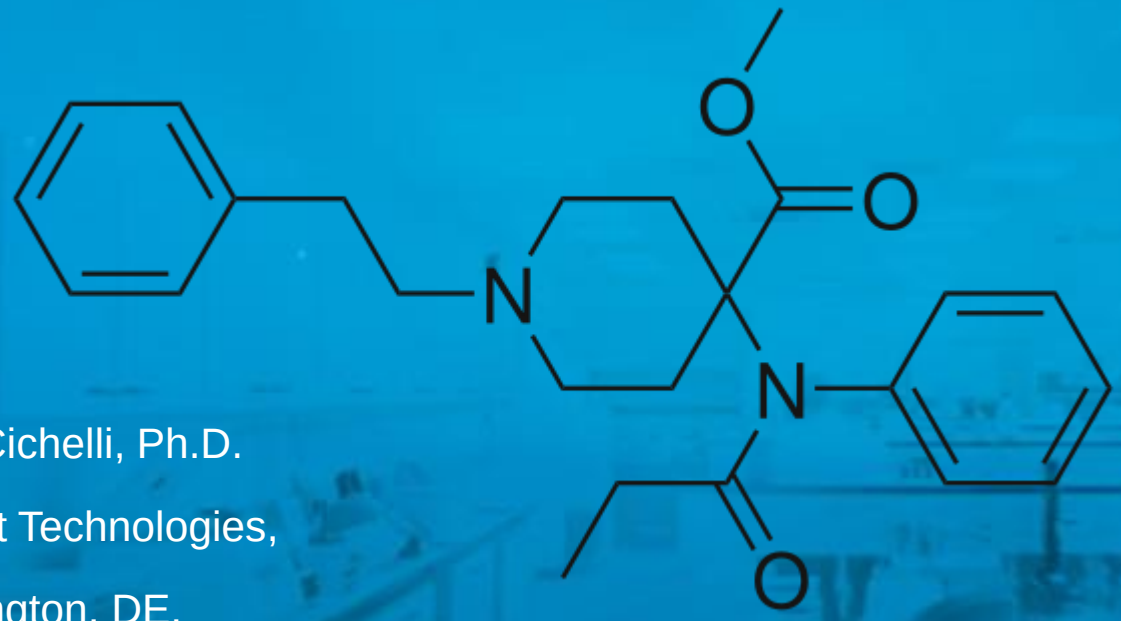
New Synthetics and New Innovations:

Synthetic fentanyl analogues and the new Agilent Ultivo Tandem Mass Spectrometer

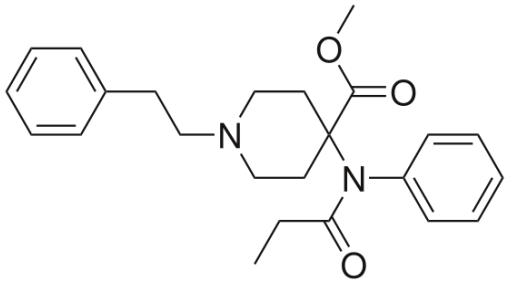


Julie Cichelli, Ph.D.

Agilent Technologies,

Wilmington, DE.

Introduction



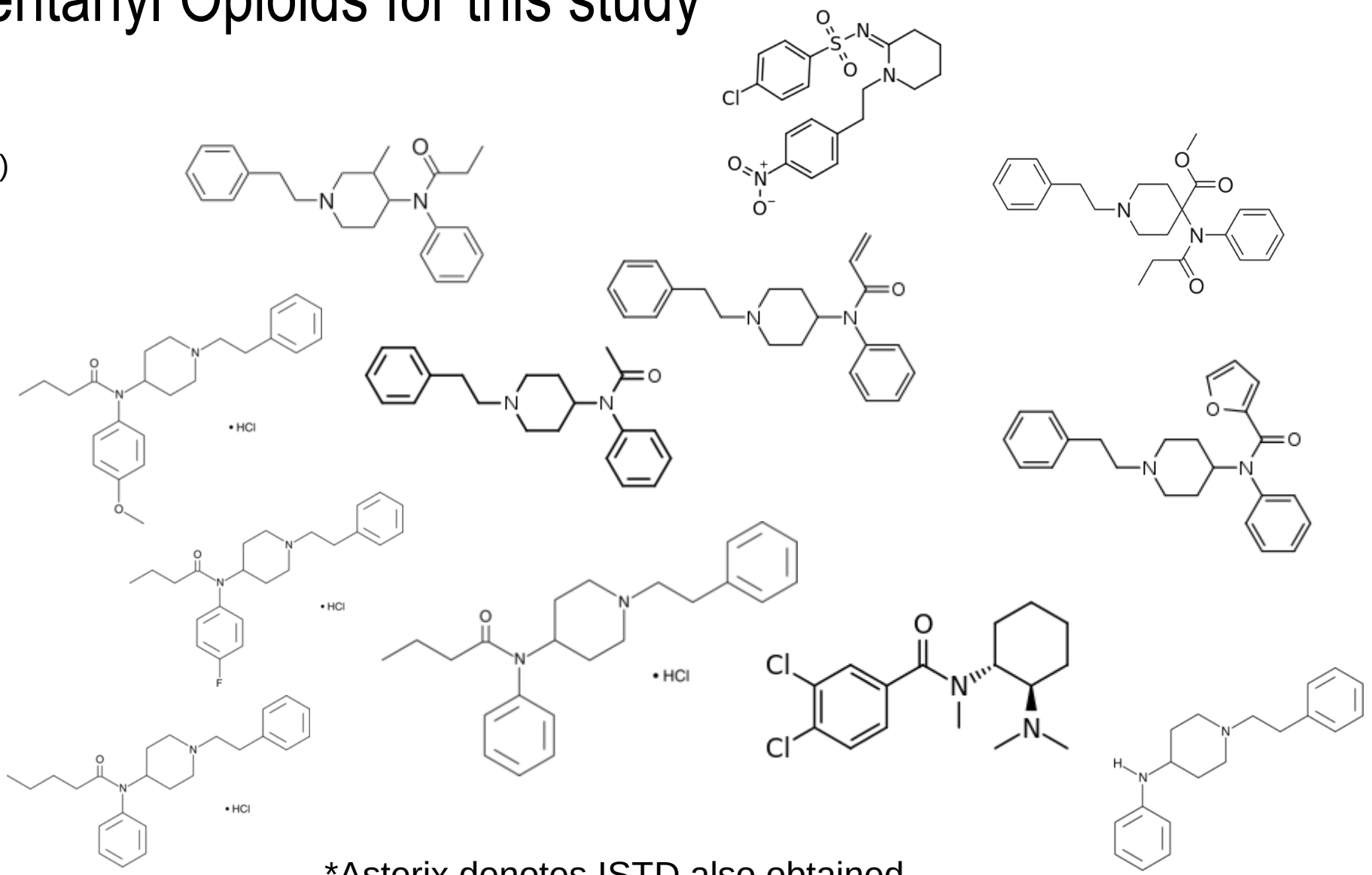
- Opioid crisis is becoming more severe, with reportedly approx. 100 deaths from OD per day across the USA.
- Intelligence suggesting that historically abused opiates such as Heroin, are being ‘cut’ with many new synthetic, more potent opioids such as Fentanyls.
- Keeping up with the new synthetic illicits and analyzing for these is difficult.
- Herein we describe a Tandem LC/MS/MS method for a list of 12x new synthetic fentanyl opioids and 4-ANPP, the precursor scheduled by the DEA.
- Details of the methodology will be presented together with results obtained from Serum and Urine matrices using the new Innovative Agilent Ultivo Tandem Mass Spectrometer.

Coming together of New Innovations, some good, some bad!

- The recent emergence of synthetic fentanyl-type opioids has let to the development and continued development of new, innovative certified standards for the forensic laboratories;
- Thankfully, we are constantly and increasingly able to accurately confirm and quantify more synthetics using such certified standards;
- Modern LC/MS Instrumentation for such analyses is also being introduced;
- Agilent Ultivo Tandem MS is such instrumentation, and therefore, it was put through this test to see how well this new innovation would prove to the new and emerging situations of the forensic toxicology community;
- The following summary will outline typical results obtained from such an application using the Agilent Ultivo TMS and will detail it's own innovations.

List of Synthetic Fentanyl Opioids for this study

- 4-ANPP* (Synthetic precursor)
- 3-methylfentanyl
- Acetylfentanyl*
- Acetylnorfentanyl*
- Acrylfentanyl*
- Butyrylfentanyl*
- Carfentanil
- Furanylfentanyl
- N-desmethyl U-47700
- Norcarfentanyl
- Para-fluorobutyrylfentanyl
- Valerylfentanyl*
- U-47700*
- **W-18 RM**



*Asterix denotes ISTD also obtained

Sample Preparation

(Standards and ISTDs obtained from Cerilliant & Cayman Chemicals)
(Serum and negative urine obtained from Golden West Biologicals)

Urine Matrix:

1. Negative Urine diluted 1/10 using deionized water;
2. 1/10 diluted negative Urine Centrifuged at 10,000 RPM through nanosep 3K filters then supernatant combined as a stock solution;
3. ISTDs Spiked into diluted negative urine stock at a concentration of 25ng/ml;
4. Serial Dilutions made for calibrators from 500ng/ml down to 1pg/ml using ISTD-spiked filtered negative Urine stock. (Equivalence dilution = 1/10)

Serum Matrix:

1. Gold Serum (10mL) mixed with 20mL of cold acetonitrile;
2. Vortexed for 2 minutes and centrifuged at 5.000 RPM for 15 minutes;
3. Supernatant (20mL) spiked with ISTD at a concentration of 50ng/ml;
4. Supernatant & ISTDs diluted with 20mL deionized water. ISTD concn. 25ng/ml.
5. Serial Dilutions made for calibrators 500ng/ml to 1pg/ml.
6. (Equivalence dilution = 1/6)

LC Conditions - 1290 UHPLC

HPLC: **Agilent 1290 Binary pump, well plate autosampler, thermostatted column compartment**

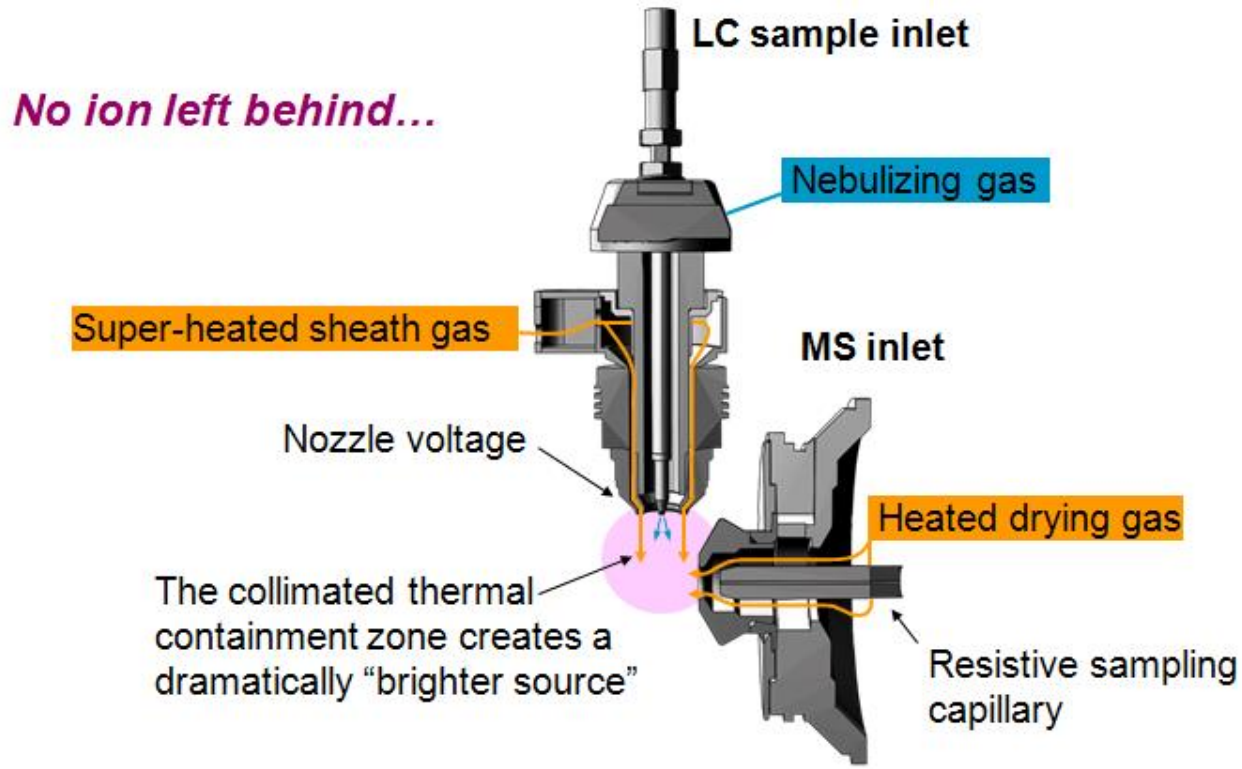
LC Conditions			
Column	Poroshell 120 EC-C18, 2.7um, 2.1x100 mm		
Column temperature	55 ºC		
Injection volume	5.0 µL (injector loop 20uL)		
Draw speed (uL/min)	100		
Eject speed (uL/min)	100		
Autosampler temperature	6 ºC		
Needle wash	5 seconds in wash port (100% methanol)		
Mobile phase	A = 5mM Ammonium Formate/0.01% Formic acid in water B = 0.01% formic acid in Methanol		
Gradient program	Time 0.00 0.50 3.50 5.50 6.00	B (%) 10 15 50 95 95	Flow rate (mL/min) 0.500 0.500 0.500 0.500 0.500
Stop time	6.0 min		
Post time	1,0 min		

MS Conditions- Agilent Ultivo TMS

Instrument and Source conditions:

Parameters	
Ion mode	AJS ESI, positive
Gas temperature	325 ºC
Drying gas flow	9 L/min
Nebulizer gas	35 psi
Sheath gas temperature	350ºC
Sheath gas flow	11 L/min
Capillary voltage	3500 V
Nozzle voltage	0 V

MRM Parameters	
Delta EMV	N/A
Q1 and Q3 resolution (amu)	0.7/0.7 (unit/unit)
Fragmentor Voltage	Various – See MRM Table
Cell Accelerator voltage	9 V
Cycle time	400 msec
Total MRMs	38
Dwell Time each MRM Transition	10 msec



