Detection and Quantitation of Emerging Opioids in Complex Matrices

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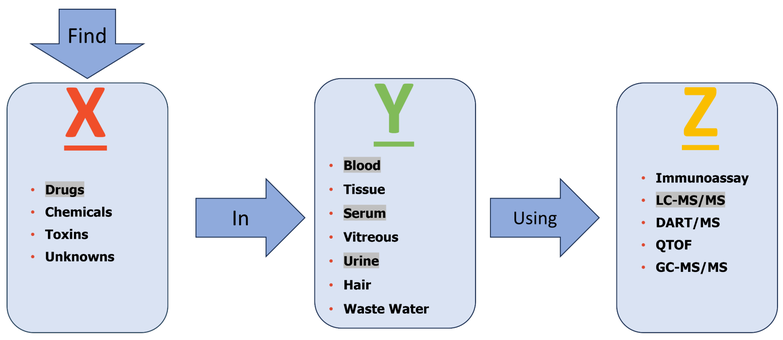
INTRODUCTION

- Informational gaps associated with the recent emergence of new designer opioids and unexplained overdose cases is creating many public health and safety challenges.
- For example, new analytical burdens being placed on clinical, public health, forensic, and research laboratories are creating an unstainable testing environment capable of keeping pace with the ever-changing analytical landscape.
- An accredited analytical method that is easily validated for multiple matrices is needed to adequately address analytical complexities associated with these new drugs.

GOAL

This study follows recently published laboratory validation requirements and guidelines from the Academy Standards Board (ASB) to develop a high-throughput, liquid chromatography tandem mass spectrometry (LC-MS/MS) test kit for detection and quantitation of emerging designer opioids in blood, serum, and urine samples.

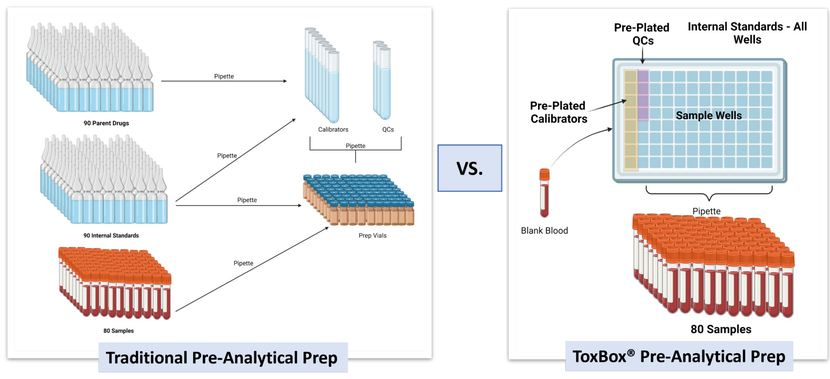


EXTRACTION METHOD

- Standards, QC material, and unknown samples were processed identically by mixing 100 µL of samples in appropriate wells of ToxBox® analytical plate.
- 100 µL of ToxBox® Solution D (NH_4OH) was added and mixed for 15 min at 500 rpm at room temp.
- 200 µL of the alkaline blood mixture was loaded on the SLE extraction plate.
- Analytes of interest were extracted using two 900 µL aliquots of ToxBox® Solution E (EtOAc).
- Each individual aliquot was allowed to flow under gravity for 5 min before applying an approximate 15 sec pulse of positive pressure.
- The organic eluent was evaporated to dryness under N_2.
- Drug residue was reconstituted in 200 µL ToxBox® Solution F (ACN & H_2O) and analyzed by LC-MS/MS.

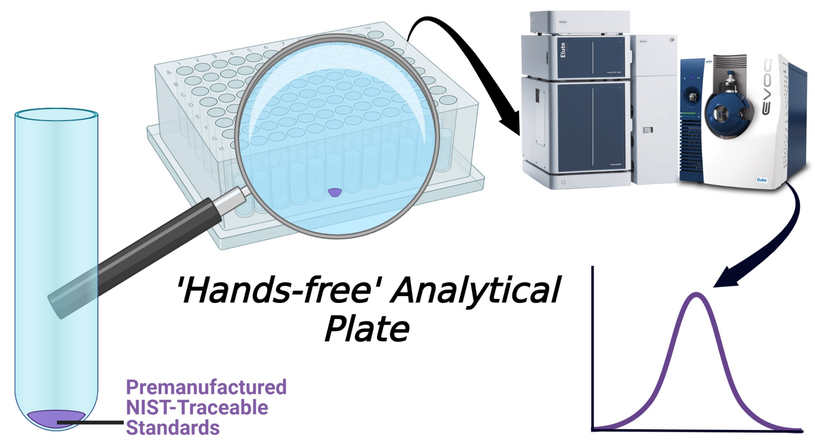
SAMPLE COLLECTION:

