



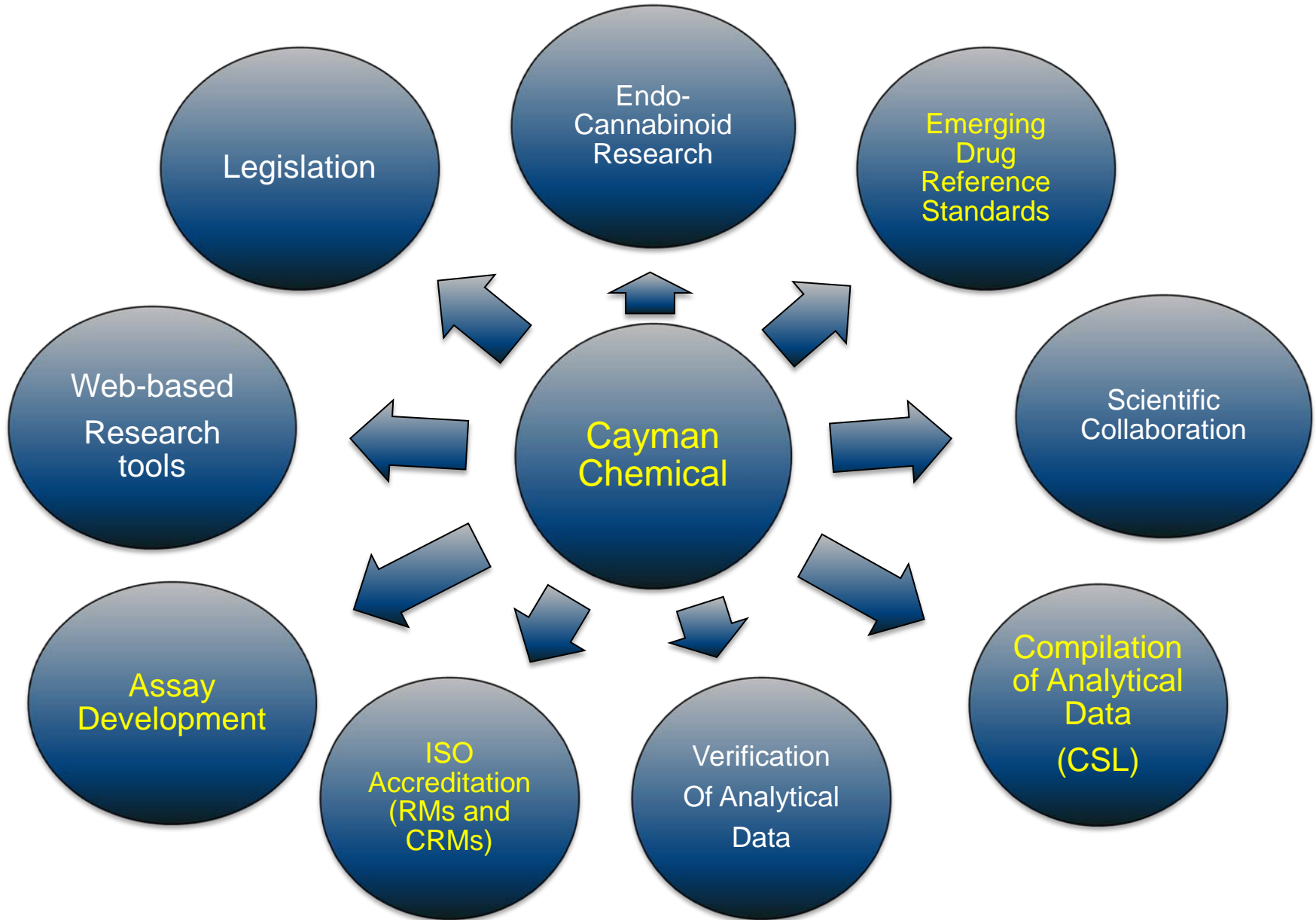
Responding to the Designer Drug Crisis:

