

Validating a Human Test for Acetyl Fentanyl Using the LRN-C Platform

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Branch Chief

Arkansas Public Health Laboratory

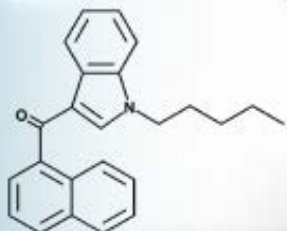
Arkansas Department of Health



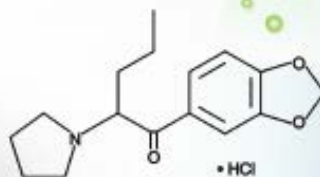
Disclaimer

- I am a Partner of PinPoint Testing, LLC.
 - UAMS BioVenture Company
 - Required for SBIR/STTR funding mechanisms
- Contents are solely the responsibility of myself and does not necessarily represent the official views of AR PHL, CDC, or FDA.

CENTER FOR DRUG DETECTION AND **RESPONSE**



JWH 018

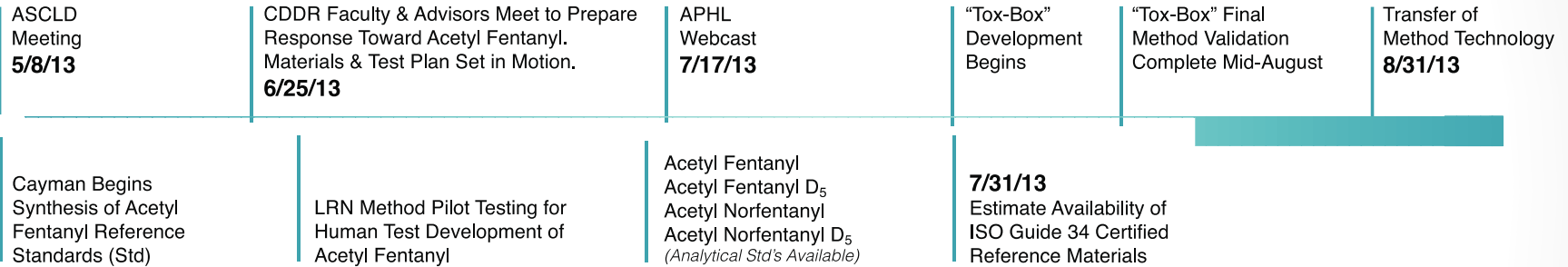


MDPV

- Founded in response to the emergence designer drugs
- Combines government, academic, clinical and private resources (ADH, ASCL, UAMS, ACH, PCC, Cayman Chemical, PinPoint Testing, LLC etc.)
- National, local advisory groups consisting of professional and community members (DEA, CDC, John W. Huffman, Nate Smith, Kermit Channell, Beth Coulson, etc.)
- Develop analytical capacity, maintains statewide surveillance and tracking, professional and community education



