Innovation Applied

10pg/mL Level Robust Quantitation of THCA and Simultaneous Detection of THC in Oral Fluid Using Oral-Eze® and LC-MS/MS

Xiang (Kevin) He and Marta Kozak

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Overview

- Assay background
- Our approach
- Key features
- Configuration
- Results
- Why it works now
- Next steps
- Conclusion

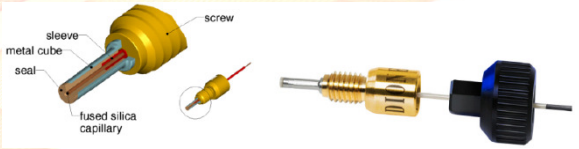
Background

- THCA in oral fluid – "*Cannabis abuse OR passive exposure*"
- Present at low pg/mL in oral fluid (OF)
- Wider adoption of the approach held up due to method complexity
- Current methods - extensive sample prep + 2D GC-MS or GC-MS/MS
- Published LC-MS methods extremely complex

Our approach

Oral-Eze®
Oral Fluid Collector

Nano Viper Fittings



DIONEX RSLC

Q Exactive System

TraceFinder 2.0

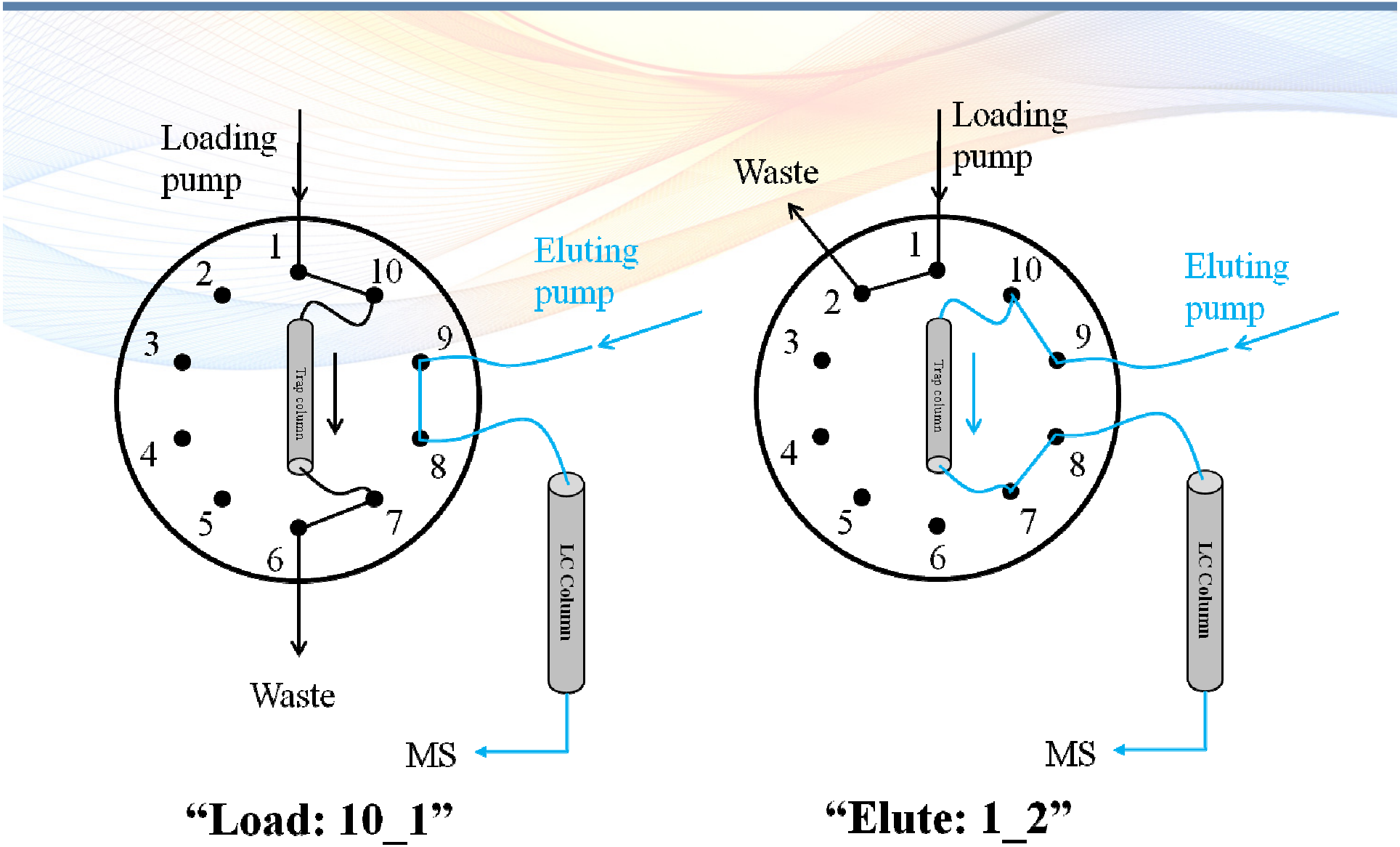
Key Features

- Online sample preparation
- OF collected injected as such
- THCA LOQ ~ 10 pg/mL
- Dynamic range up to ~300 pg/mL
- 13 min per sample
- THC analyzed along with THCA

Configuration, reagents and samples

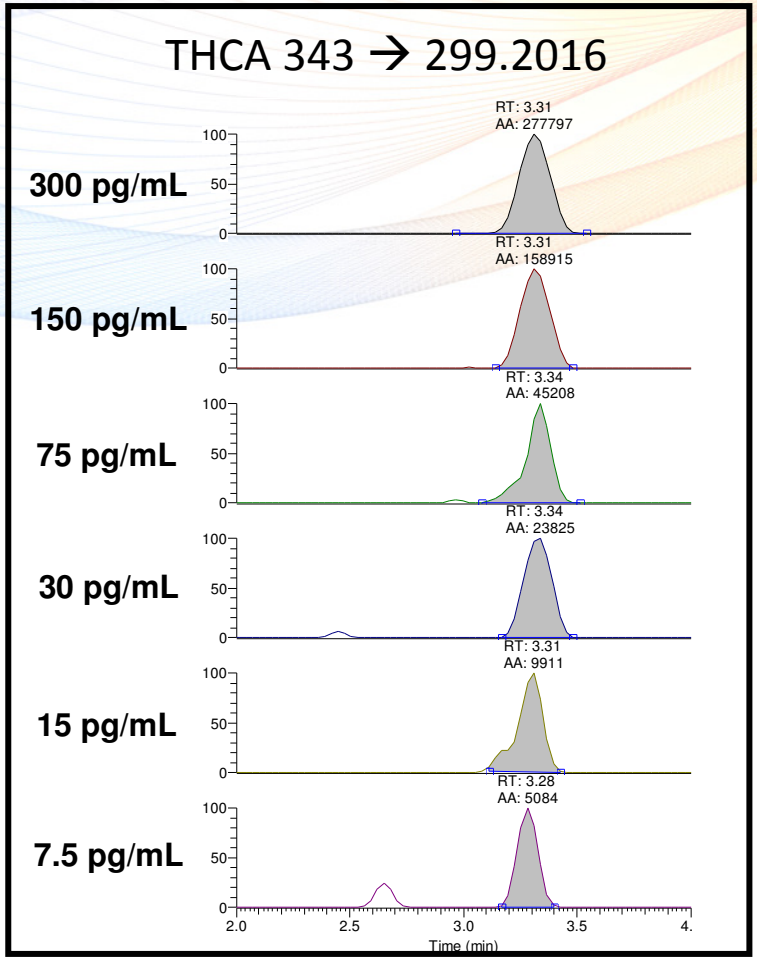
- LC
  - Ultimate 3000 RSLCnano operated in Micro mode
  - Injection volume = 40uL
- Q Exactive with HESI-II (micro capillary)
  - Negative ion mode
  - Targeted MS/MS
  - Resolution of up to 140,000
- Analytes and internal standards (IS)
  - THCA and THCA-d9
  - THC and THC-d3
- Calibrator blank matrix (synthetic blank oral fluid from CDD)
- Real oral fluid samples