# Creating a Material Program to Sustain Acetyl Fentanyl and Other Drug Testing Applications

Gregory W. Endres, PhD  
Vice President of Chemistry  
Cayman Chemical

May 20, 2015

# Cayman Response to Designer Drug Epidemic

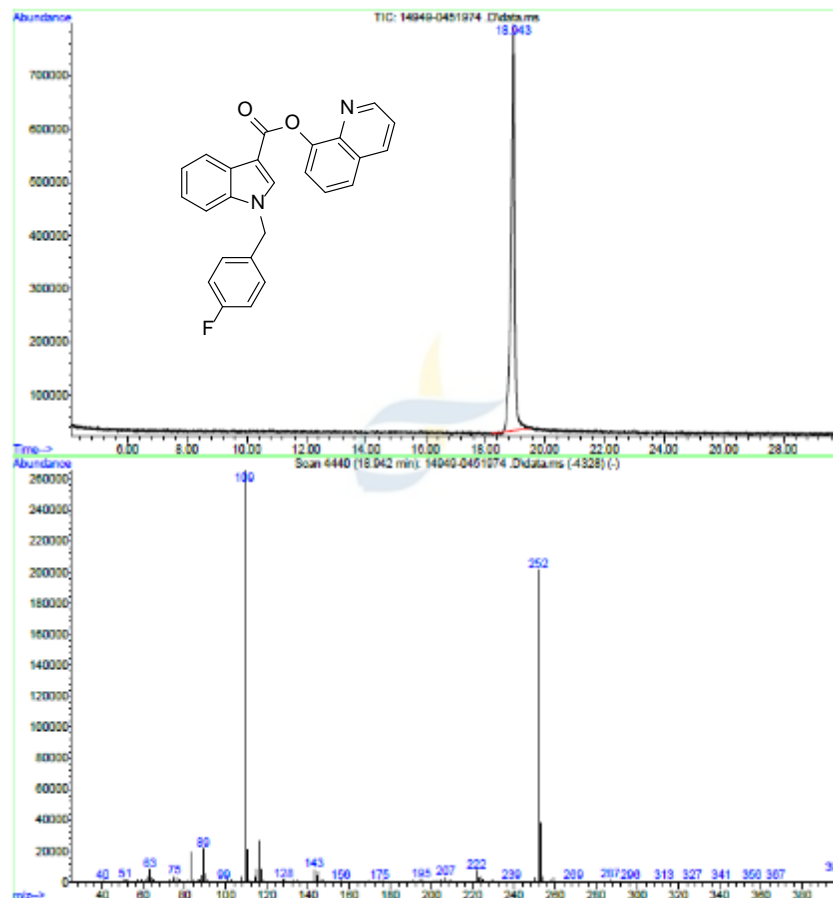


# Leads through spectral unknowns

Customer request for help in identifying an unknown analyzed by GCMS:

- 63 – lowest intensity
- 83
- 109 –greatest intensity
- 145 \*same intensity, about half of 83
- 222 \*
- 252 – second greatest intensity
- 396 – 7-10% of 252

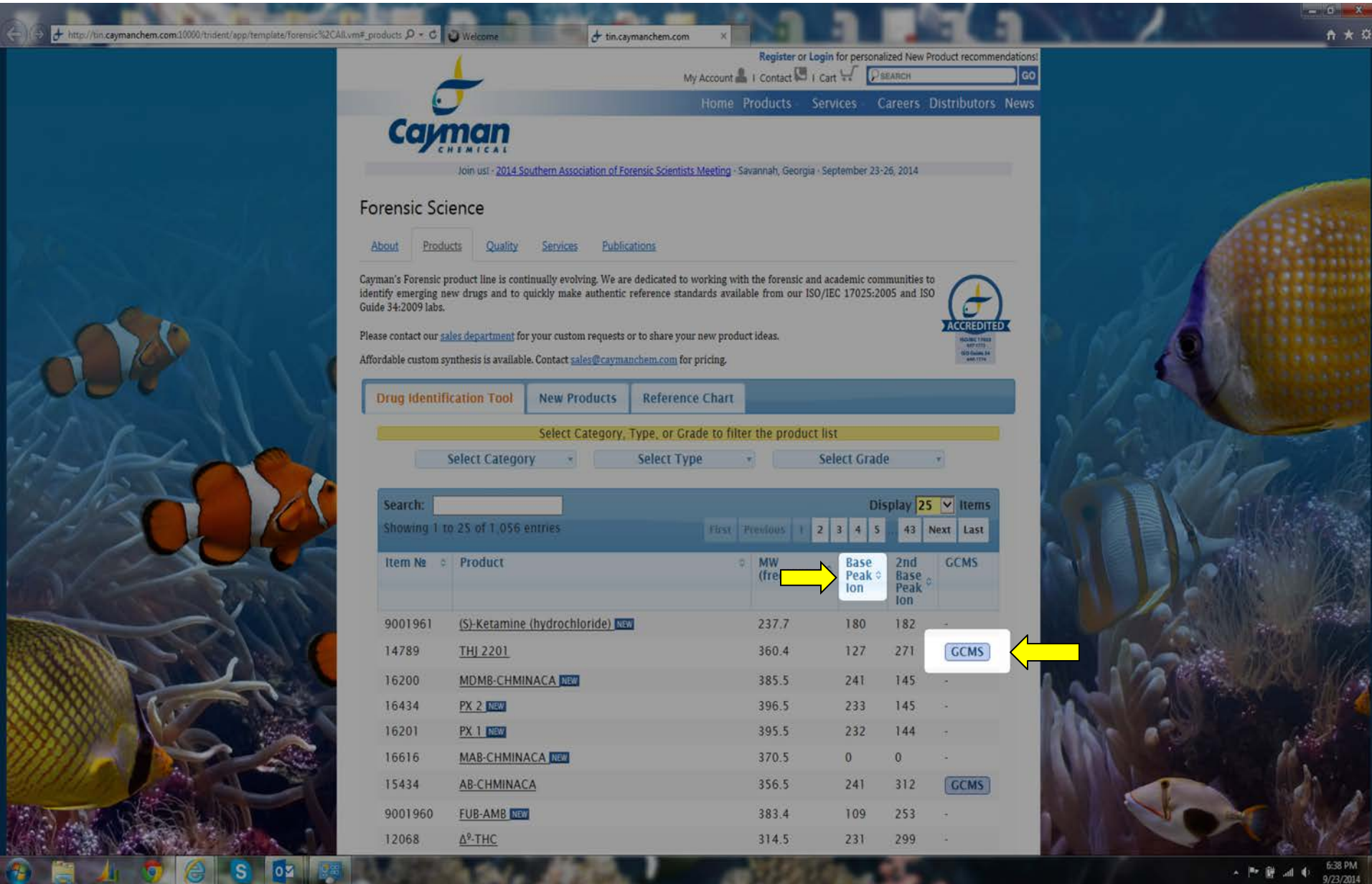
Cayman GCMS:



FUB-PB-22 was already in progress at Cayman in response to introductions by off-shore vendors



# Web Tool for Unknown Identification



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## Forensic Science

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Cayman's Forensic product line is continually evolving. We are dedicated to working with the forensic and academic communities to identify emerging new drugs and to quickly make authentic reference standards available from our ISO/IEC 17025:2005 and ISO Guide 34:2009 labs.