Center for Drug Detection and Response: Acetyl Fentanyl Timeline



Final Method Published

analytical
chemistry

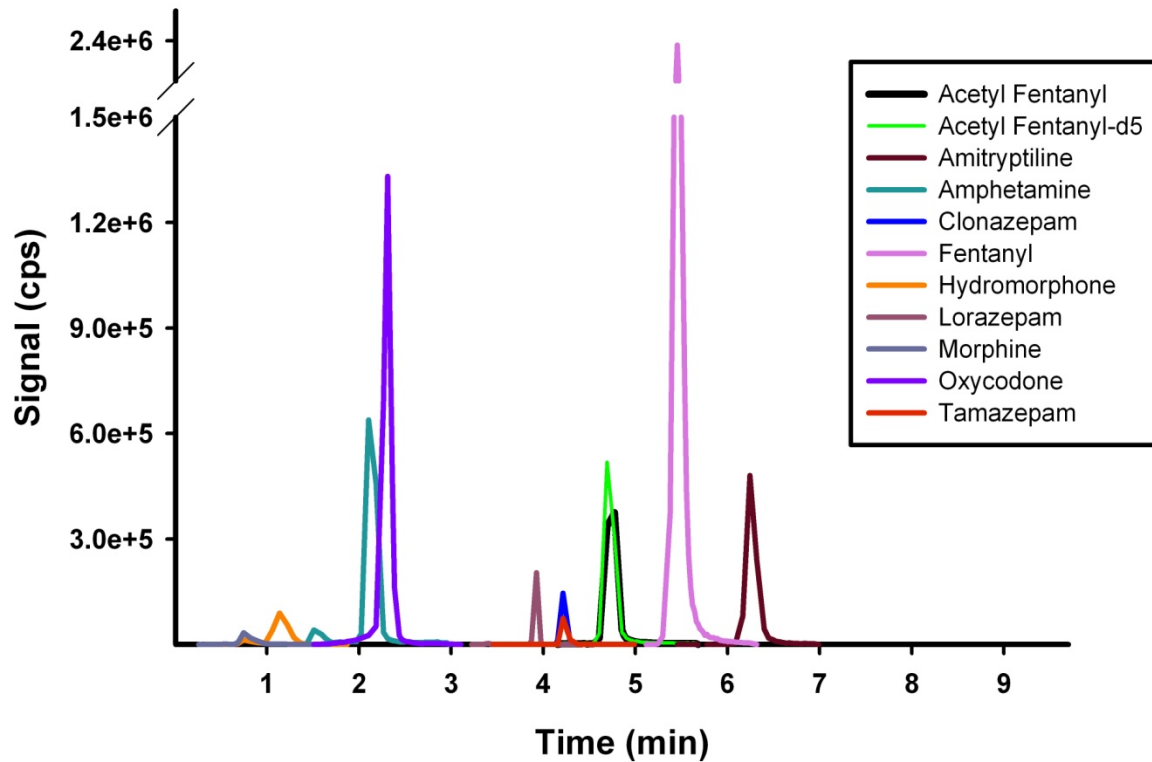
Article

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Quantitative Measurement of Acetyl Fentanyl and Acetyl Norfentanyl in Human Urine by LC-MS/MS

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Gregory W. Endres,[∇] and Jeffery H. Moran^{*,†,‡}

Acetyl Fentanyl Response: LC-MS/MS Development – LRN-C Platform



- Agilent 1200 – ABSCIEX 4000 Q-Trap
- Allure PFPP Column
 - 50 x 2.1 x 5
- 14.5 minute method
 - 90% A to 10% A over 9 minutes
 - Hold for 3 minutes
 - 2.5 minute equilibration
- sSRM –IDA-EPI
 - Acetyl Fentanyl: 323 → 188
 - ISTD: 328 → 188

SOP and Method Validation Plan

Date: 06/26/13
SOP14-Opiate
Page 1 of 31

Arkansas Department of Health
Public Health Laboratory
Environmental Chemistry Section
Chemical Terrorism Laboratory

Standard Operating Procedure

for

Acetyl Fentanyl and Metabolites
In Urine and Blood by LC-MS/MS
SOP14-Opiate

June 26, 2013

07/01/13
Page 1 of 16
SOP 14-Opiate VAL

**Arkansas Department of Health
Public Health Laboratory
Environmental Chemistry Section
Chemical Terrorism Laboratory**

Standard Operating Procedure

for

**Method Validation for Acetyl Fentanyl and Metabolites
In Urine and Blood by LC-MS/MS
SOP 14-Opiate VAL**

July 1, 2013

SO
Pla

Method Validation: Summary of Results

Method: Opiates in Urine

(AF: Acetyl Fentanyl ANF: Acetyl Norfentanyl)