- Adult male Sprague Dawley rats were surgically implanted with indwelling intravenous catheters under isoflurane anesthesia.
- Rats were injected intraperitoneally with doses of fentanyl, acrylfentanyl, furanyl fentanyl, methoxyacetyl fentanyl or tetrahydrofuran fentanyl, alone or in the presence of xylazine.
- 170 blood, 174 serum, and 133 urine samples were collected from these rats.
- At 0, 20, 60, 180, and 360 min after opioid injection, blood samples were collected through an indwelling intravenous catheter and pooled urine samples were collected in a tray beneath each rat's mesh cage floor.
- Half of each collected blood sample was left as whole blood, and the other half was centrifuged to collect serum.



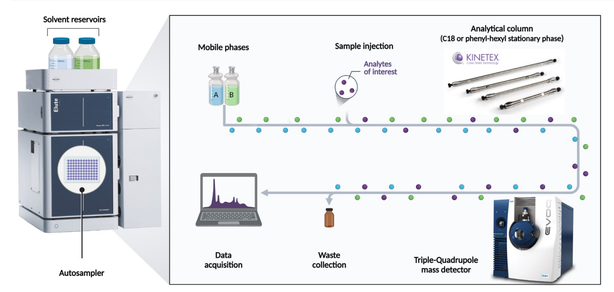
LC-MS/MS METHOD

- LC-MS/MS method utilized 10 µL injections on Phenomenex Kinetex 2.6 µm C-18 100 Å (50 x 2.1 mm) heated to 50°C
- Analytes were resolved at 0.4 mL/min using mobile phase A (25 mM ammonium formate and 0.1% formic acid in ultrapure 18.2 MΩ•cm water) and mobile phase B (100% methanol)
- The gradient included a wash step and an equilibration step that ramped the organic content to 95% for 2 min and then back to starting conditions for an additional 2 min. These added measures controlled carryover and stabilized analyte retention.
- MS/MS source ionization and analyte specific parameters selected for each analyte maximized analytical measurement ranges and were specifically selected to limit interferences.
- Nitrogen source gas temperature and flow rate were 350˚C and 12 L/min, respectively. Nebulizer gas pressure was 50 psi. Capillary voltage was +2000 V