Please contact our [sales department](#) for your custom requests or to share your new product ideas.

Affordable custom synthesis is available. Contact [sales@caymanchem.com](mailto:sales@caymanchem.com) for pricing.

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Select Category, Type, or Grade to filter the product list

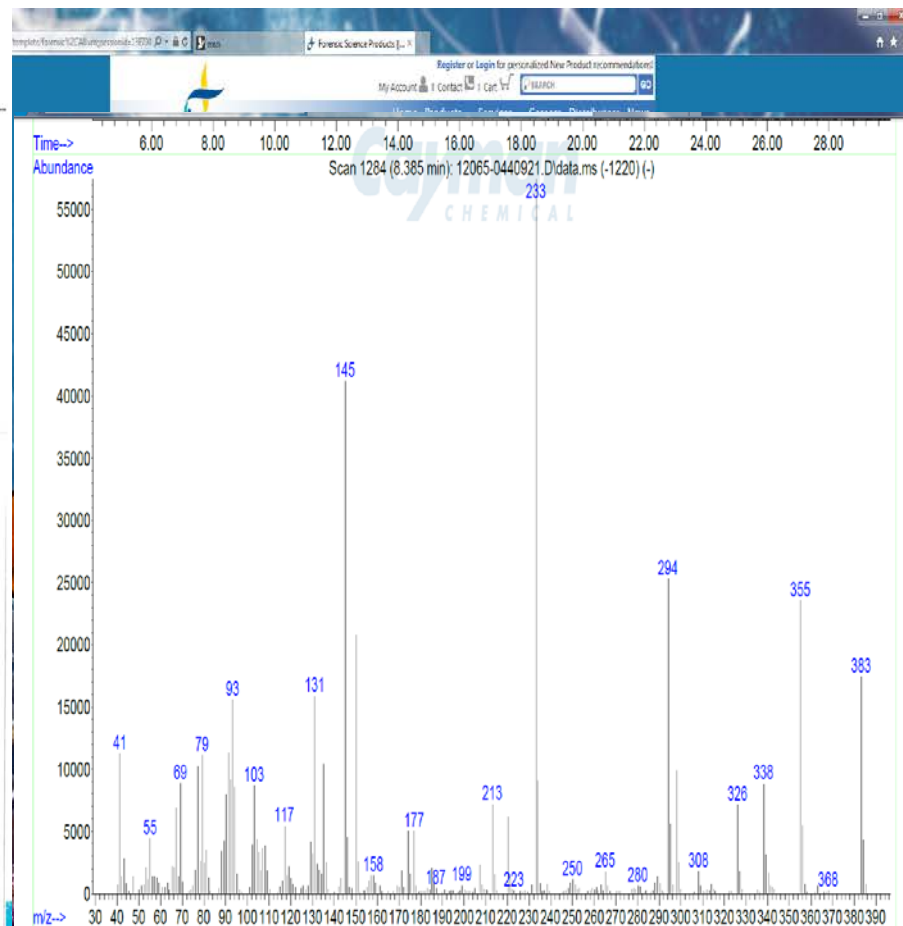
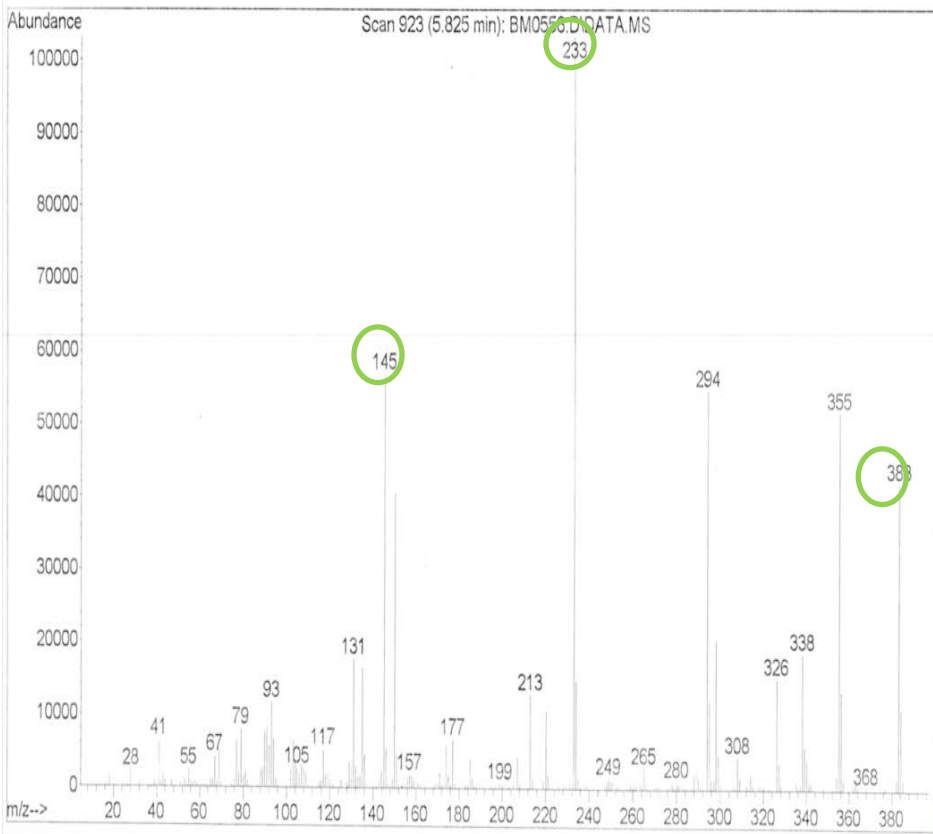
Select Category  Select Type  Select Grade