MS Conditions (Contd.) - Agilent Ultivo TMS

MRM Instrument Settings:

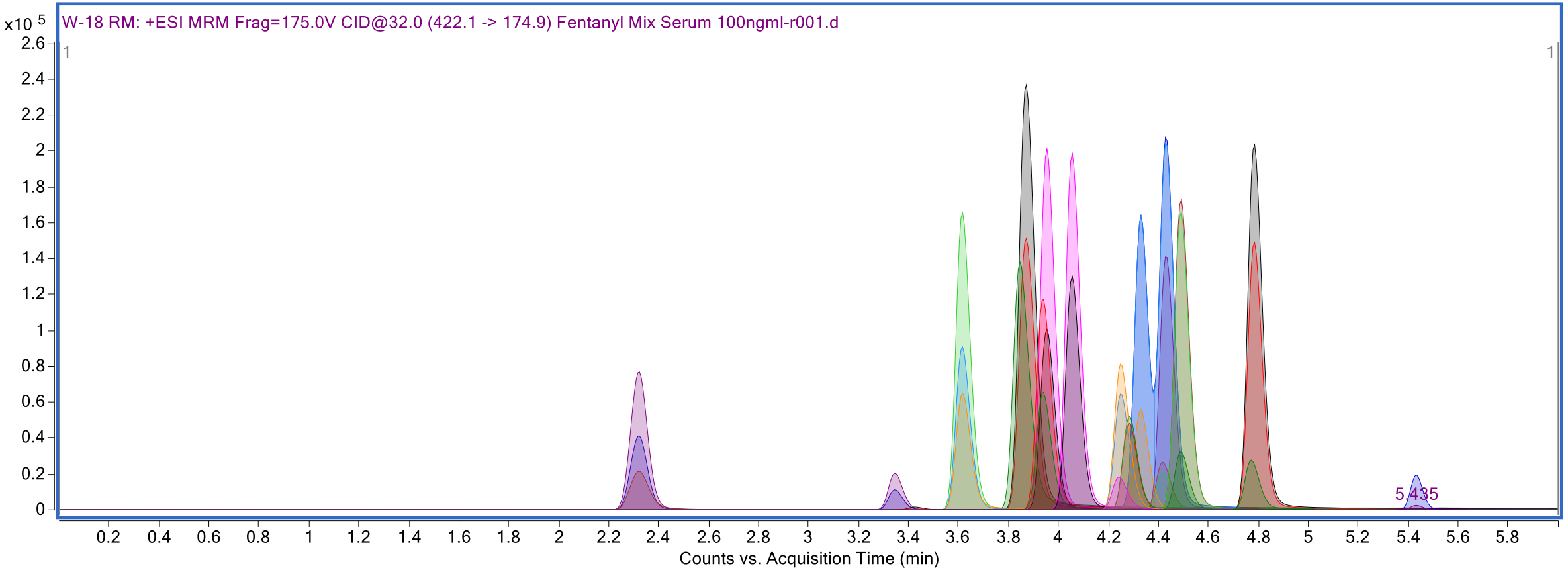
	Compound	Compound Name	ISTD	Precursor Ion	MS1 Resolution	Product Ion	MS2 Resolution	Dwell	Fragmentor	CAV	Collision Energy	Polarity
1		W-18 RM	No	422.1 m/z	Unit	174.9 m/z	Unit	10 ms	175 V	9 V	32 V	+
2		W-18 RM	No	422.1 m/z	Unit	110.9 m/z	Unit	10 ms	175 V	9 V	56 V	+
3		Furanyl fentanyl	No	375.2 m/z	Unit	188.1 m/z	Unit	10 ms	170 V	9 V	24 V	+
4		Furanyl fentanyl	No	375.2 m/z	Unit	105 m/z	Unit	10 ms	170 V	9 V	48 V	+
5		Valeryl fentanyl-D5	Yes	370.3 m/z	Unit	105 m/z	Unit	10 ms	180 V	9 V	48 V	+
6		para-fluorobutyrylfentanyl	No	369.2 m/z	Unit	188.1 m/z	Unit	10 ms	180 V	9 V	24 V	+
7		para-fluorobutyrylfentanyl	No	369.2 m/z	Unit	105 m/z	Unit	10 ms	180 V	9 V	52 V	+
8		Valeryl fentanyl	No	365.3 m/z	Unit	188.1 m/z	Unit	10 ms	180 V	9 V	24 V	+
9		Valeryl fentanyl	No	365.3 m/z	Unit	105 m/z	Unit	10 ms	180 V	9 V	48 V	+
10		Butyrylfentanyl-D5	Yes	356.3 m/z	Unit	188.1 m/z	Unit	10 ms	180 V	9 V	24 V	+
11		Butyrylfentanyl-D5	Yes	356.3 m/z	Unit	105.1 m/z	Unit	10 ms	180 V	9 V	48 V	+
12		cis-3-methyl fentanyl	No	351.2 m/z	Unit	202.1 m/z	Unit	10 ms	180 V	9 V	24 V	+
13		Butyrylfentanyl	No	351.2 m/z	Unit	188.1 m/z	Unit	10 ms	180 V	9 V	24 V	+
14		Butyrylfentanyl	No	351.2 m/z	Unit	105 m/z	Unit	10 ms	180 V	9 V	48 V	+
15		cis-3-methyl fentanyl	No	351.2 m/z	Unit	105 m/z	Unit	10 ms	180 V	9 V	48 V	+
16		Acrylfentanyl-D5	Yes	340.2 m/z	Unit	188.1 m/z	Unit	10 ms	165 V	9 V	24 V	+
17		Acrylfentanyl-D5	Yes	340.2 m/z	Unit	105 m/z	Unit	10 ms	165 V	9 V	44 V	+
18		U-47700-D6	Yes	335.2 m/z	Unit	284 m/z	Unit	10 ms	120 V	9 V	16 V	+
19		Acrylfentanyl	No	335.2 m/z	Unit	188.1 m/z	Unit	10 ms	165 V	9 V	24 V	+
20		Acrylfentanyl	No	335.2 m/z	Unit	105 m/z	Unit	10 ms	165 V	9 V	44 V	+

MS Conditions (Contd. 2) - Agilent Ultivo TMS

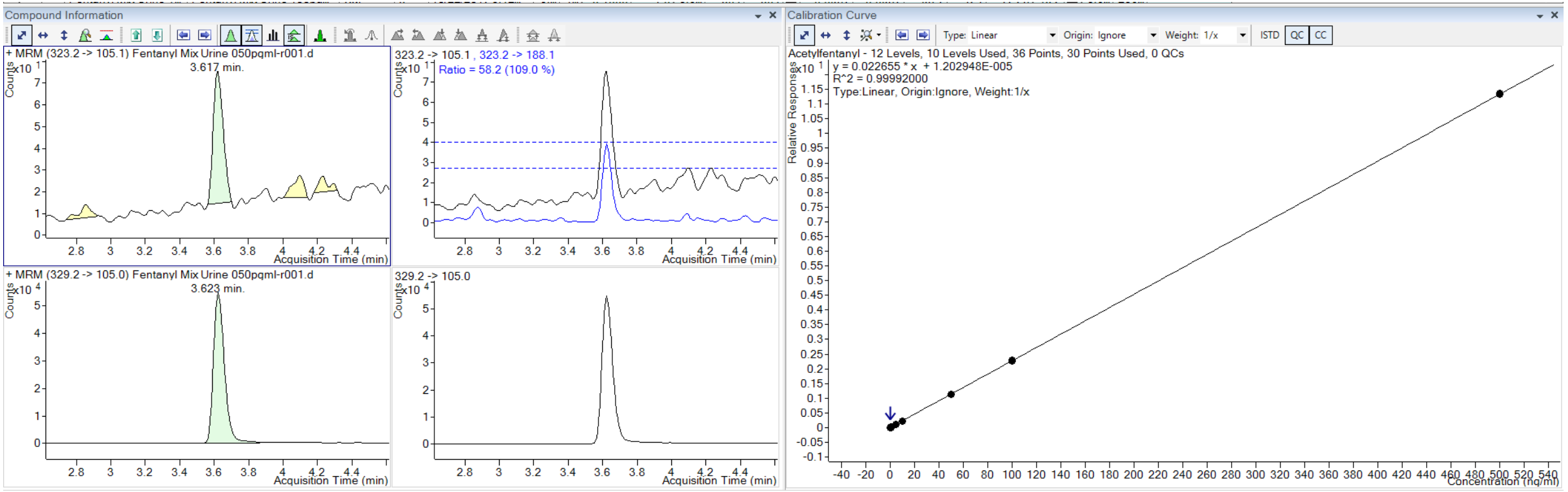
MRM Instrument Settings:

21		Acetylfentanyl-13C6	Yes	329.2 m/z	Unit	105 m/z	Unit	10 ms	170 V	9 V	44 V	+
22		U-47700	No	329.1 m/z	Unit	284 m/z	Unit	10 ms	120 V	9 V	16 V	+
23		U-47700	No	329.1 m/z	Unit	172.9 m/z	Unit	10 ms	120 V	9 V	36 V	+
24		Acetylfentanyl	No	323.2 m/z	Unit	188.1 m/z	Unit	10 ms	170 V	9 V	24 V	+
25		Acetylfentanyl	No	323.2 m/z	Unit	105.1 m/z	Unit	10 ms	170 V	9 V	44 V	+
26		N-desmethyl U-47700	No	315.1 m/z	Unit	284 m/z	Unit	10 ms	120 V	9 V	16 V	+
27		N-desmethyl U-47700	No	315.1 m/z	Unit	172.9 m/z	Unit	10 ms	120 V	9 V	36 V	+
28		Norcarfentanil	No	291.2 m/z	Unit	231.1 m/z	Unit	10 ms	95 V	9 V	12 V	+
29		Norcarfentanil	No	291.2 m/z	Unit	142 m/z	Unit	10 ms	95 V	9 V	16 V	+
30		4-ANPP-D5	Yes	286.2 m/z	Unit	105.1 m/z	Unit	10 ms	140 V	9 V	36 V	+
31		4-ANPP	No	281.2 m/z	Unit	188.1 m/z	Unit	10 ms	140 V	9 V	16 V	+
32		4-ANPP	No	281.2 m/z	Unit	105 m/z	Unit	10 ms	140 V	9 V	36 V	+
33		Acetyl norfentanyl-13C6	Yes	225.2 m/z	Unit	84.1 m/z	Unit	10 ms	120 V	9 V	20 V	+
34		Acetyl norfentanyl	No	219.1 m/z	Unit	84.1 m/z	Unit	10 ms	120 V	9 V	20 V	+
35		Acetyl norfentanyl	No	219.1 m/z	Unit	55.2 m/z	Unit	10 ms	120 V	9 V	44 V	+
36		Carfentanil-D5	Yes	400.3 m/z	Unit	113 m/z	Unit	10 ms	130 V	9 V	36 V	+
37		Carfentanil	No	395.2 m/z	Unit	335.2 m/z	Unit	10 ms	135 V	9 V	16 V	+
38		Carfentanil	No	395.2m/z	Unit	113 m/z	Unit	10 ms	135 V	9 V	36 V	+

Overlaid EIC of each Fentanyl Opioid Transition
(Serum Mix Calibrant 100ng/ml)



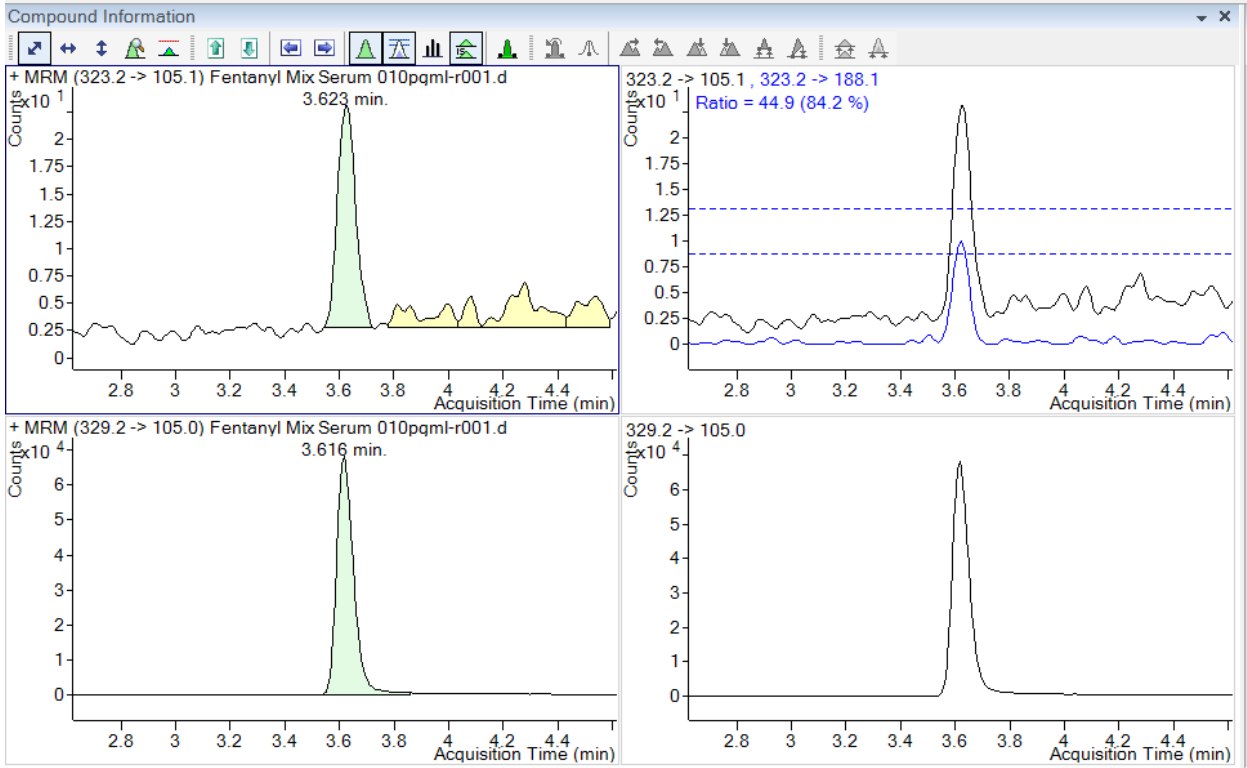
Typical Results – acetyl fentanyl in Urine (1pg/ml→500ng/ml)