Parameter	Result						
Accuracy		AF	ANF				
	QCL (%)	99.04 ✓	97.73 ✓				
	QCM (%)	98.57 ✓	98.81 ✓				
	QCH (%)	96.35 ✓	97.66 ✓				
Precision		AF			ANF		
		QCL (%CV)	QCM (%CV)	QCH (%CV)	QCL (%CV)	QCM (%CV)	QCH (%CV)
	Within-Run	7.52 ✓	5.00 ✓	4.26 ✓	3.84 ✓	3.77 ✓	7.85 ✓
	Between-Run	9.50 ✓	7.94 ✓	9.42 ✓	14.08 ✓	5.77 ✓	7.72 ✓
Analytical Sensitivity (LOD and LOQ)		AF	ANF		AF	ANF	
	Lower Detection Limit (ng/mL)	0.5	1.0	Lower Quantitation Limit (ng/mL)	1.06 ✓	1.62 ✓	
Analytical Interferences	This method uses low resolution tandem mass spectrometry; therefore, it eliminates most analytical interferences. No interferences are observed with this method.						
Reportable Range		AF	ANF				
	Low (ng/mL)	1.06	1.62				
	High (ng/mL)	100	100				
				<ul style="list-style-type: none"> • This method is linear within this range. • The low value is the calculated LOQ. 			
Uncertainty Budget*		Uncertainty Value			Uncertainty Value		
	QC Charts		1.5785 ✓	SPE Cartridges		0.0250 ✓	
	Acetyl Fentanyl Std.		0.0100 ✓	Sodium Acetate		0.0033 ✓	
	Acetyl Fentanyl-d5 Std.		0.0100 ✓	Acetonitrile		0.0003 ✓	
	Acetyl Norfentanyl Std.		0.0250 ✓	Formic Acid		0.0033 ✓	
	Acetyl Norfentanyl-d5 Std.		0.0250 ✓	Ammonium Formate		0.0033 ✓	
	Analytical Balance		0.0006 ✓	Methanol		0.0033 ✓	
	All Pipettes		0.0498 ✓	Ammonium Hydroxide		0.0033 ✓	

This validation study has been reviewed and the performance of all analytes measured in this method is considered acceptable for testing.

Analyst(s):
Supervisor:
Branch Chief:
Laboratory Director:
QA Officer:

Amey J. Patkar 9-20-13
Kathy Kelly 9/26/13
Pat Karney 10-7-13
Pat Karney 10-4-13
Pat Karney 10-3-13

If changes are made to this method, a new validation may be necessary.

*Operator and instrument uncertainty is measured within the method by evaluation of QC elements.

Introducing ToxBox[®] Products and Services

Everything you need to quickly run validated analysis on emerging drugs of abuse

- Analytical Run Packs
- Column and Extraction Cartridges
- Standard Operating Procedures
- Validation Plan
- On-site Consulting



Choose the solution that best fits your needs!

Acetyl Fentanyl ToxBox - Bronze	Acetyl Fentanyl ToxBox - Silver	Acetyl Fentanyl ToxBox - Gold	Acetyl Fentanyl ToxBox - Platinum
5 Analytical Run Packs	5 Analytical Run Packs	5 Analytical Run Packs	5 Analytical Run Packs
	1 HPLC Column	1 HPLC Column	1 HPLC Column
		500 SPE Cartridges	500 SPE Cartridges
		1 SOP	1 SOP
		1 Validation Plan	1 Validation Plan
			80 hrs. of on-site consulting and training from PinPoint Testing, LLC

ToxBox has been created through a partnership between PinPoint Testing and Cayman Chemical. Please contact PinPoint Testing today for more information on ToxBox products and services.