Search:  Display  Items

Showing 1 to 25 of 1,056 entries

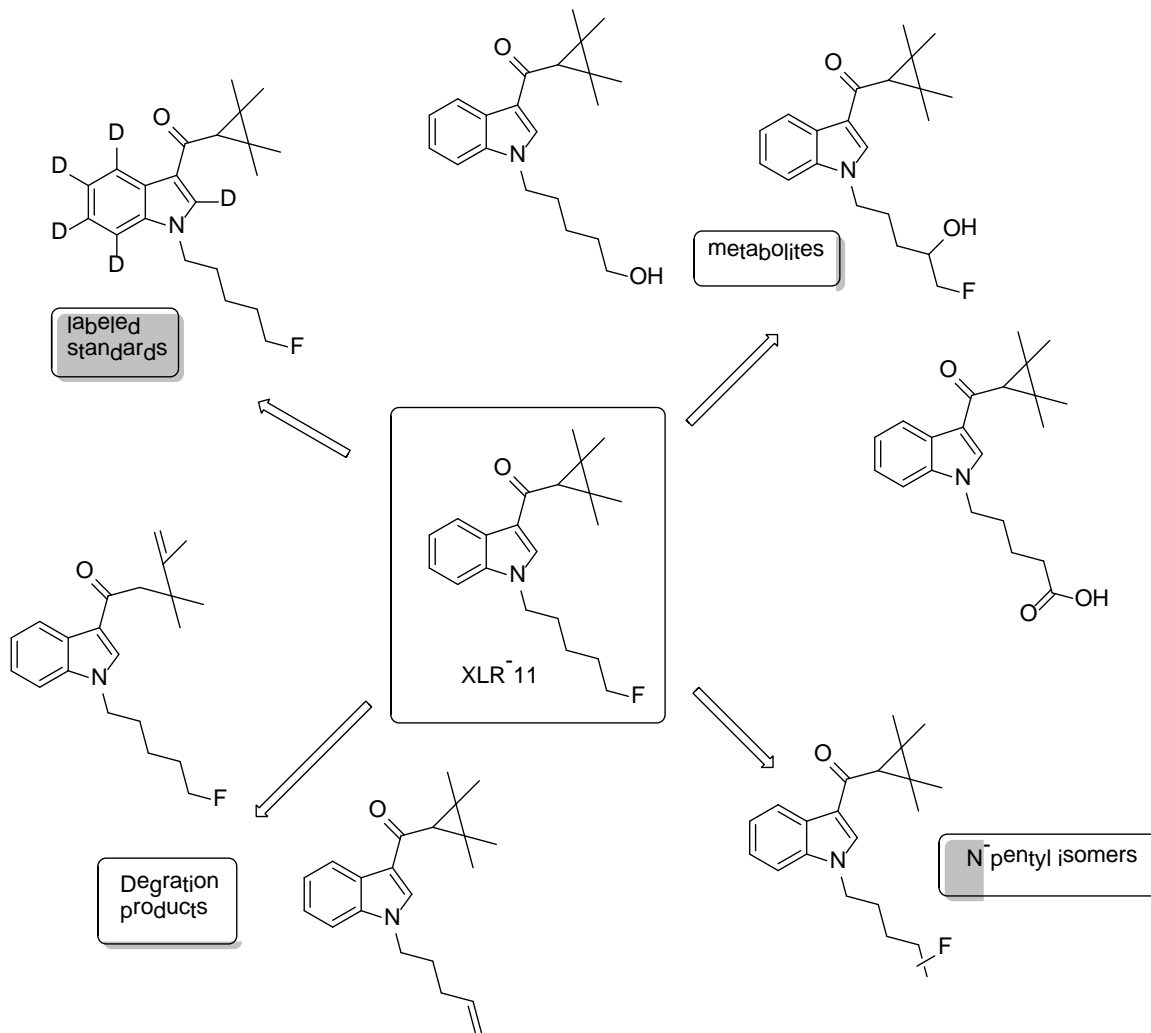
Item No	Product	MW (fre)	Base Peak Ion	2nd Base Peak Ion	GCMS
9001961	<a href="#">(S)-Ketamine (hydrochloride)</a> <small>NEW</small>	237.7	180	182	-
14789	<a href="#">THJ 2201</a>	360.4	127	271	<input type="button" value="GCMS"/>
16200	<a href="#">MDMB-CHMINACA</a> <small>NEW</small>	385.5	241	145	-
16434	<a href="#">PX 2</a> <small>NEW</small>	396.5	233	145	-
16201	<a href="#">PX 1</a> <small>NEW</small>	395.5	232	144	-
16616	<a href="#">MAB-CHMINACA</a> <small>NEW</small>	370.5	0	0	-
15434	<a href="#">AB-CHMINACA</a>	356.5	241	312	<input type="button" value="GCMS"/>
9001960	<a href="#">FUB-AMB</a> <small>NEW</small>	383.4	109	253	-
12068	<a href="#">Δ<sup>9</sup>-THC</a>	314.5	231	299	-

# ID by Cayman base peak tool



- Parent ion of 383, base peak of 233, secondary peak of 145 suggests **5F-AKB48**
- Click the GCMS button to compare unknown and authentic reference standard