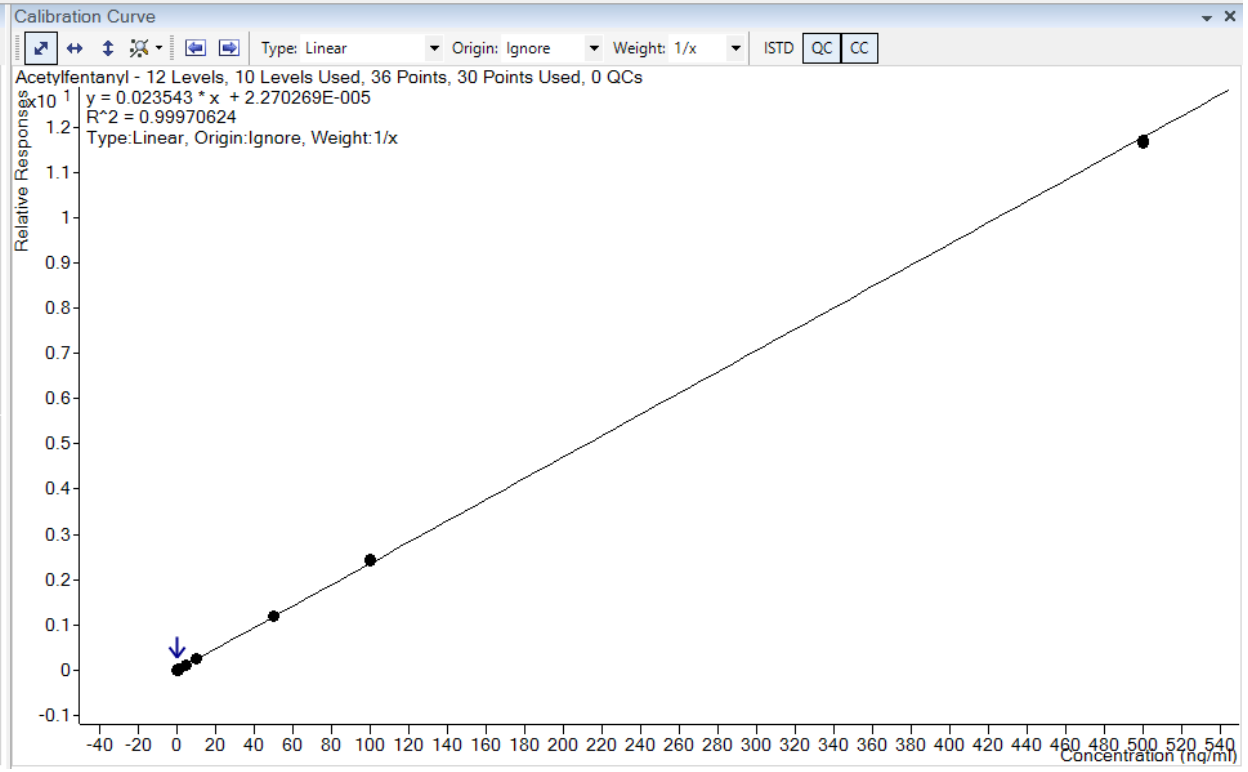


LLOQ of 50pg/mL

R^2=0.9999, N=3

Typical Results – acetyl fentanyl in Serum (1pg/ml→500ng/ml)

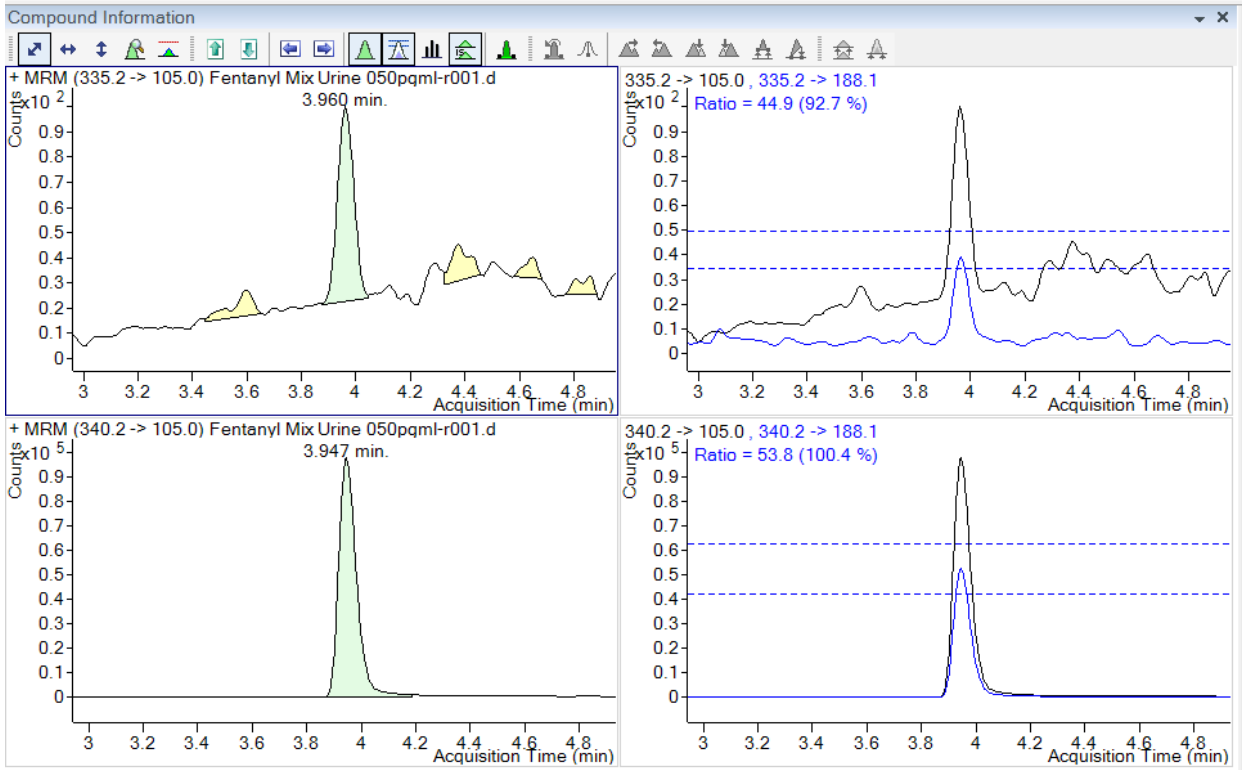


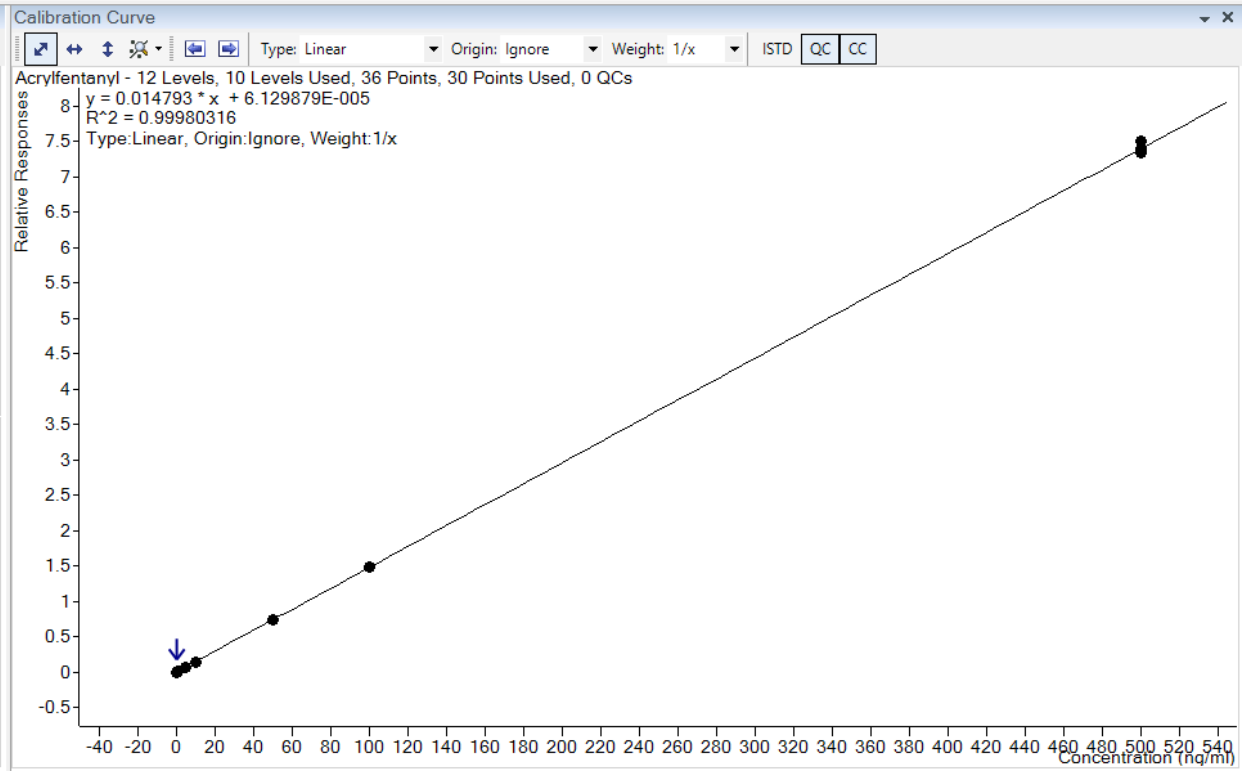


LLOQ of 10pg/mL

R^2=0.9997, N=3

Typical Results – acrylfentanyl in Urine (1pg/ml→500ng/ml)

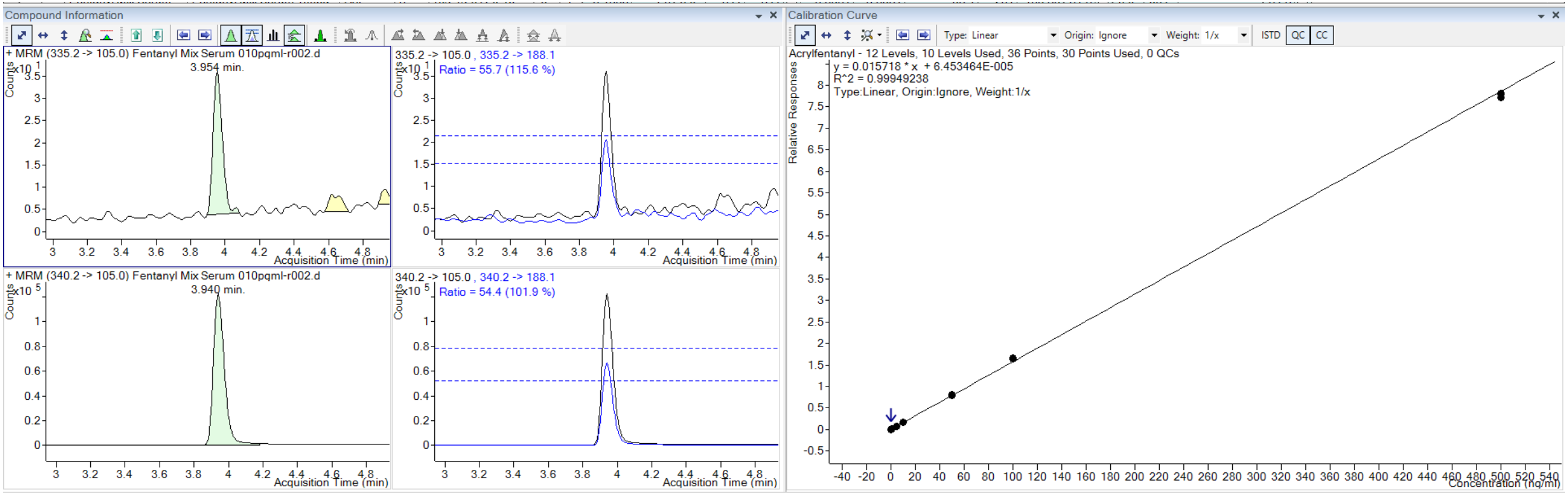




LLOQ of 50pg/mL

R^2=0.9998, N=3

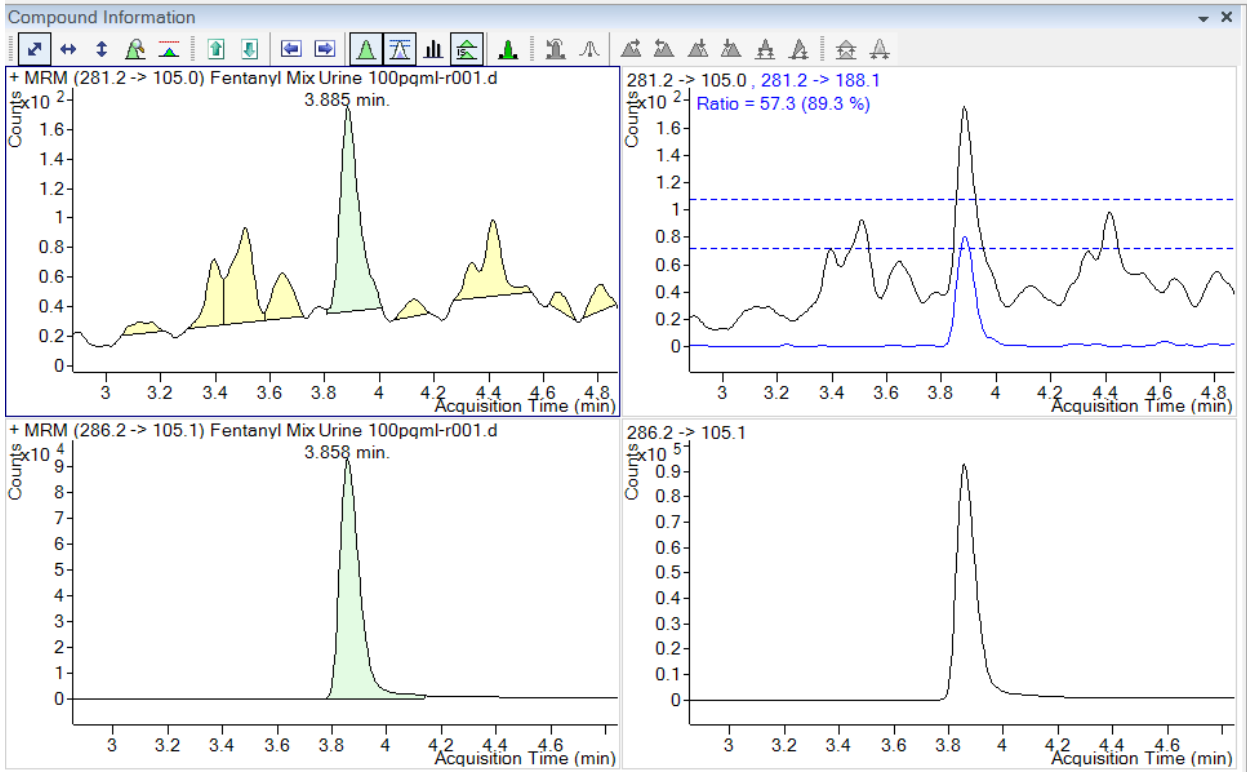
Typical Results – acrylfentanyl in Serum (1pg/ml→500ng/ml)