PINPOINT TESTING, LLC

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Now available - Acetyl Fentanyl ToxBox[®]

Continued Improvement and Sustainability

- Acetyl Fentanyl:
 - Waning Popularity
 - Too Focused (Analyte and Matrix)
 - Commercial markets outside of Public Health Laboratories did not grasp concept
- Next steps:
 - Drug Panel Expansion (1 method for urine/blood supported by LC-MS/MS and/or LC-TOF/MS)
 - Move to a fully customizable format
 - Implement 'Green Chemistry Concepts'
 - Miniaturize & reduce solvents
 - Use 'safer/environmentally friendly' solvents

Next Generation ToxBox®:

Expanded Testing Menu

- **Opiates**

- Morphine & metabolite
- Acetyl fentanyl & metabolite
- Buprenorphine & metabolite
- Carisoprodol (Soma)
- Codeine
- Cyclobenzaprine (Flexeril)
- Dextromethorphan
- Dihydrocodeine
- Fentanyl & metabolite
- Heroin
- Hydrocodone
- Hydromorphone (Dilaudid)
- Meperidine (Demerol) & metabolite
- Meprobamate
- Methadone & metabolite
- Morphine
- Naloxone
- Naltrexone
- Oxycodone
- Oxymorphone
- Propoxyphene & metabolite
- Sufentanil
- Tramadol (Ultram)

- **Benzodiazepines**

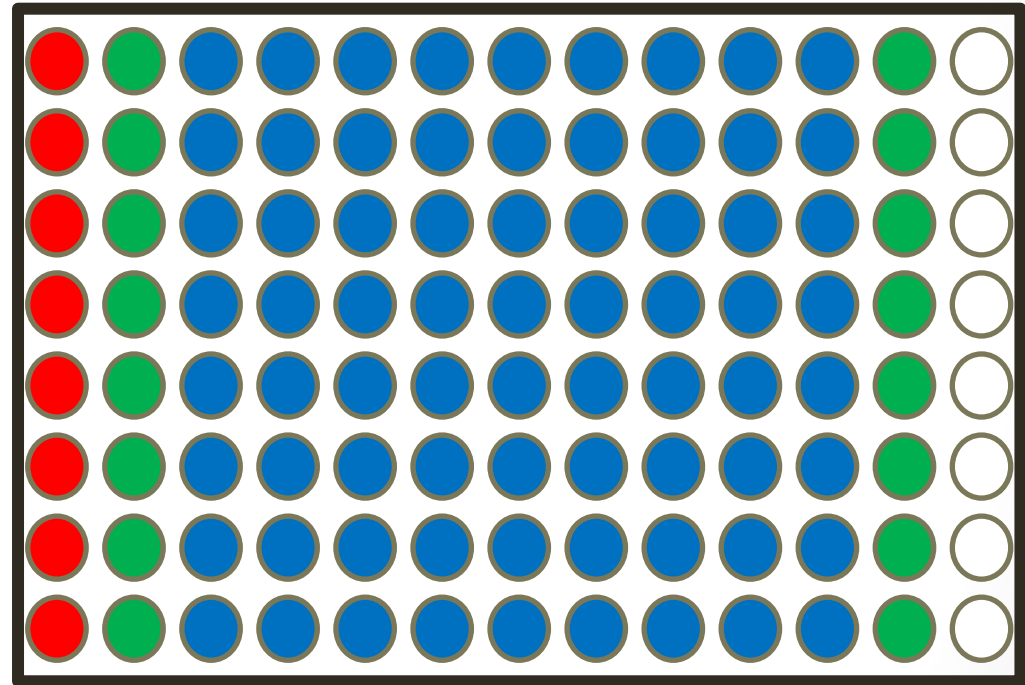
- Alprazolam (Zanax) & metabolite
- Clonazepam (Clonapin) & metabolite
- Diazepam (Valium) & metabolite
- Flunitrazepam (Rohypnol)
- Flurazepam (Dalmane)
- Lorazepam (Ativan)
- Midazolam (Versed)
- Nitrazepam (Nitrados)
- Oxazepam (Serepax)
- Phenazepam
- Temazepam (Restoril)

- **Other Drugs of Abuse**

- Amphetamine
- Cocaine & metabolite
- MDA
- MDEA
- MDMA
- Methamphetamine
- PCP
- Phentermine