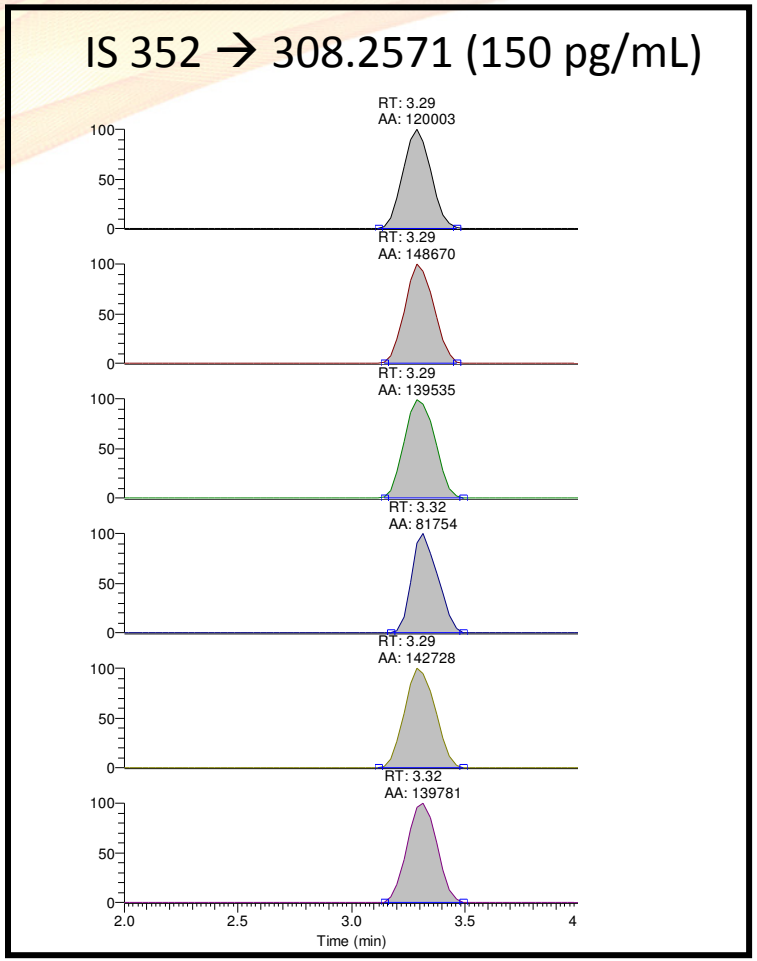
Online Sample Prep – TrapElute Mode



THCA - Synthetic Oral Fluid Matrix

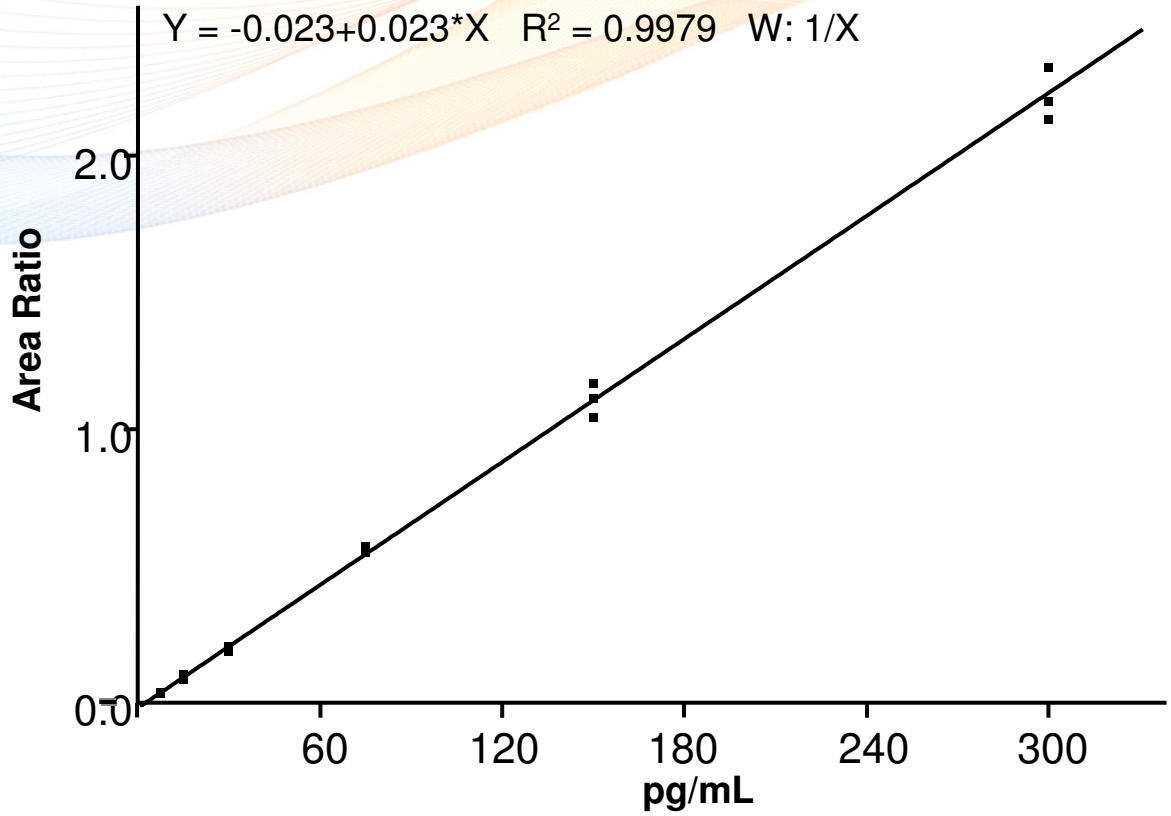
Injection volume = 40uL





THCA - Synthetic Oral Fluid Matrix

Calibration Curve – m/z 299.2006



Note: Device dilution factor of 3

THCA - Preliminary Performance Summary

Synthetic Oral Fluid

LLOQ: **7.5 pg/mL**
Dynamic range: **7.5 to 300 pg/mL**
Accuracy: **95.8 to 103.3%**
Precision: **3.7 to 12.5 (intra and inter)**
Carryover: **Not detected**
Ion Suppression: **max 35%**

Accuracy, Dynamic range and LLOQ