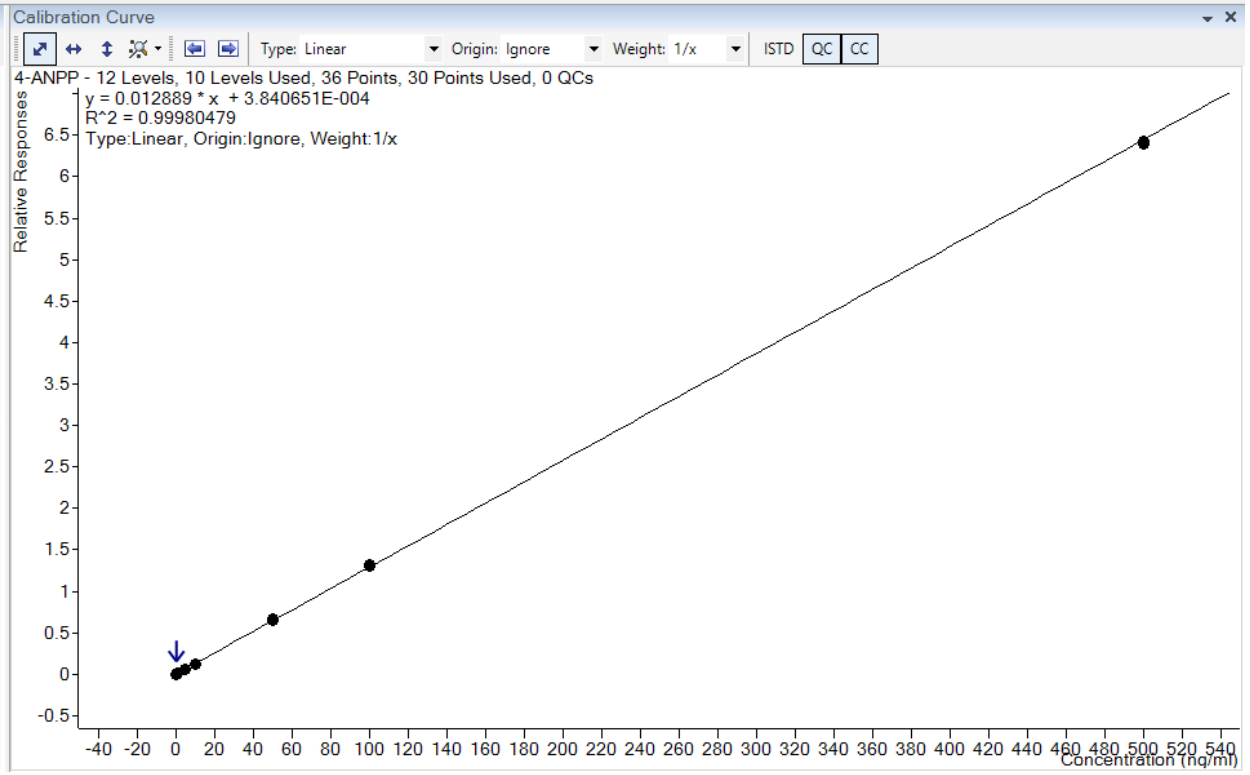


LLOQ of 10pg/mL

R^2=0.9994, N=3

Typical Results – 4-ANPP in Urine (1pg/ml→500ng/ml)

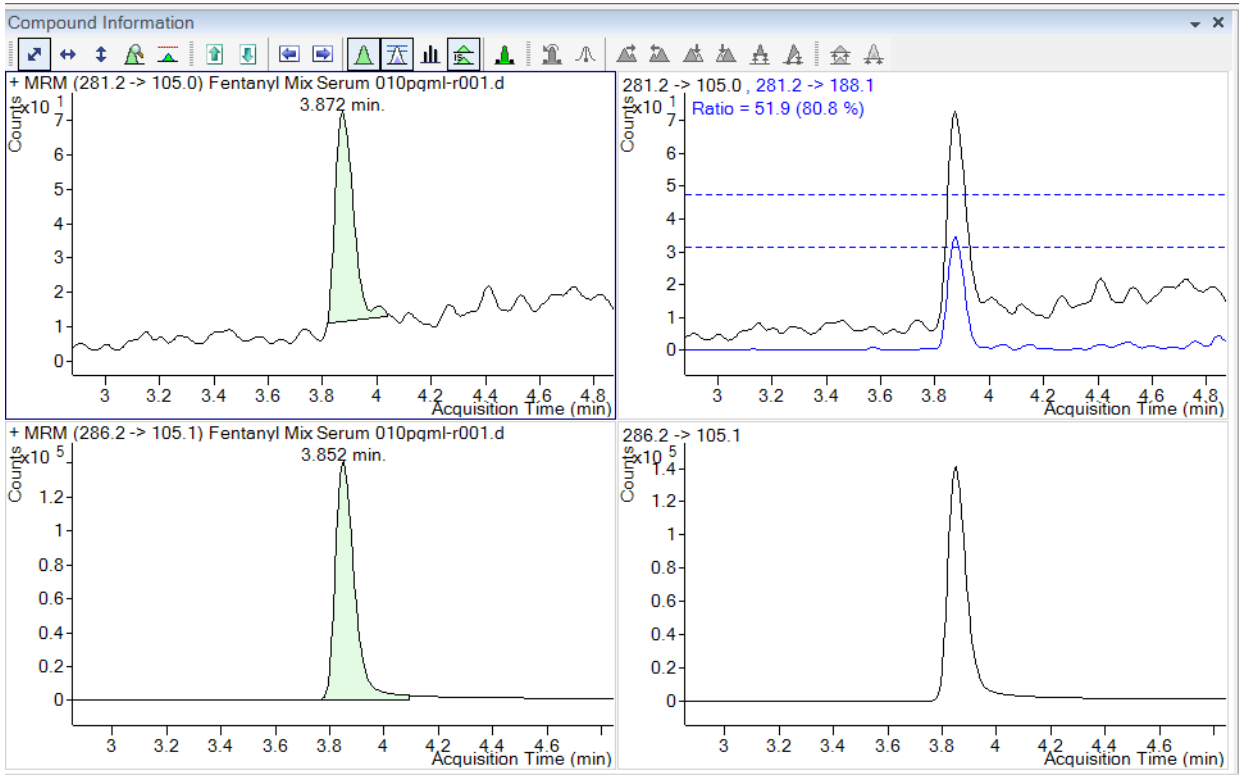


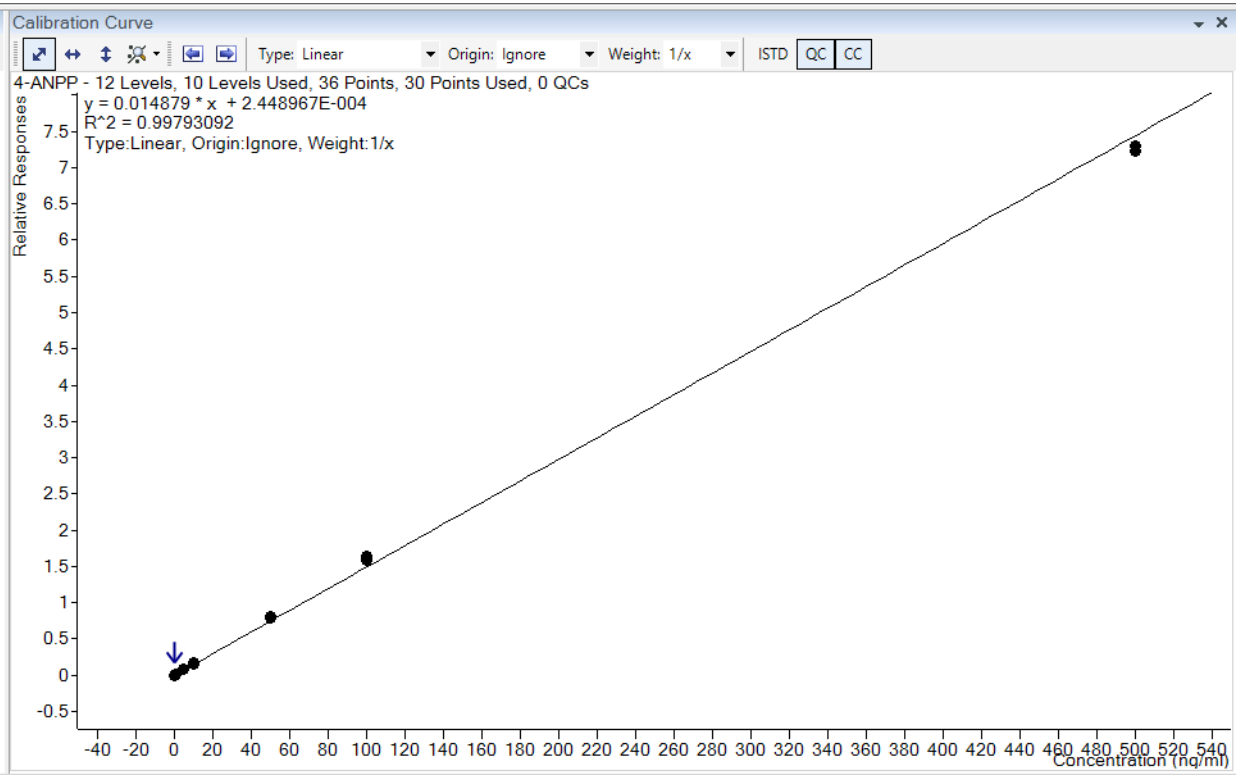


LLOQ of 100pg/mL

R^2=0.9998, N=3

Typical Results – 4-ANPP in Serum (1pg/ml→500ng/ml)

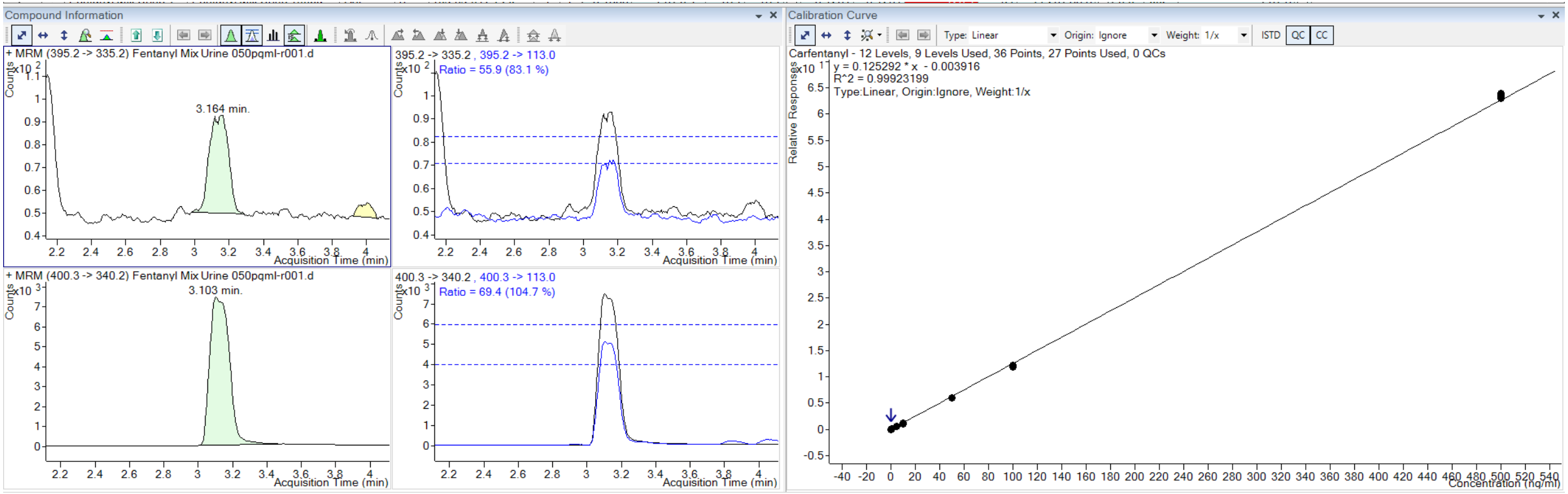




LLOQ of 10pg/mL

R^2=0.9979, N=3

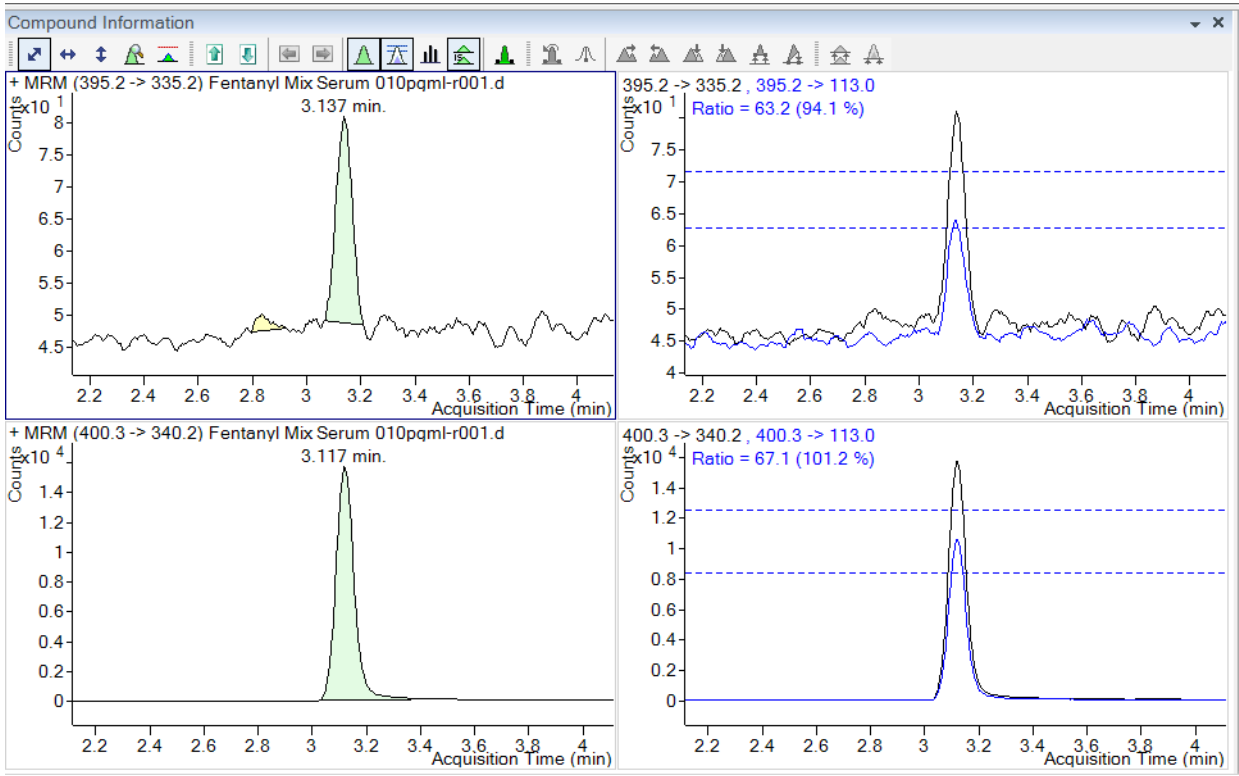
Typical Results – Carfentanyl in Urine (1pg/ml→500ng/ml)