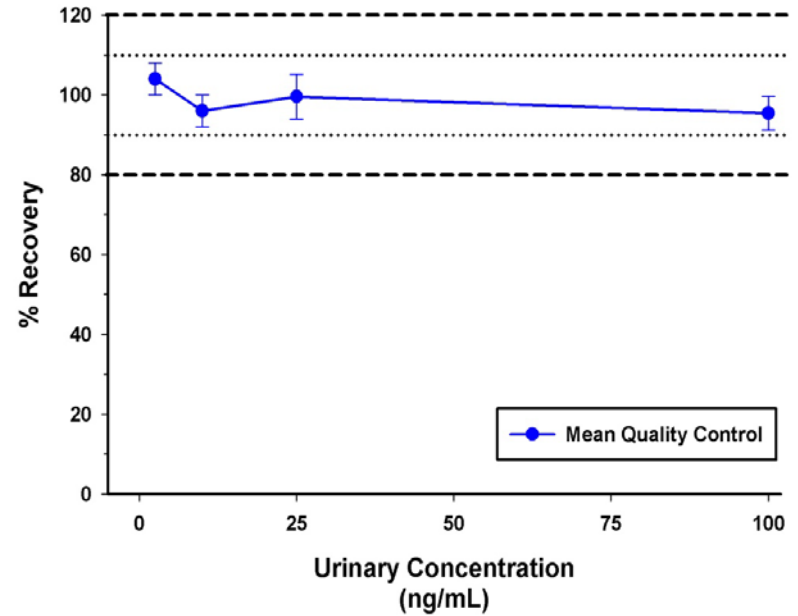
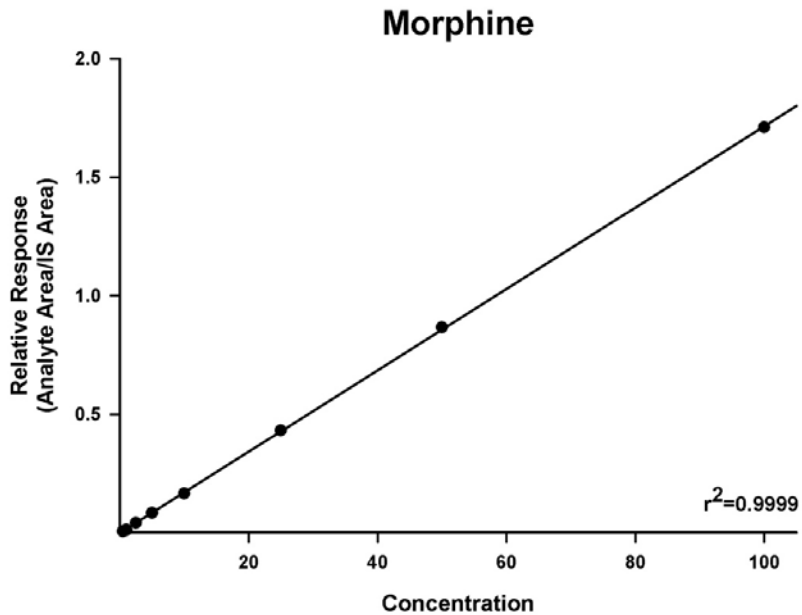
Next Generation ToxBox[®]: ToxBox[®] Analytical Plates*

- 96-Well plate processing and high throughput capacity (1 plate/hr/person)
- Whole blood and urine
- Small volume (175 ul)
- Low level quantification
- Low cost
- No liquid waste
- Validated 55 total drugs of abuse (traditional and designer) in human urine and blood
- Completely customizable