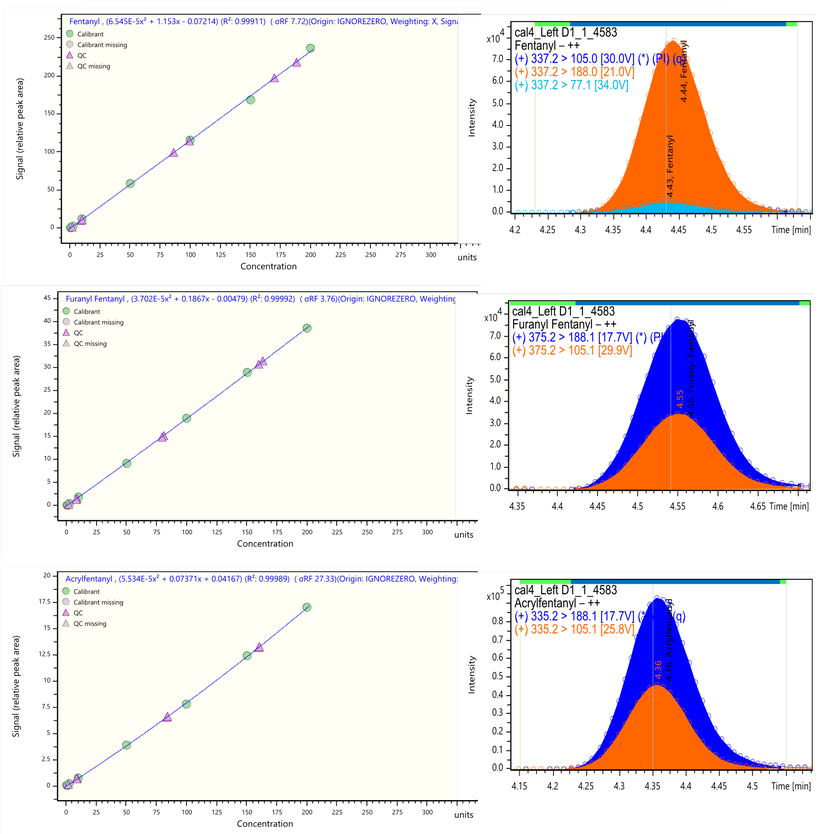
Analyte List

4-ANPP	Carfentanyl	MDEA	para-Fluorobutyrylfentanyl
4-Fluoroisobutyryl Fentanyl (FIBF)	Cyclohexyl Fentanyl	MDPV	para-Fluorofentanyl
6-MAM	Cyclopropyl Fentanyl	Mephedrone	Pentylone
8-Aminoclonazolam	Eutylone	Methcathinone	Protonitazene
AB-CHMINACA	Fentanyl	Methoxyacetyl Fentanyl	Sufentanil
Acetyl Fentanyl	Flualprazolam	Methylone	Tetrahydrofuran Fentanyl
Acetyl Norfentanyl	Flubromazepam	Mitragynine	U-47700
Acrylfentanyl	Flubromazolam	Naloxone	Valeryl Fentanyl
ADB-BUTINACA	Furanyl Fentanyl	Naltrexone	W-15
beta-Hydroxythiofentanyl	Isotonitazene	Norbuprenorphine	W-18
Bromazolam	JWH-018	Norfentanyl	Xylazine
Buprenorphine	Lidocaine	N-Pyrrolidino Etonitazene	
Butyryl Fentanyl	MAB-CHMINACA	Ocfentanil	

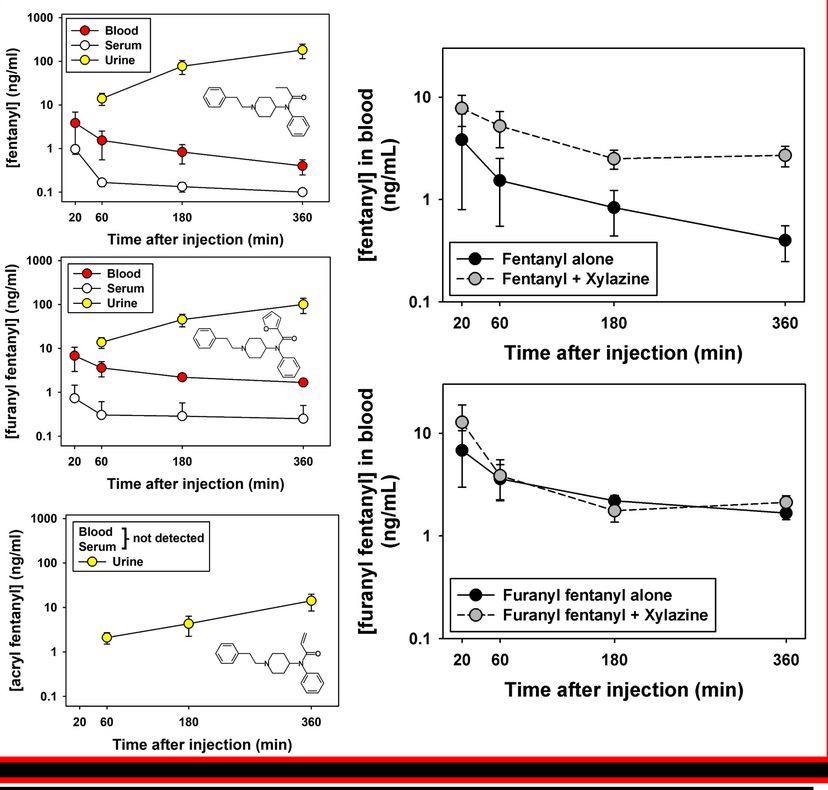


RESULTS

Calibration Curves & Chromatography



Pharmacokinetics of selected fentanyl analogs



CONCLUSIONS AND ACKNOWLEDGEMENTS

- This streamlined testing procedure is suitable for testing numberous fentanyl analogs in multiple complex matrices (blood, serum, urine)
- The tested analytical procedure provides wide analytical measurement range (0.5 – 200 ng/mL) and linear calibration curves for multiple opioids in a single specimen
- Time- and matrix-dependent factors are important in the detection of specific drugs and combinations
- For fentanyl and furanyl fentanyl, drugs more prevalent in blood than in serum throughout time course. For methoxyacetyl and tetrahydrofuran fentanyl, blood and serum were similar. For acrylfentanyl, urine was the only useful matrix to detect exposure
- In the presence of xylazine, more fentanyl is found in the blood at all time points, but xylazine does not alter amounts of furanyl fentanyl at any time point