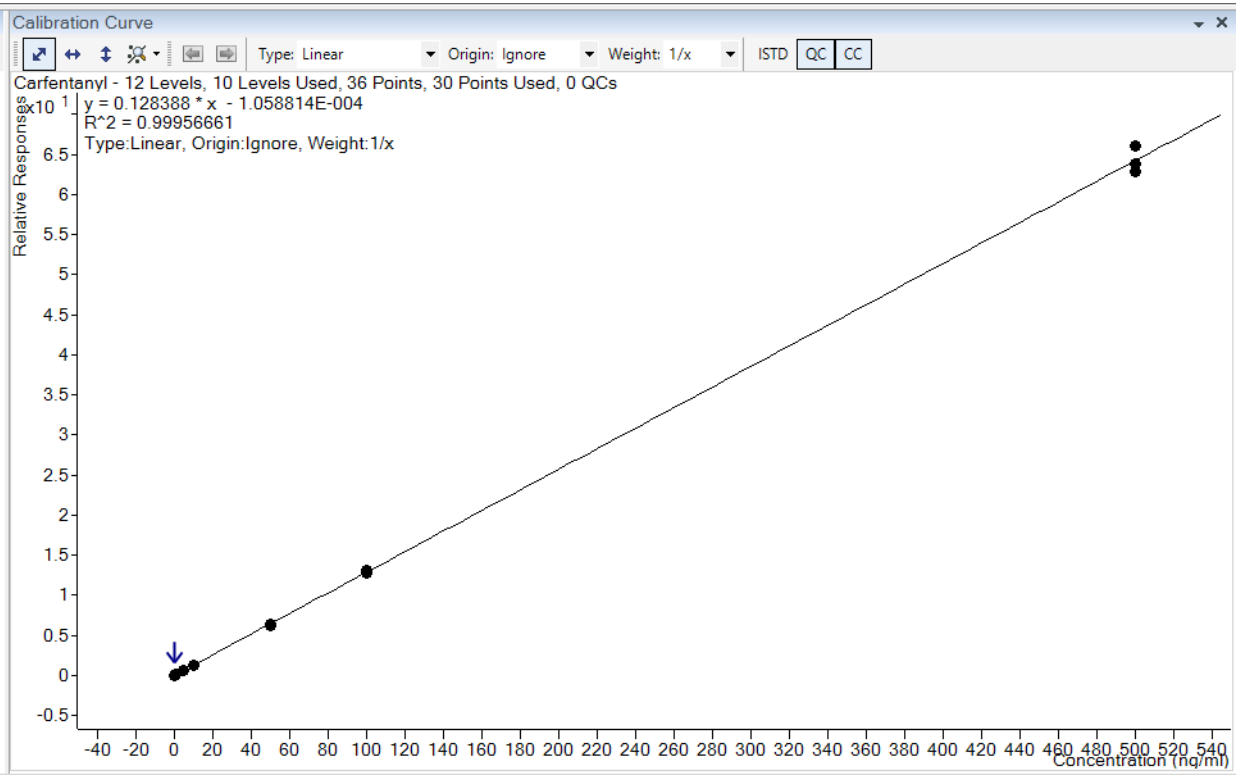


LLOQ of 50pg/mL

R^2=0.9992, N=3

Typical Results – Carfentanyl in Serum (1pg/ml→500ng/ml)





LLOQ of 10pg/mL

R^2=0.9995, N=3

LLOQ Results Table for Fentanyl synthetics – Agilent Ultivo TMS

Analyte	Actual LLOQ (Urine) pg/ml	Actual LLOQ (Serum) pg/ml	Equivalence LLOQ (Urine) pg/ml (1/10 dilution)	Equivalence LLOQ (Serum) pg/ml (1/6 dilution)
4-ANPP	100	10	1000	60
3-methylfentanyl	100	50	1000	300
Acetylfentanyl	50	10	500	60
Acetylnorfentanyl	100	50	1000	300
Acrylfentanyl	50	10	500	60
Butyrylfentanyl	100	50	1000	300
Carfentanil	50	10	500	60
Furanylfentanyl	50	50	500	300
Para-fluorobutyrylfentanyl	50	50	500	300
Norcarfentanyl	100	50	1000	300
N-desmethyl U-47700	100	50	1000	300
Valerylfentanyl	100	50	1000	300
U-47700	100	50	1000	300
W-18 RM	50	50	500	300

*Equivalence refers to the actual concentration of the Urine or Serum prior to dilution

Innovations: Ultivo Triple Quad LC/MS

AGILENT IS RESHAPING THE FUTURE OF MASS SPECTROMETRY.

JOIN US AND WELCOME IN A NEW ERA OF "FIT FOR PURPOSE" MASS SPECTROMETRY.

Typical LC/TQ Lab Layout

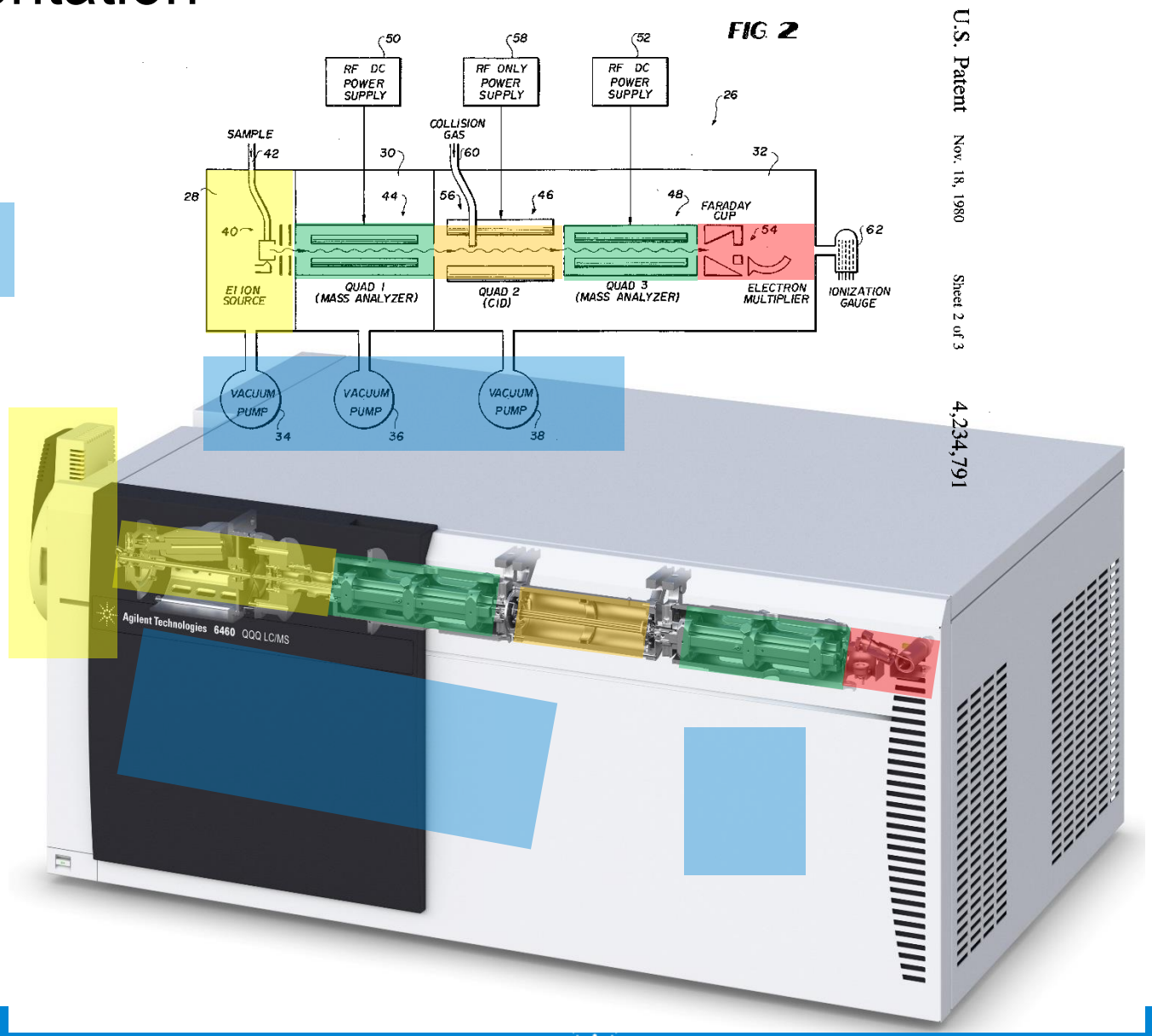
6 feet
1.83 meters

LC/TQ Instrumentation

Vacuum ~10^{-5} torr

Source &
Ion Transfer Optics

Quadrupoles

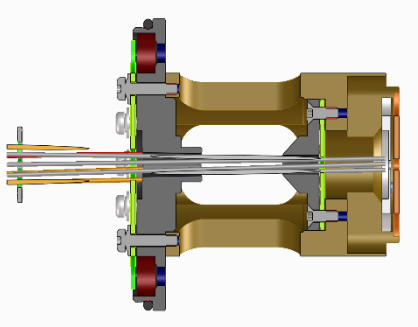


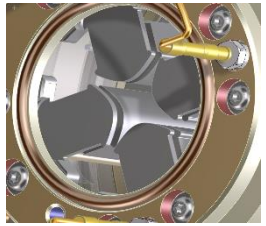
Collision Cell

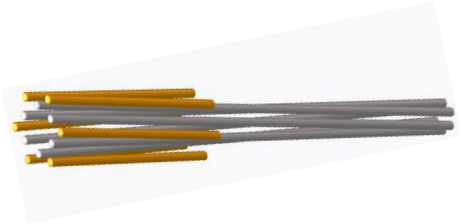
Detector

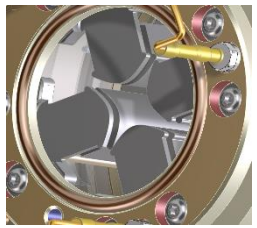
Electronics

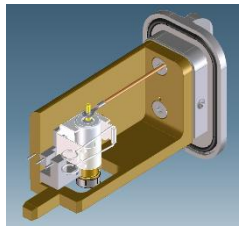
Ultivo Ion Guide, Mass Analyzer and Detector