# Additional Research Products



XLR-11 reference standards and research tools

# Metabolite detection by EIA (limitations)

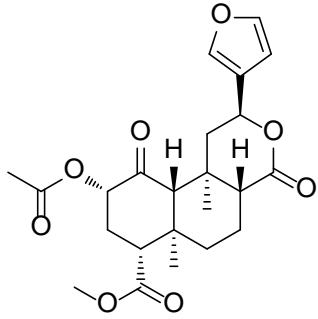
Compound	Detection	Compound	Detection
JWH 018 (immunogen)	++	JWH 015	+
JWH 018 5-O-glucuronide (std)	++	JWH 020	+
JWH 018 4-O-glucuronide	+++	JWH 081 5-OH	+
JWH 018 4-OH	+++	JWH 122	+
JWH 018 5-OH	+++	JWH 398	+
JWH 018 5-COOH	+++	JWH 210	-
JWH 019 6-OH	+++	JWH 250 5-COOH	-
JWH 022	++	STS 135	-
JWH 073	+++	XLR11	-
JWH 073 3-OH	+++	2 Arachidonoyl Glycerol	-
JWH 073 4-OH	+++	Anandamide	-
JWH 073 4-COOH	+++		
JWH 122 5-OH	++		
JWH 200	+++		
JWH 210 5-COOH	++		
JWH-398 5-OH	++	+++ High detection	
AM2201	++	++ Moderate detection	
AM2201 4-OH	+++	+ Low detection	
MAM2201	++	- Not recognized	



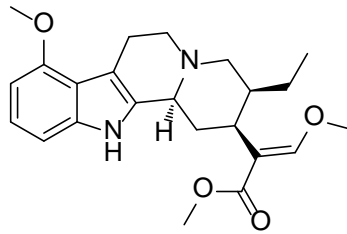
## JWH Metabolite EIA Kit (Developed in 2011)

- Qualitative urinary assay, 100pg/ml limit
- Glucuronide detection w/o enzymatic cleavage
- Good cross-reactivity among similar analogs.
- Does not detect SC metabolites observed post 2011
- Alternative assay is needed for quantitative results for multi-component mixtures

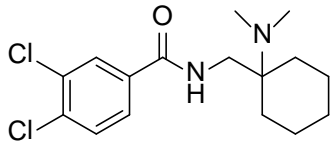
# Emerging Trend: Opiates



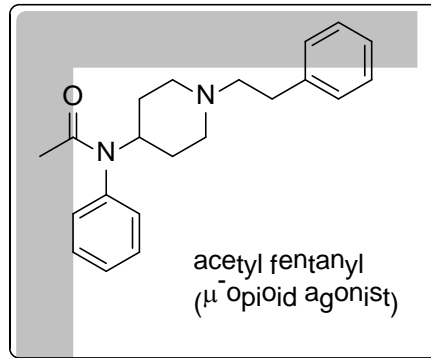
Salvinorin A  
( $\kappa$ -opioid agonist)



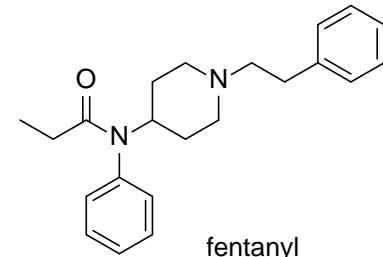
Miltiragynine  
( $\mu$ -opioid agonist)



AH 7921  
( $\mu$ -opioid agonist)



acetyl fentanyl  
( $\mu$ -opioid agonist)



fentanyl  
( $\mu$ -opioid agonist)



- Opiate abuse is expanding into new, non morphine-based chemical classes
- Acetyl fentanyl is a “designer” form of fentanyl and responsible for several deaths
- ELISA (available) will detect **fentanyl** but will not distinguish from **acetyl fentanyl**
- Positive results for fentanyl exposure need to be confirmed by MS
- Certified Reference Material (CRMs) are needed for MS-based kit development



# ISO 17025 / Guide 34 Reference Standards



- Cayman Forensic/Toxicology product line is transitioning from research grade to reference materials (RMs)
- RMs (solids) needed by crime labs
- CRMs (solutions) needed for quantitation

**Cayman Chemical Co.**  
1180 E. Ellsworth Rd  
Ann Arbor, MI 48108  
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1-800-364-9897 or (734)971-3335  
Fax: (734)971-3640  
Email: crmquality@caymanchem.com

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## Certificate of Analysis

**Acetyl fentanyl (hydrochloride) CRM**  
N-phenyl-N-[1-(2-phenylethyl)-4-piperidinyl]-acetamide, monohydrochloride

Item #: ISO60128  
Batch #: 0451384  
CAS Registry Number: 117332-89-5

ISO/IEC 17025  
#AT-1773  
ISO Guide 34  
#AR-1774

Expiry: 07FEB2016 (valid from date of certification)  
Provided as: 100µg/mL (nominal, as free base) solution in Methanol  
Volume per ampule: not less than 2mL  
\*Ampules are overfilled. It is advised that labs use measured volumes.  
Storage and Handling: Store unopened in freezer. Warm to RT prior to opening.  
Safety: Flammable, Poison

AClass  
ACCREDITED  
REFERENCE MATERIAL PRODUCER

**Compound Information**

Chemical Formula	C <sub>21</sub> H <sub>26</sub> N <sub>2</sub> O • HCl
Formula Weight	358.90

**Certified Concentration**

Certified Concentration (as free base)	100.0 ± 0.7 µg/mL
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Concentration is calculated based on product mass (as free base), solution mass, corrected purity and density at 20°C.  
Uncertainty of concentration is expressed as and expanded uncertainty in accordance with ISO 17025 and Guide 34 at the approximate 95% confidence interval using a coverage factor of k=2 and incorporates uncertainties from the corrected purity, solution preparation, homogeneity, long and short term stability.  
Concentration was verified by comparison to an independently prepared calibration standard.