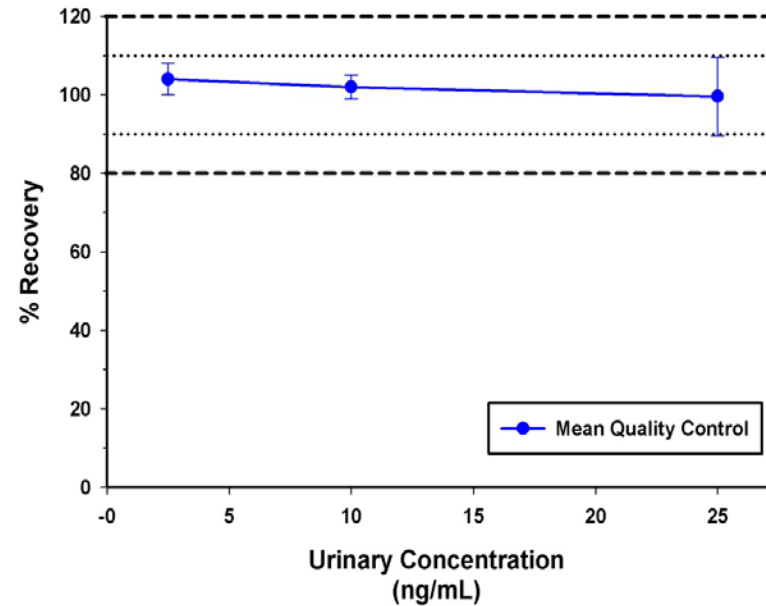
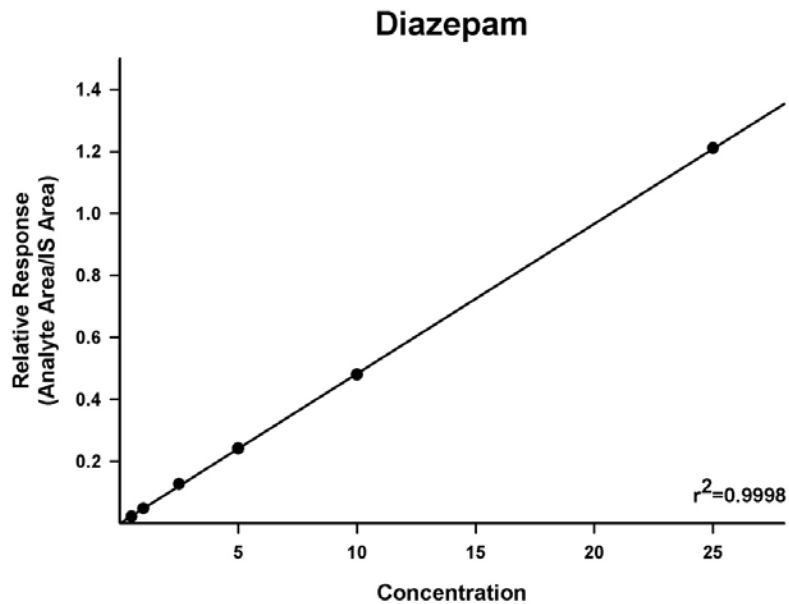


*Patent Pending

Example Results: Opiates



Example Results: Benzodiazepines



Resources for Clinical Laboratories

ISSUES IN BRIEF: LRN-C ANALYTICAL METHOD VALIDATION PLAN & TEMPLATE

DECEMBER 2013

CLIA-Compliant Analytical Method Validation Plan and Template FOR LRN-C LABORATORIES

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LIST OF ACRONYMS

EQA	External Quality Assessment
HPLC-MS/MS	High-Performance Liquid Chromatography Tandem Mass Spectrometry
IS	Internal Standard
LDT	Laboratory Developed Test(s)
LOD	Limit of Detection
LOQ	Limit of Quantitation
PK/PD	Pharmacokinetic/Pharmacodynamic modeling
TE_a	Total Allowable Error
ULOL	Upper Limit of Linearity

- Product from a National Workgroup

- Arkansas, Texas, New York, Utah, Wisconsin, Massachusetts, CMS, APHL, CDC



TOXBOX[®]

BLOOD & URINE VALIDATION TIMELINE



- Method Development and/or Technology Transfer
- Development and Approval of Standard Operation Procedures
- Training and Initial Demonstration of Capability