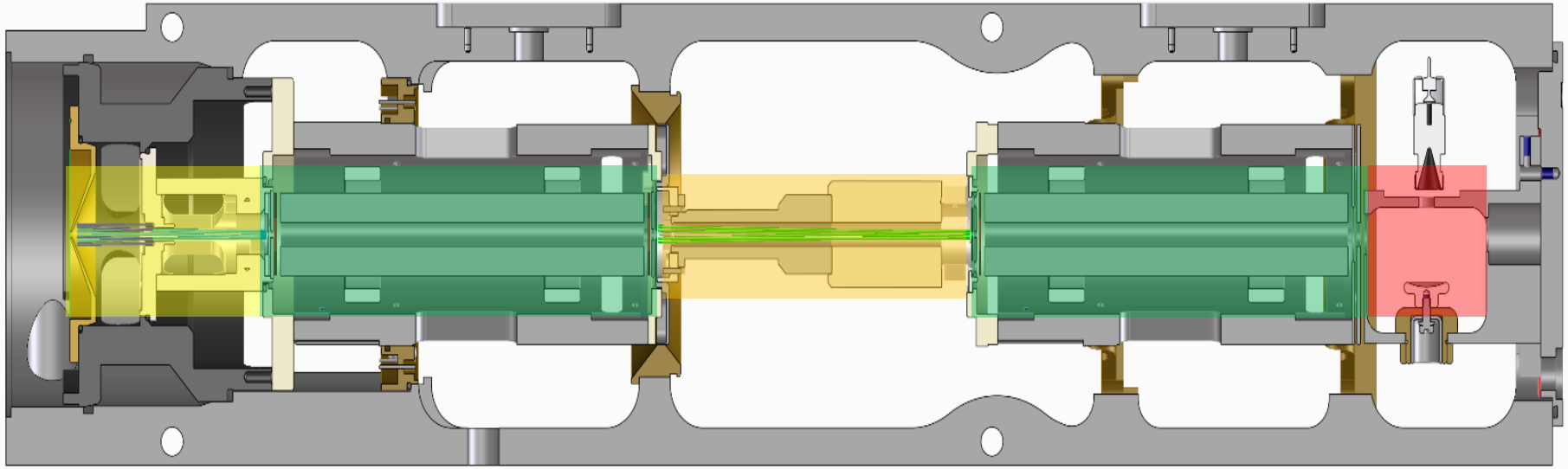




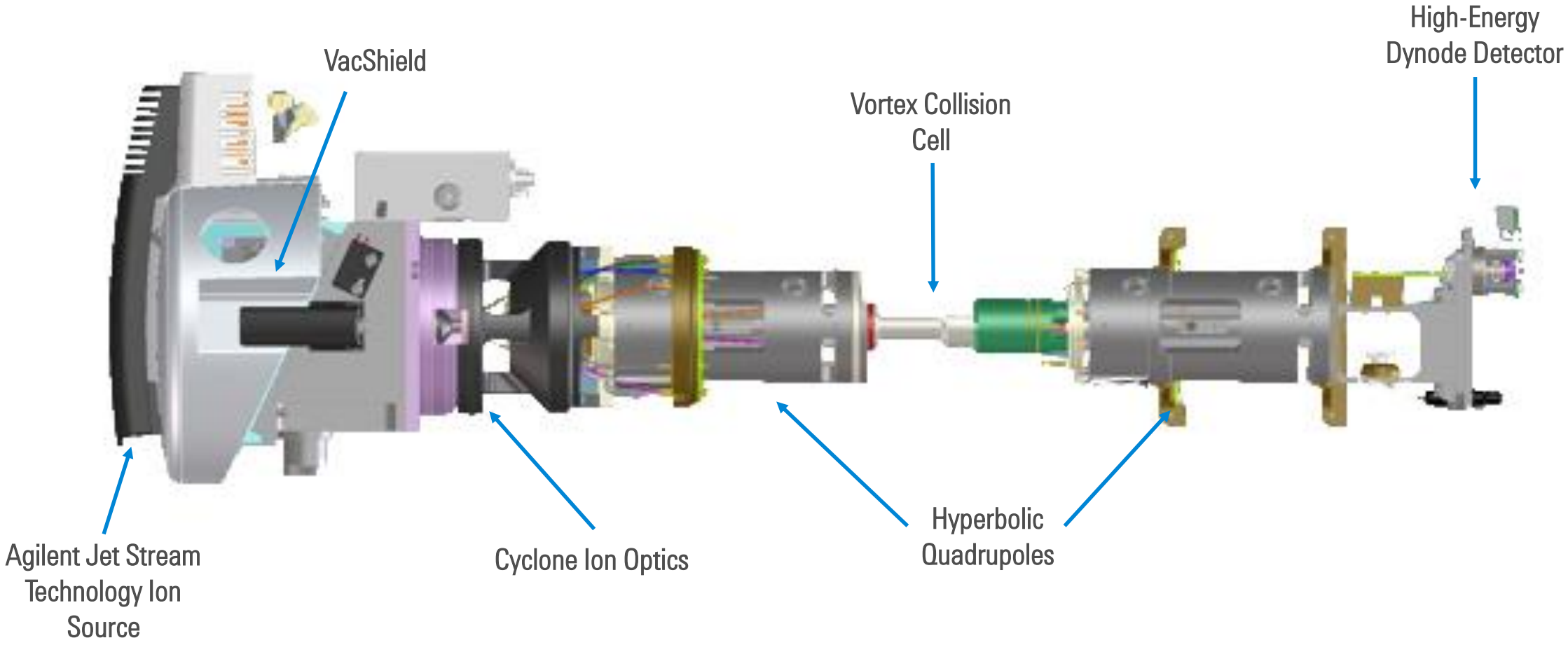








ULTIVO: Designed for Robust Performance

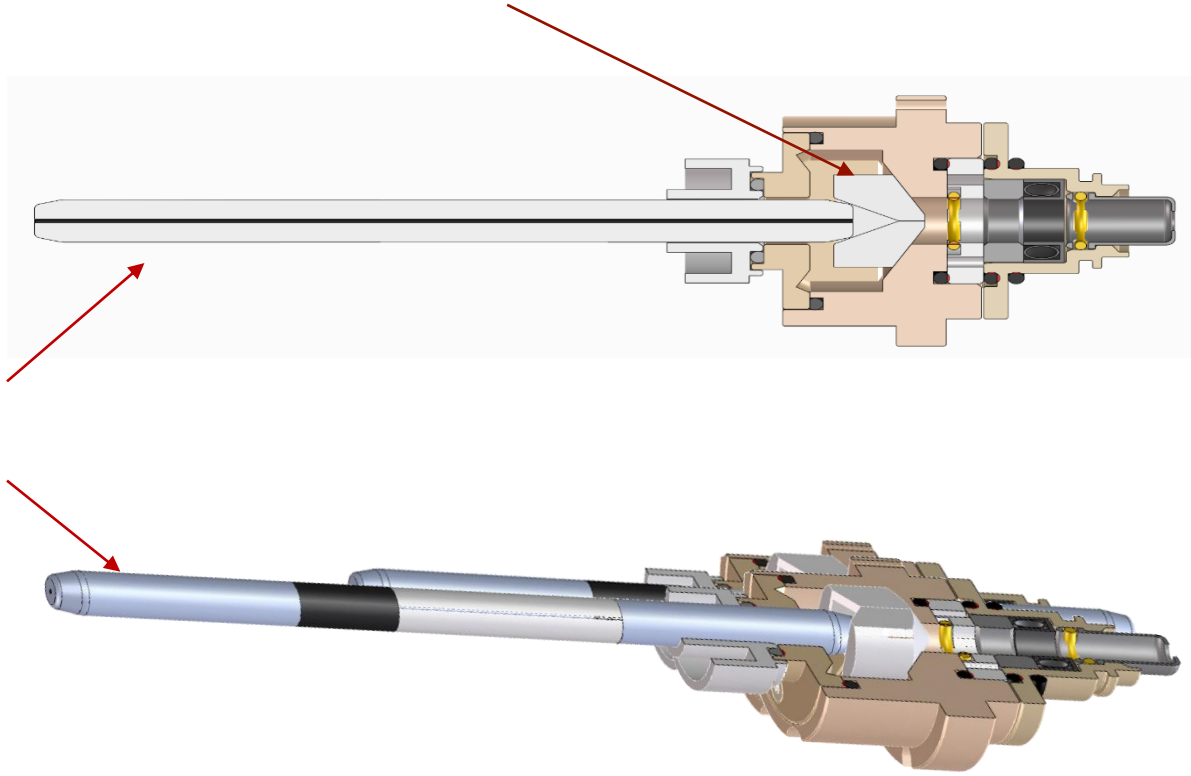


Ultivo: VacShield

- Fast, ion injector exchange without system venting
- Easy-to-use, increased throughput, less downtime for maintenance

Ion Injector Puller Tool

VacShield




Agilent Technologies

Pre MS1 Ion Beam Compressor – *"Cyclone Ion Guide"*

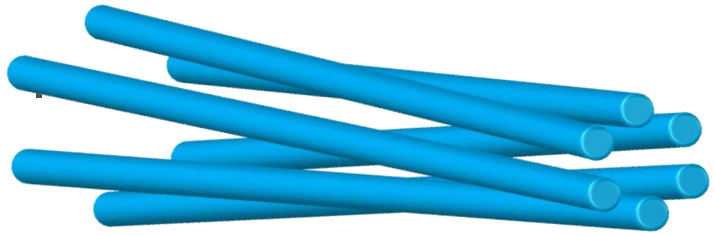
Shorter than octopole.

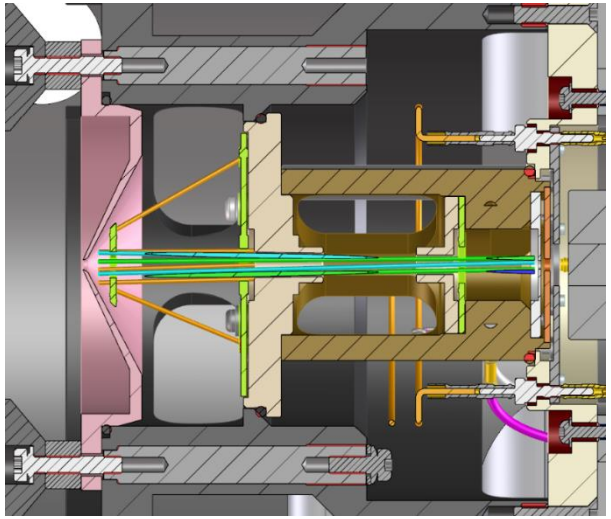
Transport ions through multiple Vacuum stages

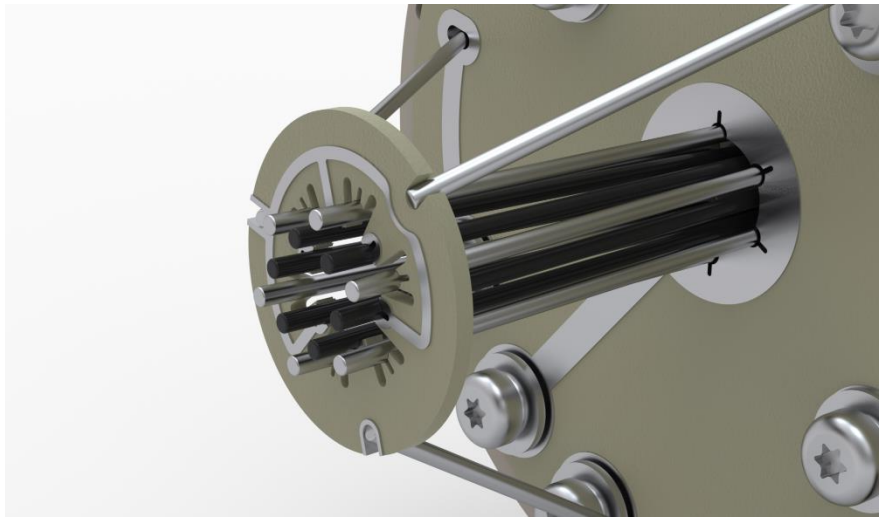
Transport Ions with minimal ion losses

Twisted and tapered

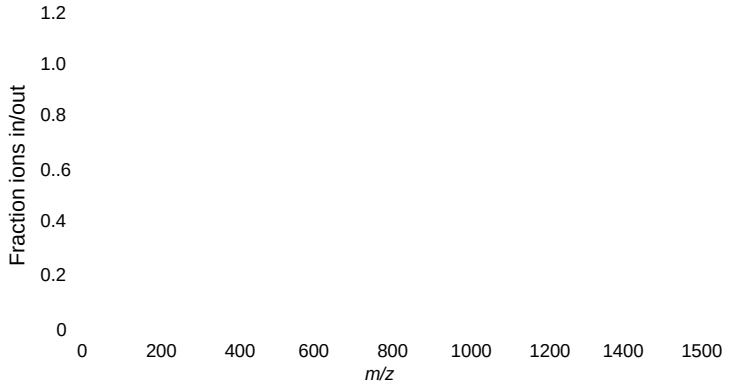
Reduce ion beam size



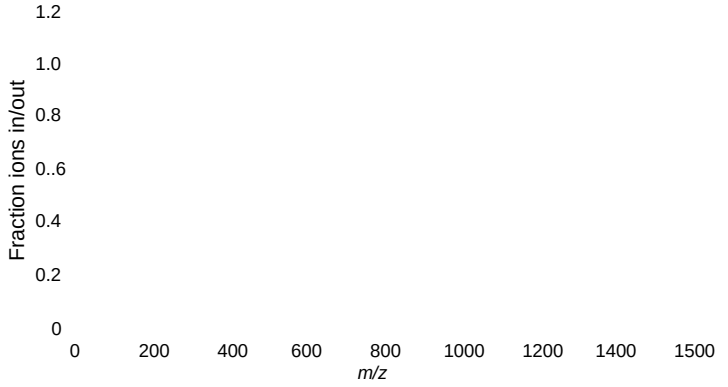




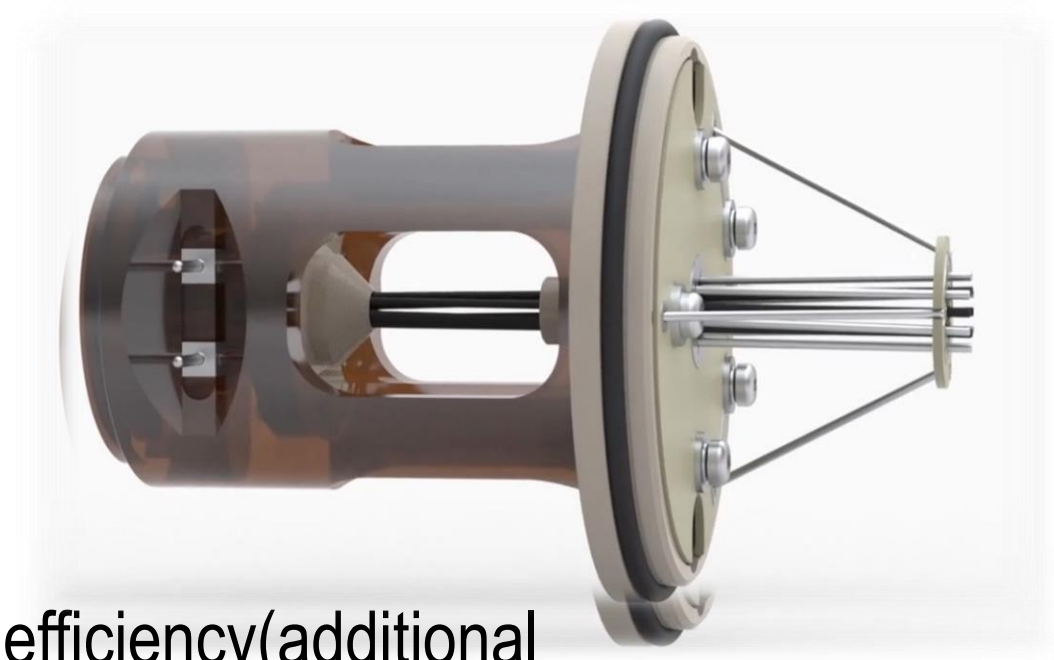
Cyclone Transmission vs *m/z* – outer rods off