**Neat Material Quality Information (Item #ISO00128 Batch #0450197)**

Qualifier	Method	Limit	Result	Meets Specification
Chromatographic Purity, HPLC	Cayman Method TST SD60	≥98.00%	99.36% ± 0.17%	Y
Identity, LCMS	Cayman Method TST SD13, +ESI	323.2 ± 0.5 amu	323.2 amu	Y
Identity, GCMS	Cayman Method TST SD12	Conforms	Conforms	Y
FTIR	USP <851> (diamond ATR)	Conforms	Conforms	Y
Melting Point	USP <741>	252.0 ± 5.0°C	247.8°C	Y
% LOD	Cayman Method TST SD24	<3.00%	<0.10% ± 0.15%	Y
% ROI	Cayman Method TST SD06	<2.00%	0.71% ± 0.12%	Y
*Identity, NMR	<sup>1</sup> H NMR	Conforms	Conforms	Y
**Corrected Purity			98.56% ± 0.26%	

\*NMR is provided as supplemental info but is not within scope of ISO accreditation  
\*\*Corrected purity is determined as follows: Corrected Purity = [(100 - % LOD - % ROI) \* Chromatographic Purity / 100]

**Measurement Uncertainty**  
All measurement uncertainties are expressed as expanded uncertainties in accordance with ISO 17025 and Guide 34 at the approximate 95% confidence interval using a coverage factor of k=2.

Cayman Chemical certifies that this standard meets the specifications stated in this certificate and warrants this product to meet the stated acceptance criteria through the expiration date when stored unopened as recommended.

Approval:

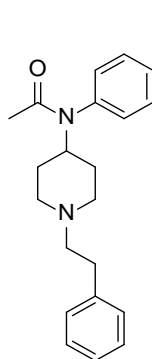
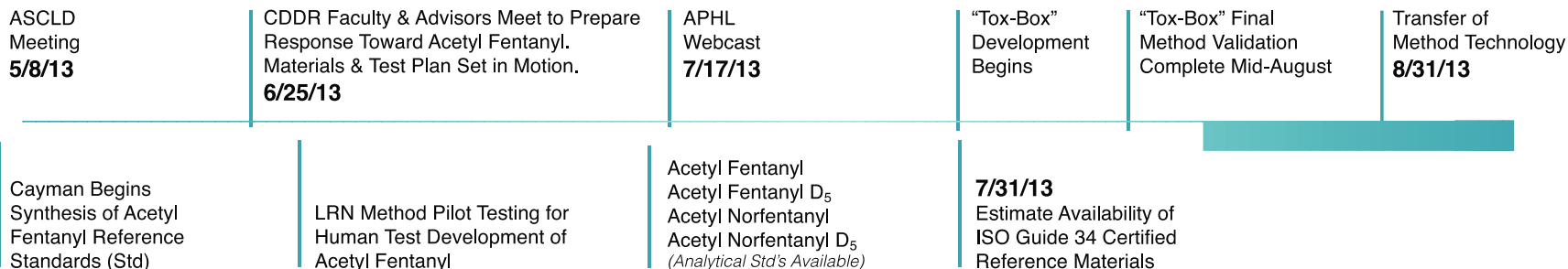
Title: Cayman Chemical ISO Quality Manager

Certification Date: 07FEB2014

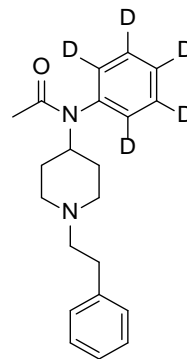
Page 1 of 4

Certificate 60128-0451384-02

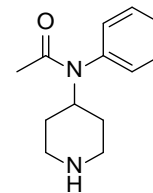
# Acetyl Fentanyl Outbreak: May 2013



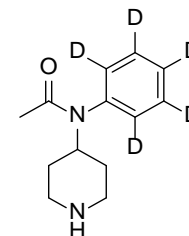
Acetyl Fentanyl



Acetyl Fentanyl-d<sub>5</sub>



Acetyl Norfentanyl



Acetyl Norfentanyl-d<sub>5</sub>

## Response:

1. Quickly Produce the AF Reference Standard for Confirmation.
2. Produce CRMs for MS-based Assay Development
3. Method Development and Validation (CDDR)
4. Manufacture MS assay for Quantitative Analysis (Cayman Chemical)

# ToxBox® - Ampuled Solutions for Quantitation by MS



**ToxBox® Goal:** Provide laboratories with the tools and methods to acquire, evaluate, and report validated data for designer drug analysis (multi-component mixtures)

- **First Product:** Acetyl Fentanyl ToxBox®
  - Produced in response to an outbreak first identified in RI
  - Scope and extent of outbreak was unknown
  - Customer feedback: **Flexibility in design, analytes, and concentration needed.**

# ToxBox<sup>®</sup> - New Format: Ready-to-Reconstitute Plates

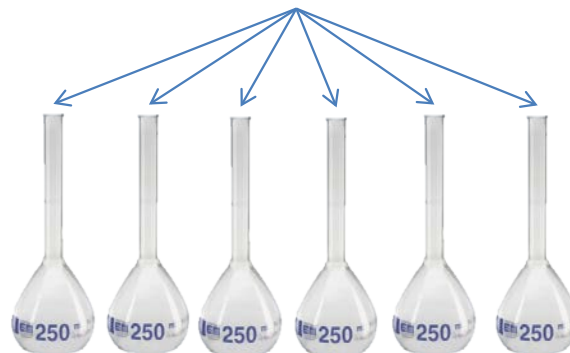
- **New Format:** Multicomponent panels of analytes formulated on 96-well plates (dry residue) per custom request
  - Calibrators (CRMs)
  - QC (2nd source CRMs)
  - Internal Standards
- **Contents**
  - Prep plate
  - Analytical plate (Cayman)
  - Extraction plate (Biotage)
  - Collection plate
- **Custom Panels:**
  - Opiates
  - Benzodiazepines
  - Barbituates
  - Phytocannabinoids
  - Synthetic Cannabinoids and other Designer Drugs
  - Other testing platforms (traditional environmental / food / water / public health testing)



