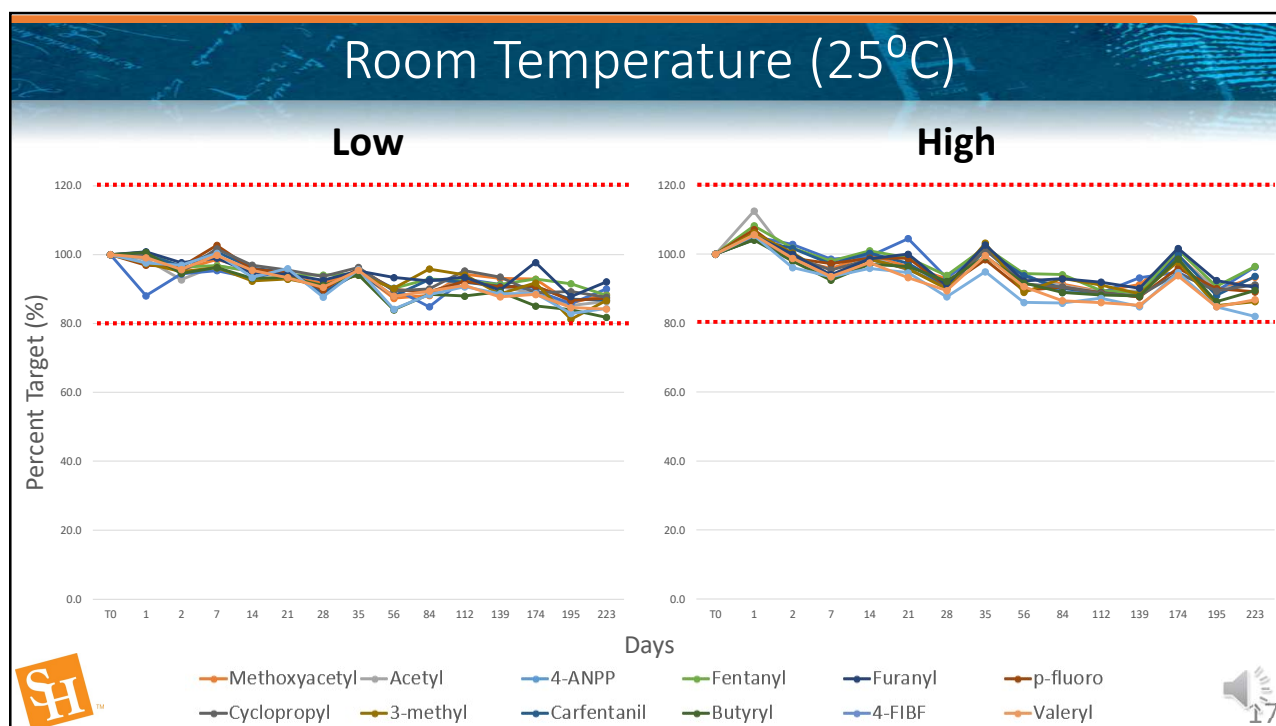
15

Long-term Stability Study

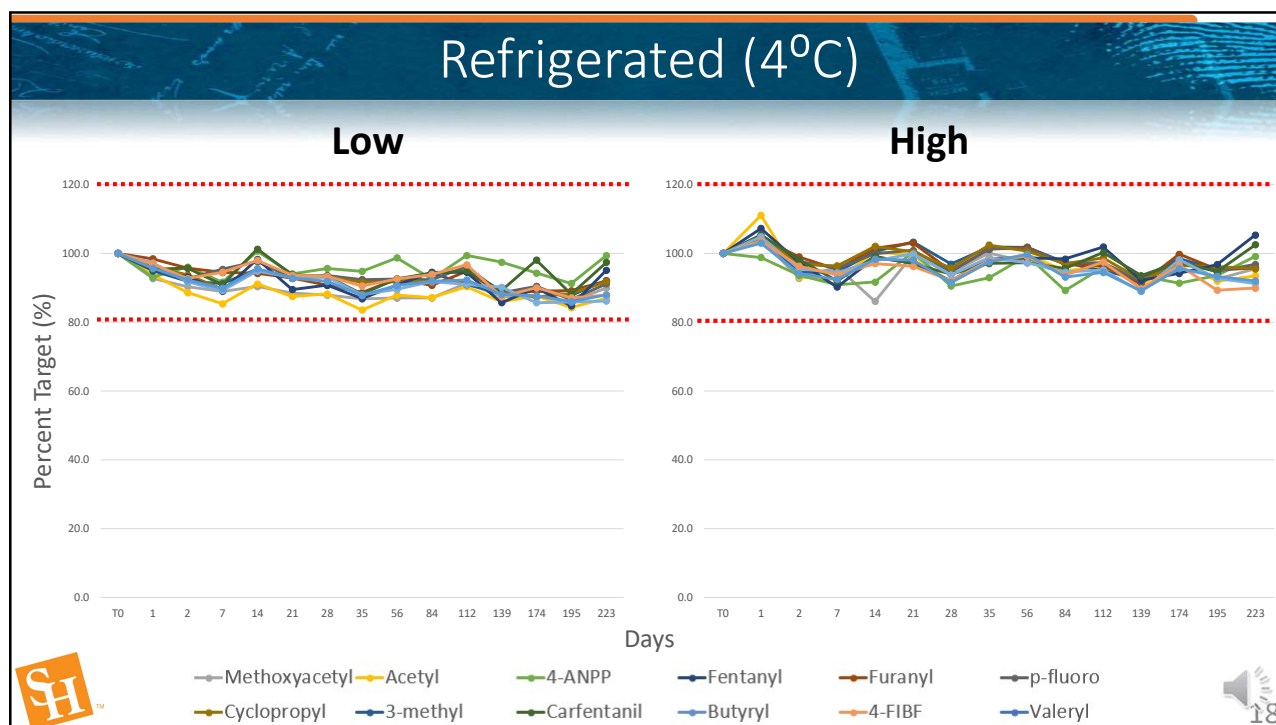
- Quality Control Preparation:
 - Bovine blood (100 mL) with potassium oxalate & sodium fluoride
 - Low QC- 10 ng/mL
 - High QC- 80 ng/mL
- Conditions:
 - Room temperature ($\sim 25^{\circ}\text{C}$)
 - Refrigerated (4°C)
 - Frozen (-20°C)
 - Elevated (35°C)
- Time points:
 - T0, 24h, 48h, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, then monthly (for 9 months)