Cyclone Transmission vs *m/z* – outer rods on



Benefits of Cyclone Ion Guide

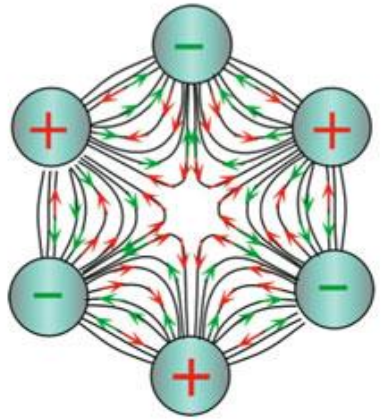


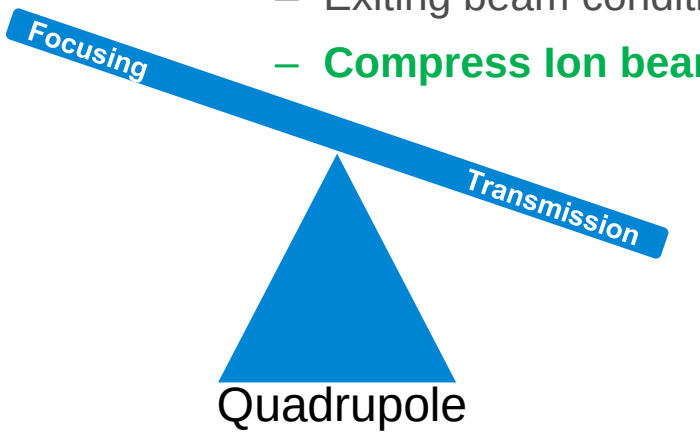
- Twisted, tapered (2) hexapole set
  - Convergence section – greater ion acceptance
  - Outer rods – increased high mass transmission efficiency(additional confinement)
  - Resistive rods – DC bias to gently pull ions towards the exit
  - Parallel section – superior collimation of the ion beam
  - ***More ions, increased signals, better results***


Agilent Technologies

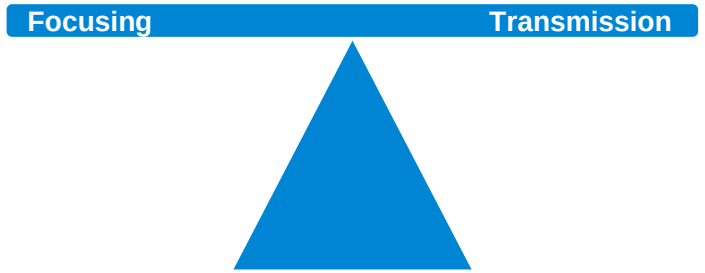
Traditional Collison Cell

- Consists of **multiple** parallel rods held at alternating positive or negative voltage
- Controls orientation before electron transfer
- Uses gas (N_2, argon) to stimulate collisions
- Design criteria:
  - Simple and **reliable** design
  - High ion transmission - **Improved**
  - Broad mass range transmission - **Improved**
  - Short ion transmission time - **Improved**
  - Exiting beam conditions indistinguishable between MS and MS/MS
  - **Compress Ion beam for improved Q2 acceptance**

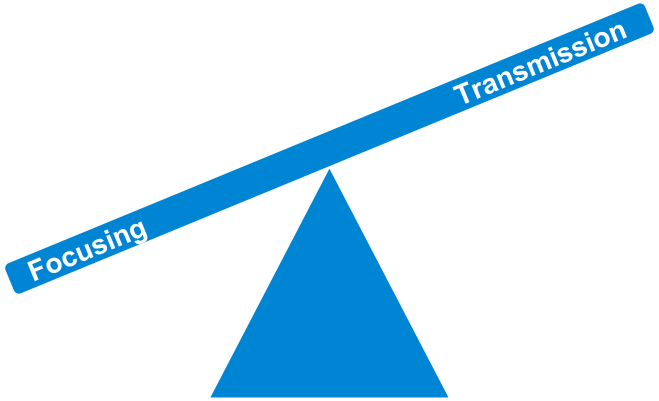




Quadrupole



Hexapole



Octopole

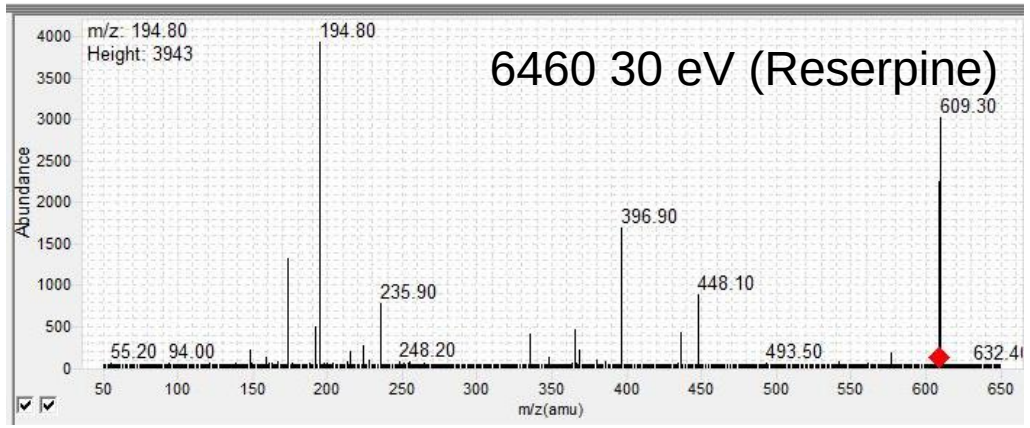
Collision Cell Results

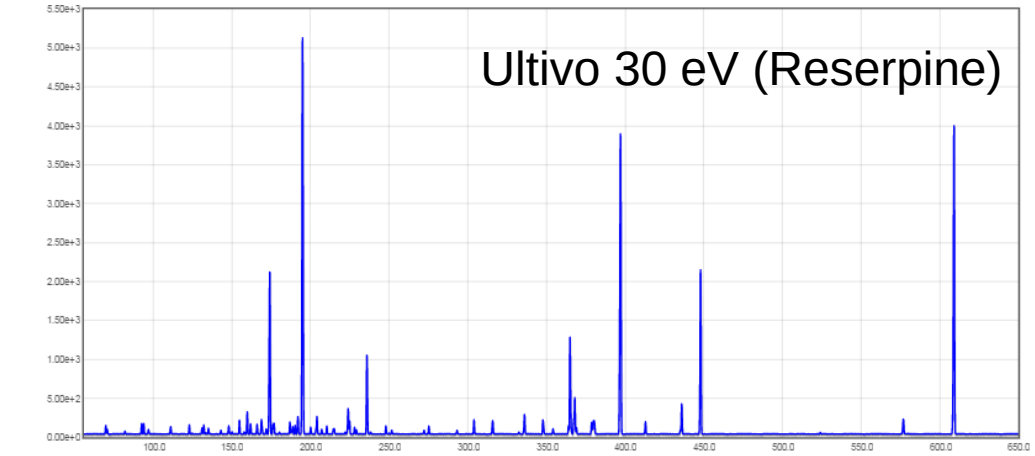
Significantly shorter (60 mm less) collision cell affords reduced bench space

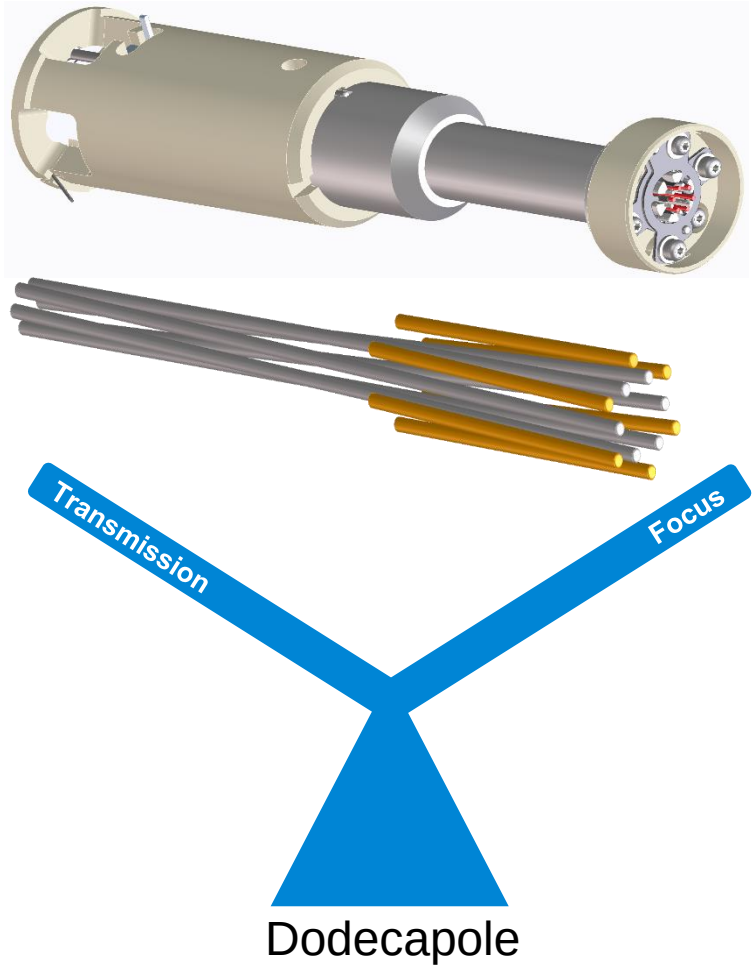
Compresses ion beam to improve transmission into smaller diameter quads

Operates at multifrequency for broad range transmission

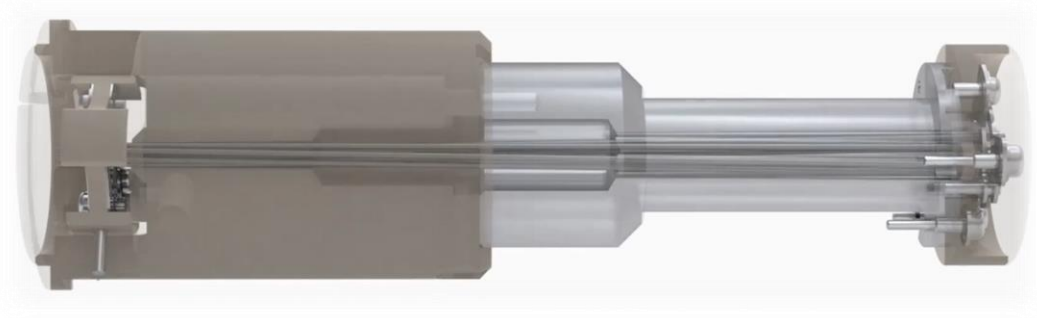
Nearly equivalent MS/MS spectra compared to 6420/60







Benefits of Vortex Collision Cell



- Twisted, tapered hexapole
  - Taper – maximum ion collection from Q1 and collimates ion beam for transmission to Q2
  - Twist – optimal pressure and fragmentation of ions at front, higher pressure for cooling and compression of ion in the middle
  - Crosstalk is prevent by applying a DC-bias to resistive rods (more kinetic energy) to prevent stalling
  - Openings at end enable a fast pressure drop, while ions are confined (prevents scattering before entry into Q2)
  - ***Optimum Ion Transmission and better MS/MS performance***


Agilent Technologies

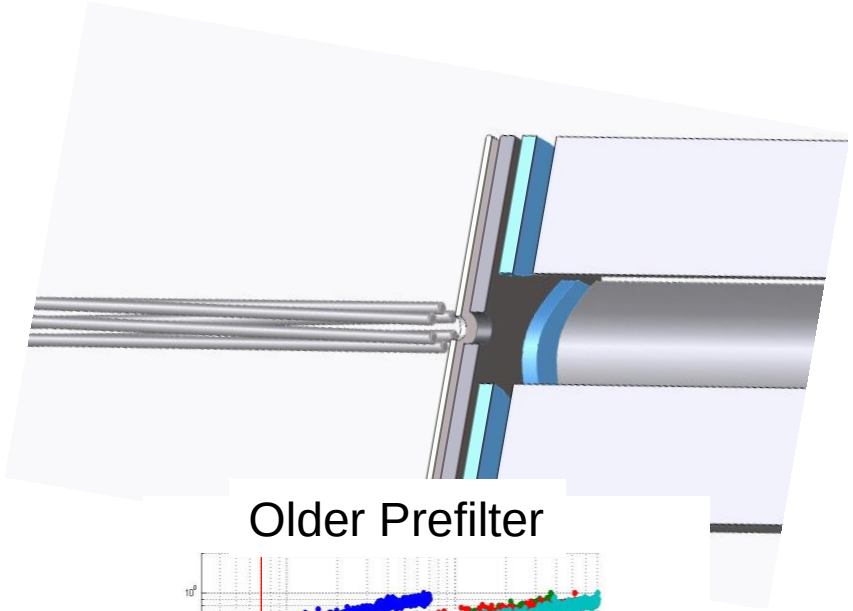
Virtual Pre/Post Filters

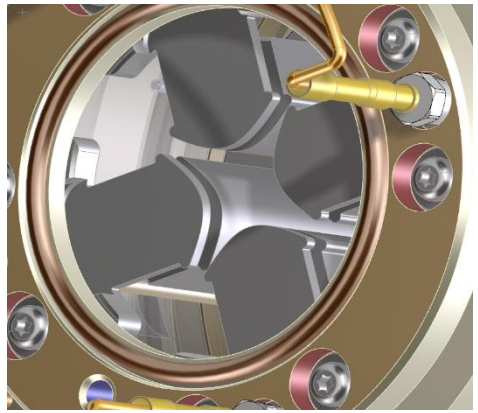
20x thinner than traditional pre/post filters

Reversed polarity U+/U- DC only, no RF required.