# Manufacturing Process



Individual CRMs



Calibrator and QC solutions



Bulk solutions onto master plate



Automation used to mass-produce, elimination of aliquot error



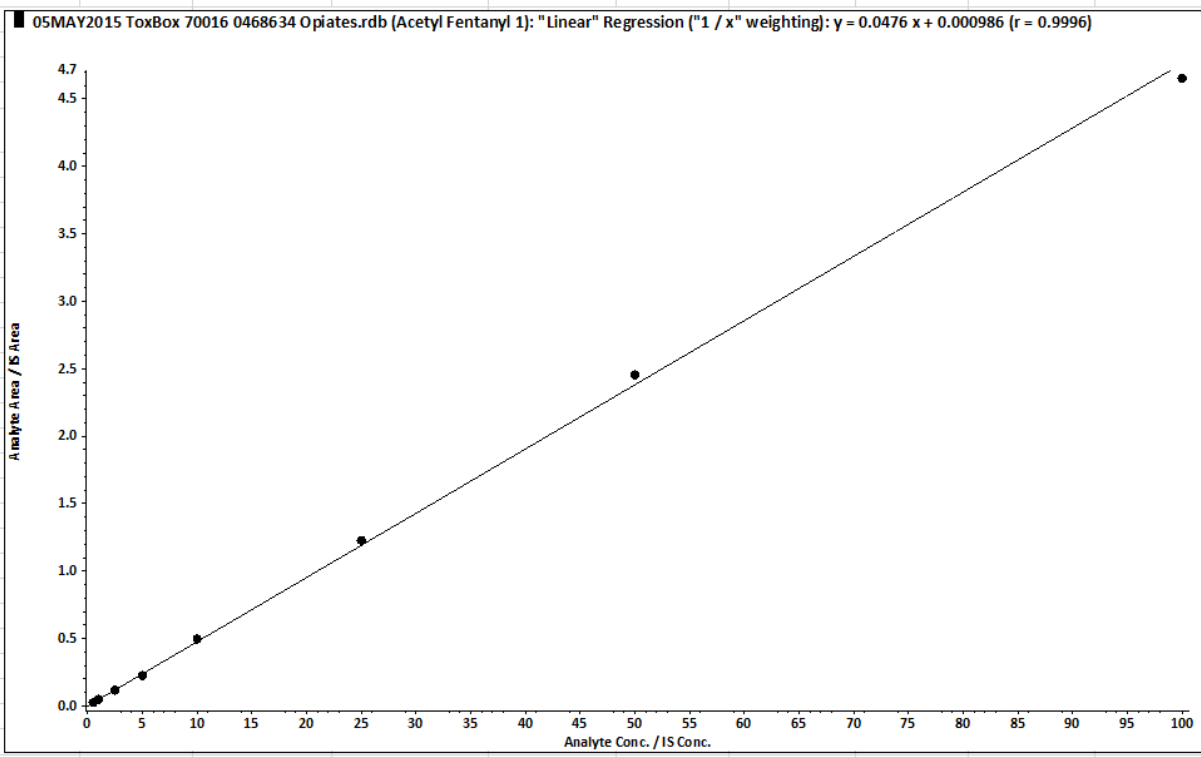
# QC Process: 1/51 Analytes (Acetyl Fentanyl)



1 Analyte: Acetyl Fentanyl

Second Source Recovery

Run 1			
Date:	5/5/2015		
Run ID:	70016 0468634		
r2=	0.9996	Pass ≥ 0.99	
Assigned Value (ng)	Concentration (ng/mL)	Recovery (Pass +/- .25%)	
Blank IS	N/A	7.58E+05	
Blank IS	N/A	7.06E+05	
Blank IS	N/A	7.17E+05	
Blank IS	N/A	7.44E+05	
QC1	2.5	2.56	102.4%
QC1	2.5	2.45	98.0%
QC1	2.5	2.56	102.4%
QC1	2.5	2.72	108.8%
QCL	2.5	2.64	105.6%
QC2	10	10.5	105.0%
QC2	10	10.5	105.0%
QC2	10	10.2	102.0%
QC2	10	10.6	106.0%
QC2	10	10.6	106.0%
QC3	25	24	96.0%
QC3	25	25.2	100.8%
QC3	25	24.4	97.6%
QC3	25	25.6	102.4%
QC3	25	25.7	102.8%
QC4	100	101	101.0%
QC4	100	94.4	94.4%
QC4	100	105	105.0%
QC4	100	98.4	98.4%
QC4	100	103	103.0%



Rep. Range (ng):	Mean	Std. Dev.	% CV	Total Error	Internal Standard	Mean	Std. Dev.	% CV
.5-100	QC1 2.6	0.1	3.9	9.88	731250.0	23935.7	3.3	
	QC2 10.5	0.2	1.6	7.39				
	QC3 25.0	0.7	3.0	5.03				
	QC4 100.4	4.2	4.2	7.31				
AVERAGE		1.3	3.2	7.4				

Notes:

6-MAM Acetyl Fentanyl Acetyl Norfentanyl Buprenorphine Codeine Dextromethorphan Dihydrocodeine EDDP Fentanyl Heroin Hydrocodone Hydromorphone Meperidine Meprobamate

# Certificate of Analysis



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Email: techserv@caymanchem.com

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## Certificate of Analysis

### ToxBox® Benzo/Opiate Panel 6

Item #: 70016  
Batch #: 0468634

Expiry: 12MAY2016 (valid from date of certification)  
Storage and Handling: Store unopened in freezer. Warm to room temperature prior to opening.