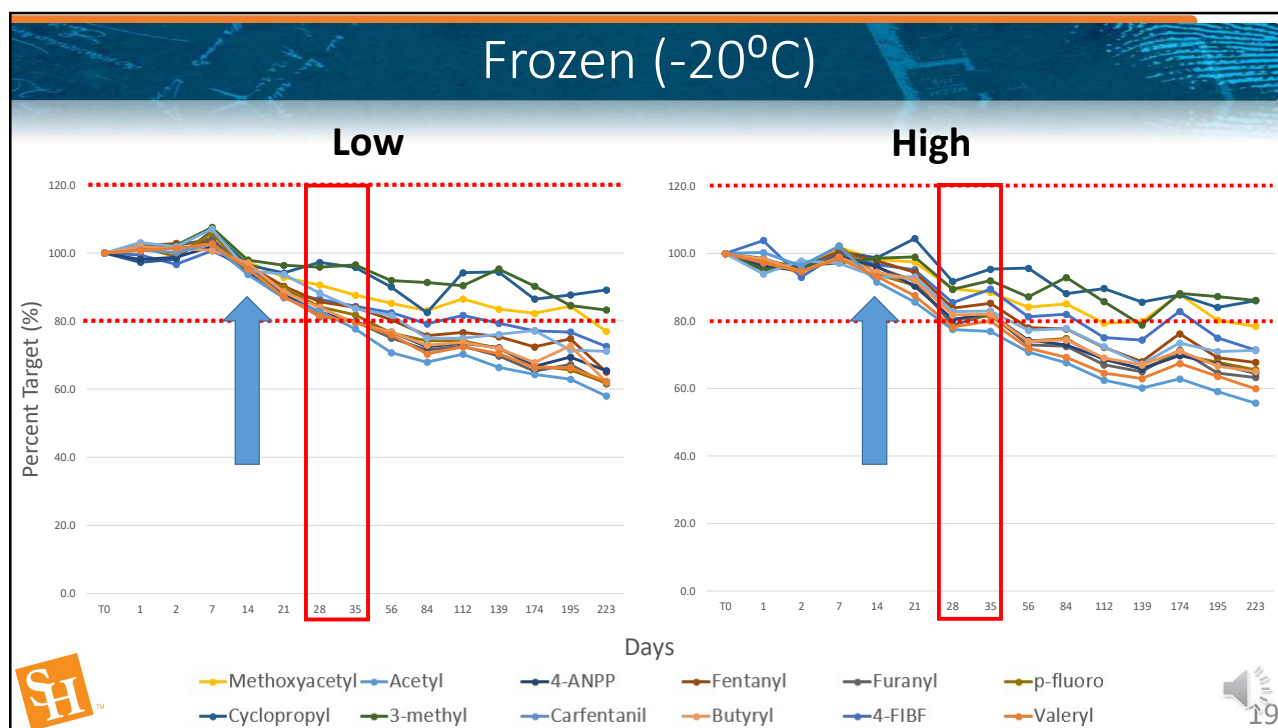
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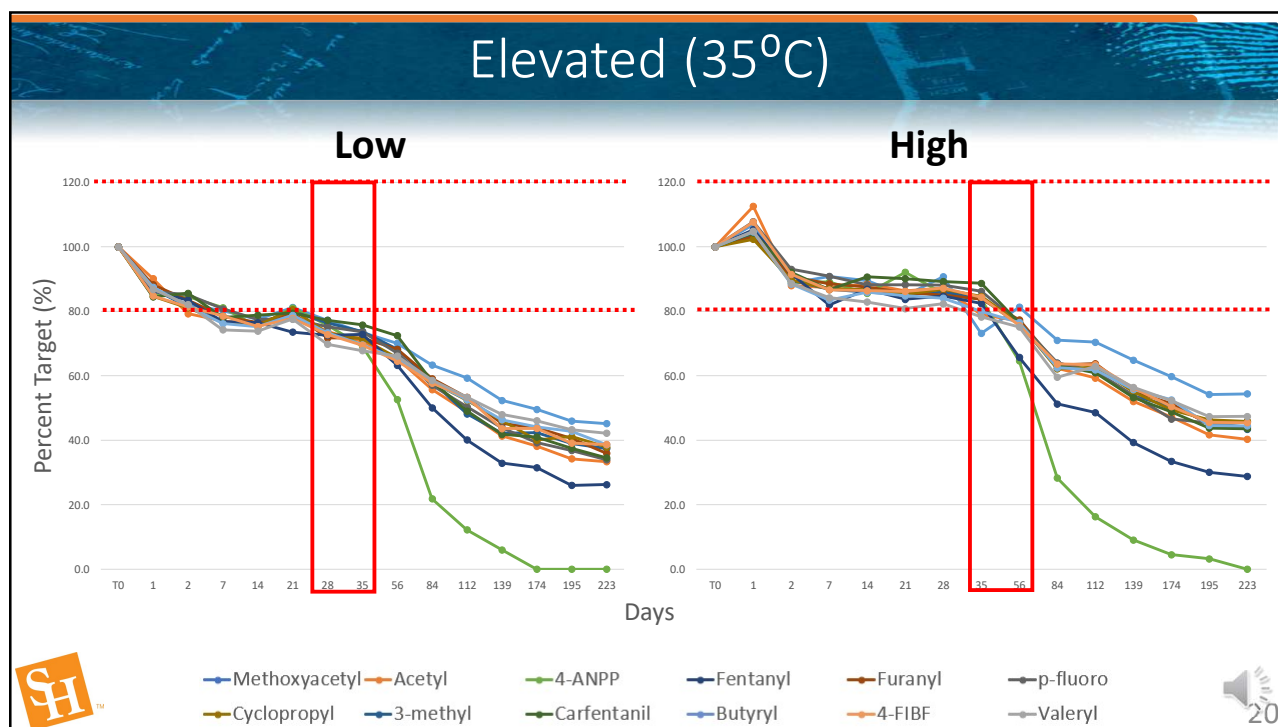
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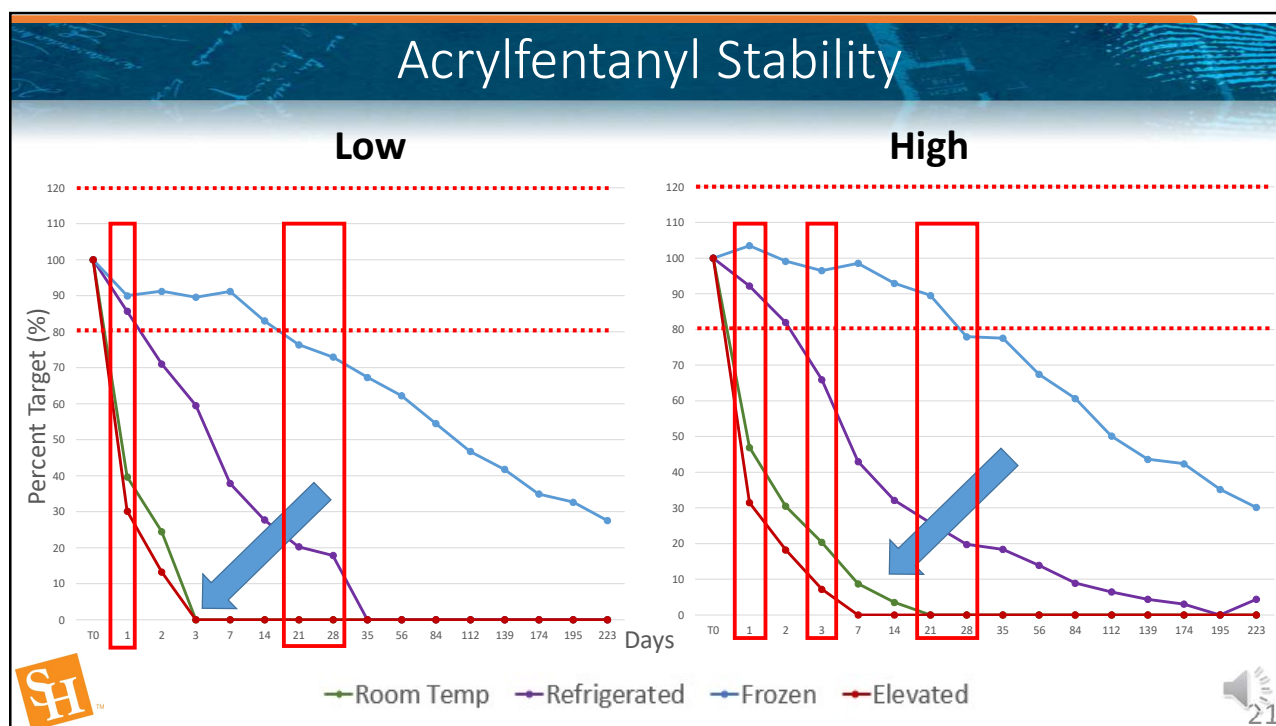
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19



20



21

Conclusions

- Validated method for the quantification of fentanyl analogs in blood using LC-QTOF-MS
- Long-term stability determination:
 - Room temperature & Refrigerated- stable for 8 months
 - Frozen should limit number of freeze/thaw cycles
 - Improper storage/handling (elevated)- stable for 1 week
 - Acrylfentanyl- unstable, requires immediate analysis

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22

22

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- Sam Houston State University- Department of Forensic Science
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23

Questions?

Kaitlyn B. Palmquist

kbp018@shsu.edu



24



Sam Houston
State University