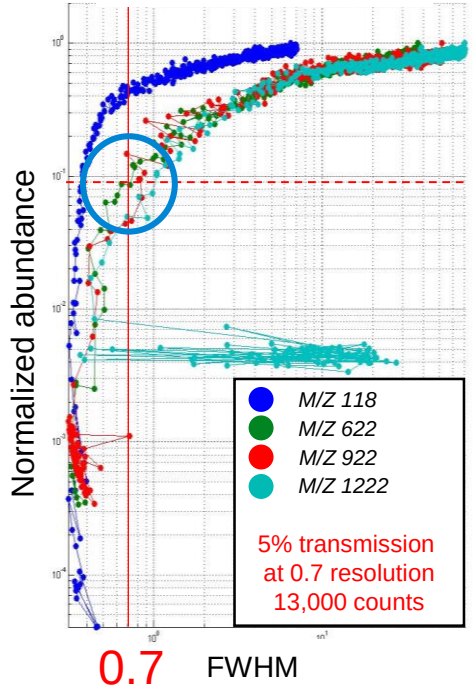
Noding is eliminated

Consistent tuning with improved instrument performance

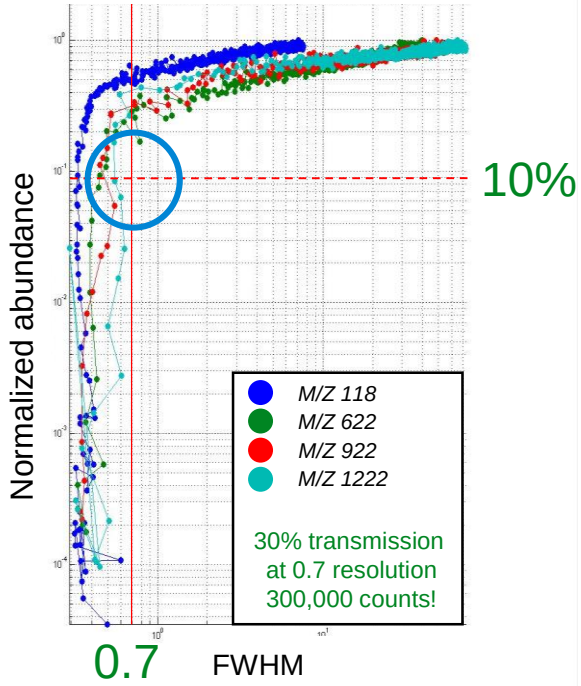




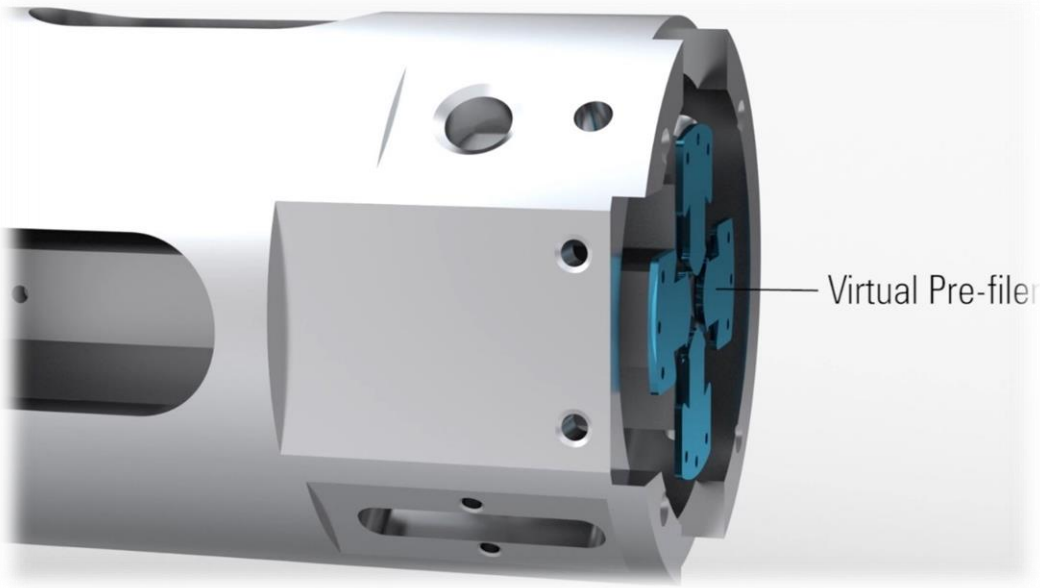
Older Prefilter



New *Virtual* Prefilter



Benefits of Virtual Pre/Post-Filters

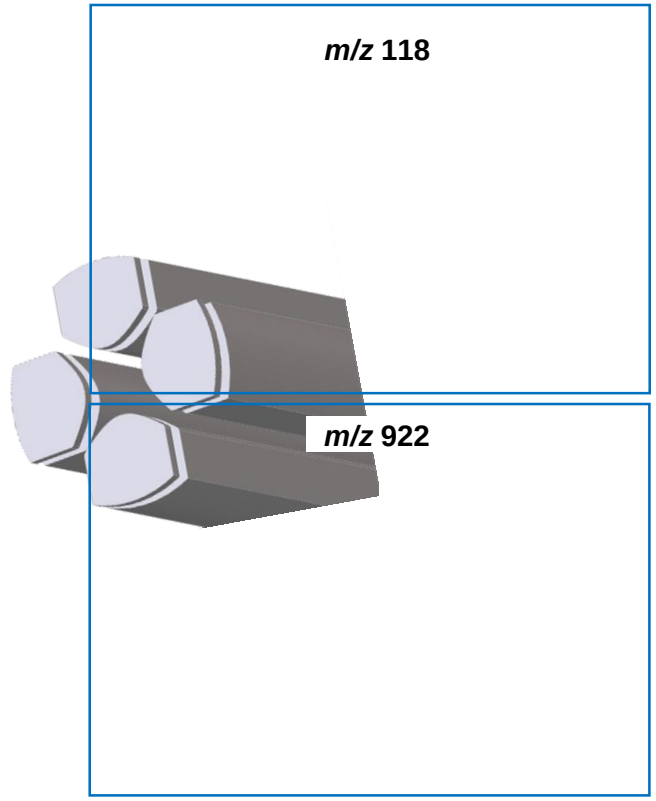


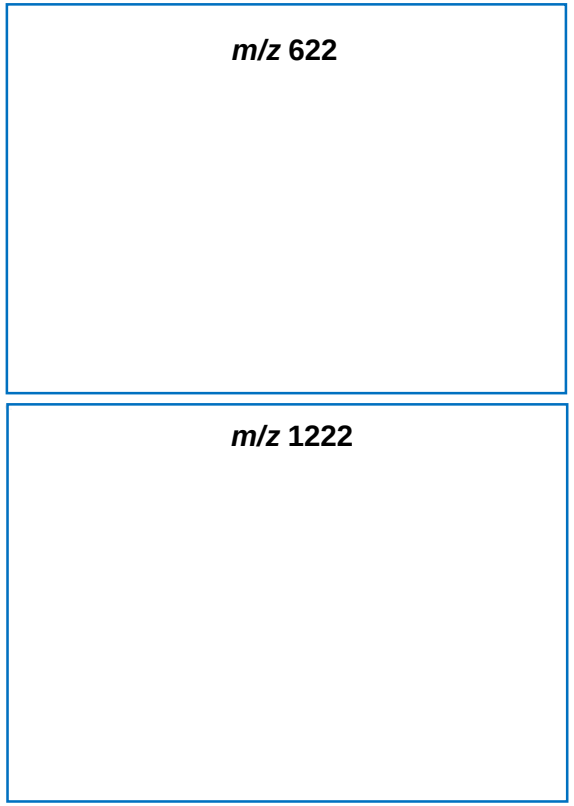
- Pre-filters are necessary to reduce fringe fields
- Virtual filters cancels the DC component in the fringe field region by applying an opposite sign DC voltage to the filter
- ***Maximum ion transmission at entrance and exit of resolving quadrupoles***
- ***Quicker switching → Faster MRMs***

Redesigned Hyperbolic Quadrupole

A quadrupole's performance are defined by the following:

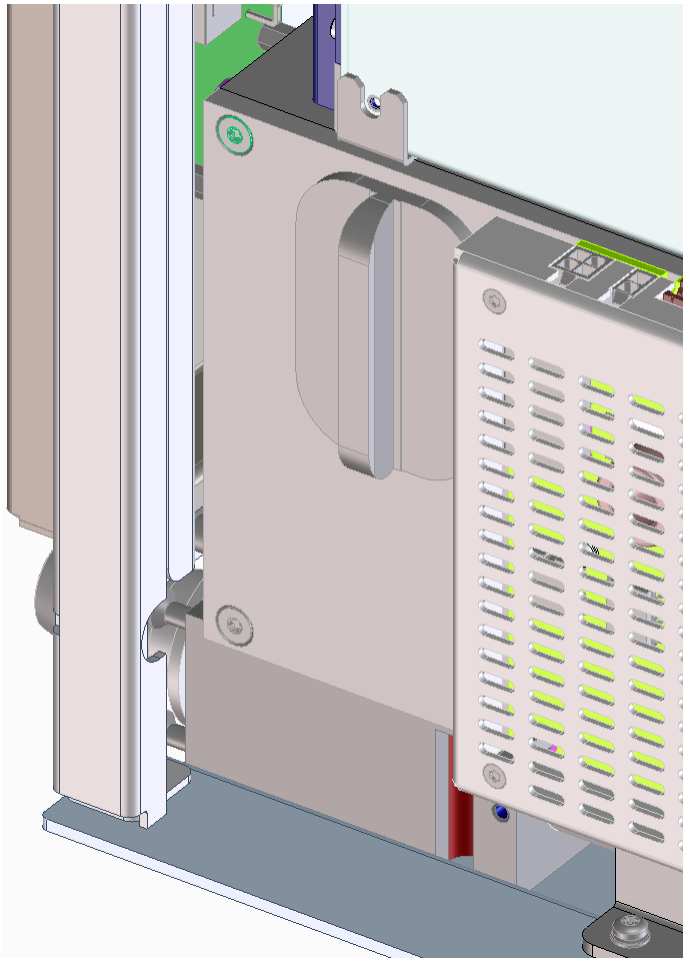
1. Electrode shape
2. Radius
3. Length
4. Frequency
5. Voltage

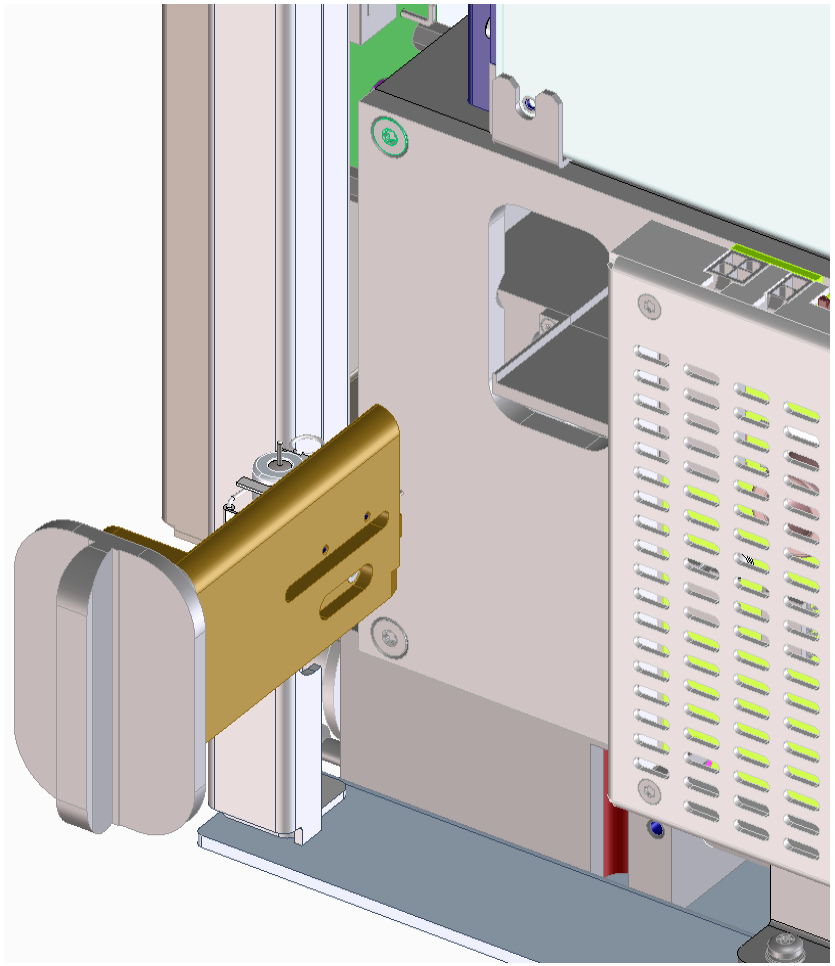


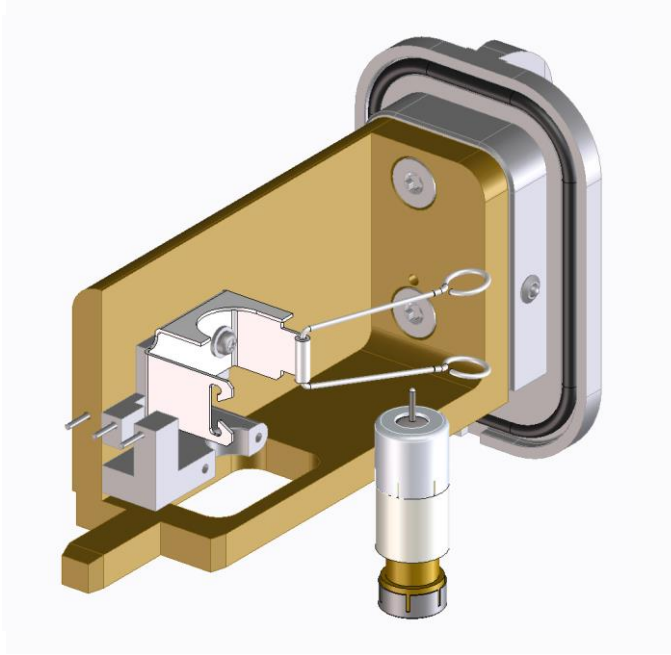


Agilent's Next Gen hyperbolic Quadrupoles operate at a higher frequency and voltage which allows a smaller sized quadrupole to perform with exceeding performance.

Easy Change Detector Assembly







Summary from Initial Ultivo TMS Synthetic Fentanyl Analyses.

- Quick 6 minute MRM method has been developed;
- Minimal sample preparation techniques, 1/10 dilution and filtering for Urine Matrix & protein precipitation with 1/6 dilution for Serum Matrix;
- Sensitivity measured as 100pg/ml or less for every analyte in both matrices (5uL injection volume);
- Precision data less than 10%RSD at the LLOQ for each analyte;
- Completely new and innovative minature Tandem Mass Spectrometer performance demonstrated herein.

Ultivo Triple Quad LC/MS

AGILENT IS RESHAPING THE FUTURE OF MASS SPECTROMETRY.

JOIN US AND WELCOME IN A NEW ERA OF "FIT FOR PURPOSE" MASS SPECTROMETRY.