**Description**  
This product consists of a 96-well plate containing custom prepared mixture of 51 drugs and their corresponding 50 internal standards. See the Plate Layout diagram for an overview of the contents in each well. The plate includes calibrators, internal standards, blanks and quality control standards which have been prepared from a separate lot of intermediate solution and added to the well plate on a separate day from the calibrator solutions. The plate has been dried to a film and must be reconstituted in an appropriate solvent prior to use. The accurate quantity of each component (reported in ng) in each well position is tabulated in the Contents section of this document and may also be found in the associated Excel document located in the provided data package. The data package also contains the lot specific certificates of analysis for each parent drug and internal standard used in the preparation of this plate.

This product may also come prepackaged with additional components such as extraction and collection plates. Please refer to the original manufacturer documentation for each of those components.


#### Traceability of intermediate solutions used to prepare the plate.

Component	Item / Lot #	Description
ToxBox – Benzo/Opiate Panel 2 Internal Standard Intermediate Solution	TOX30002/0465955	Mixture of labeled opiates/benzodiazepines used to spike all plate wells containing Internal Standards
ToxBox – Benzo/Opiate Panel 2 Calibrator #1 intermediate solution	TOX10005/0467988	Mixture of opiates/benzodiazepines used to prepare well plate calibrator #1 and #2
ToxBox – Benzo/Opiate Panel 2 Calibrator #2 intermediate solution	TOX10006/0467989	Mixture of opiates/benzodiazepines used to prepare well plate calibrator #3 and #4
ToxBox – Benzo/Opiate Panel 2 Calibrator #3 intermediate solution	TOX10007/0467990	Mixture of opiates/benzodiazepines used to prepare well plate calibrators #5 and #6
ToxBox – Benzo/Opiate Panel 2 Calibrator #4 intermediate solution	TOX10008/0467991	Mixture of opiates/benzodiazepines used to prepare well plate calibrators #7 and #8

ToxBox – Benzo/Opiate Panel 2 QC Low intermediate solution	TOX20004/0467992	Mixture of opiates/benzodiazepines used to prepare QC A wells
ToxBox – Benzo/Opiate Panel 2 QC Mid intermediate solution	TOX20005/0467993	Mixture of opiates/benzodiazepines used to prepare QC B and QC C wells
ToxBox – Benzo/Opiate Panel 2 QC High intermediate solution	TOX20006/0467994	Mixture of opiates/benzodiazepines used to prepare QC D wells

\*Solutions were prepared from traceable CRMs, using balances calibrated with NIST traceable weights and Class A volumetric glassware. Material weights were corrected for salt content and concentrations are reported as free base concentrations.

Cayman Chemical certifies that this product meets the specifications stated in this certificate and warrants this product to meet the stated acceptance criteria through the expiration date when stored unopened as recommended.

Approval:  Title: Cayman Chemical ISO Quality Manager Certification Date: 12MAY2015

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#### Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std. 1	QC_Blank1	QC_A5	QC_C3	Std. 1	QC_Blank1	QC_A5	QC_C3	Std. 1	QC_Blank1	QC_A5	QC_C3
B	Std. 2	QC_Blank2	QC_B1	QC_C4	Std. 2	QC_Blank2	QC_B1	QC_C4	Std. 2	QC_Blank2	QC_B1	QC_C4
C	Std. 3	QC_Blank3	QC_B2	QC_C5	Std. 3	QC_Blank3	QC_B2	QC_C5	Std. 3	QC_Blank3	QC_B2	QC_C5
D	Std. 4	QC_Blank4	QC_B3	QC_D1	Std. 4	QC_Blank4	QC_B3	QC_D1	Std. 4	QC_Blank4	QC_B3	QC_D1
E	Std. 5	QC_A1	QC_B4	QC_D2	Std. 5	QC_A1	QC_B4	QC_D2	Std. 5	QC_A1	QC_B4	QC_D2
F	Std. 6	QC_A2	QC_B5	QC_D3	Std. 6	QC_A2	QC_B5	QC_D3	Std. 6	QC_A2	QC_B5	QC_D3
G	Std. 7	QC_A3	QC_C1	QC_D4	Std. 7	QC_A3	QC_C1	QC_D4	Std. 7	QC_A3	QC_C1	QC_D4
H	Std. 8	QC_A4	QC_C2	QC_D5	Std. 8	QC_A4	QC_C2	QC_D5	Std. 8	QC_A4	QC_C2	QC_D5

Plate contains Internal Standard in all 96 wells.

#### Quality Control Test Results

Test	Specification	Result	Meets Specification
Linearity, r <sup>2</sup>	≥ 0.990	0.9980 ± 0.0018	Y
% Recovery, QC A	≤ 25%	106.3% ± 7.8%	Y
% Recovery, QC B	≤ 25%	106.8% ± 7.6%	Y
% Recovery, QC C	≤ 25%	101.2% ± 6.9%	Y
% Recovery, QC D	≤ 25%	99.0% ± 9.1%	Y
Precision (Internal Standards), %CV	≤ 15%	5.6%	Y

Results are expressed as average values across all analytes with the exception of the Internal Standard precision. Internal Standard precision was determined by randomly selecting 5 analytes and measuring the precision of the Internal Standard responses of QC Blank wells. The result reported is the largest %CV of these results.

#### Additional Notes

**Questions?**