01 WEEK

DAY 1 **LC MS**
SAMPLE PRE/EXTRACTION
 - Perform a plan of 30 blood urine stability study
 - Perform a plan of 30 blood urine QC data for system stability study for 10, 15, and 7 days
INSTRUMENT TIME
 - Inject 3 day blood urine stability samples

DAY 2 **MS**
DAY 3 **MS**
DATA & STATISTICAL EVALUATIONS
 - Evaluate 3 day blood urine stability samples

DAY 4 **LC MS**
INSTRUMENT TIME
 - Inject 3 day blood urine stability samples

DAY 5 **MS**
DAY 6 **MS**
DATA & STATISTICAL EVALUATIONS
 - Evaluate 5 day blood urine stability samples

DAY 7 **LC MS**
INSTRUMENT TIME
 - Inject 7 day blood urine stability samples

02 WEEK

DAY 14 **MS**
SAMPLE PRE/EXTRACTION
 - Perform blood and urine for 10 day sample stability study

DAY 12 **MS**
DAY 13 **MS**
DATA & STATISTICAL EVALUATIONS
 - Evaluate 10 day blood urine stability samples

DAY 11 **LC MS**
INSTRUMENT TIME
 - Inject 10 day blood urine stability samples
DATA & STATISTICAL EVALUATIONS
 - Evaluate 1 day urine stability samples

DAY 9 **MS**
DAY 10 **MS**
DATA & STATISTICAL EVALUATIONS
 - Evaluate 7 day blood urine stability samples and summarize all blood urine stability data

DAY 8 **LC MS**
SAMPLE PRE/EXTRACTION
 - Perform a plan of 30 urine urine QC data for system stability study for 10, 15, and 7 days
INSTRUMENT TIME
 - Inject 7 day urine urine stability samples

03 WEEK

DAY 15 **LC MS**
INSTRUMENT TIME
 - Inject 7 day urine urine stability samples

DAY 16 **MS**
DAY 17 **MS**
DATA & STATISTICAL EVALUATIONS
 - Evaluate 7 day urine urine stability samples and summarize all blood urine stability data

DAY 18 **LC MS**
SAMPLE PRE/EXTRACTION
 - Perform a plan of 30 urine urine QC data
 - Inject all 30 pre-validated urine QC data

DAY 19 **MS**
DATA & STATISTICAL EVALUATIONS
 - Evaluate blood urine and QC data

DAY 20 **LC MS**
DAY 21 **MS**
SAMPLE PRE/EXTRACTION
 - Perform a plan of 30 urine urine QC data
INSTRUMENT TIME
 - Inject all 30 pre-validated urine QC data
DATA & STATISTICAL EVALUATIONS
 - Evaluate pre-validated blood urine and QC data

04 WEEK

DAY 27 **MS**
DAY 28 **MS**
DATA & STATISTICAL EVALUATIONS
 - Evaluate and summarize all blood and urine stability data

DAY 26 **LC MS**
SAMPLE PRE/EXTRACTION
INSTRUMENT TIME
 - Inject blood and urine stability samples

DAY 25 **MS**
SAMPLE PRE/EXTRACTION
 - Perform blood and urine for 10 day sample stability study

DAY 22 **MS**
DAY 24 **MS**
DATA & STATISTICAL EVALUATIONS
 - Evaluate and summarize blood and urine stability and QC data

05 WEEK

DAY 29 **MS**
SAMPLE PRE/EXTRACTION
 - Perform blood and urine for 7 day sample stability study

DAY 30 **LC MS**
SAMPLE PRE/EXTRACTION
 - Extract all blood and urine sample stability samples (10, 15, 5, and 7 day)
INSTRUMENT TIME
 - Inject all blood and urine sample stability samples

DAY 31 **MS**
DAY 33 **MS**
DATA & STATISTICAL EVALUATIONS
 - Evaluate and summarize all blood and urine stability data

- Final Data Summary
- Compiling Data Packets for Final Review and Approval
- Submit to QA and Laboratory Director

Questions?

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