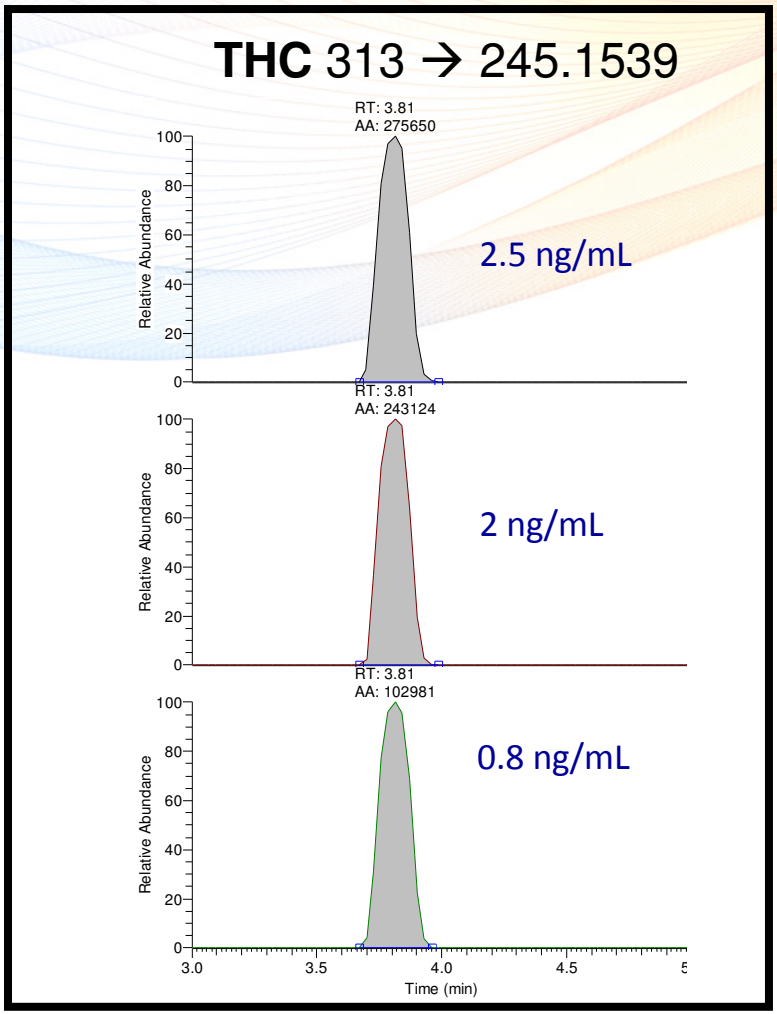
Sample	Specified (pg/mL)	Measured (pg/mL)	Accuracy (%)	Mean Accuracy (%)	Precision (%)
Cal1	7.5	7.26	96.8		
Cal1	7.5	7.30	97.3		
Cal1	7.5	7.80	104.0	99.4	4.07
Cal2	15	16.54	110.3		
Cal2	15	14.00	93.3		
Cal2	15	14.76	98.4	100.7	8.63
Cal3	30	30.13	100.4		
Cal3	30	28.48	94.9		
Cal3	30	27.58	91.9	95.8	4.50
Cal4	75	78.83	105.1		
Cal4	75	77.68	103.6		
Cal4	75	75.81	101.1	103.3	1.97
Cal5	150	141.68	94.5		
Cal5	150	150.90	100.6		
Cal5	150	158.19	105.5	100.2	5.50
Cal6	300	312.20	104.1		
Cal6	300	295.75	98.6		
Cal6	300	287.33	95.8	99.5	4.24

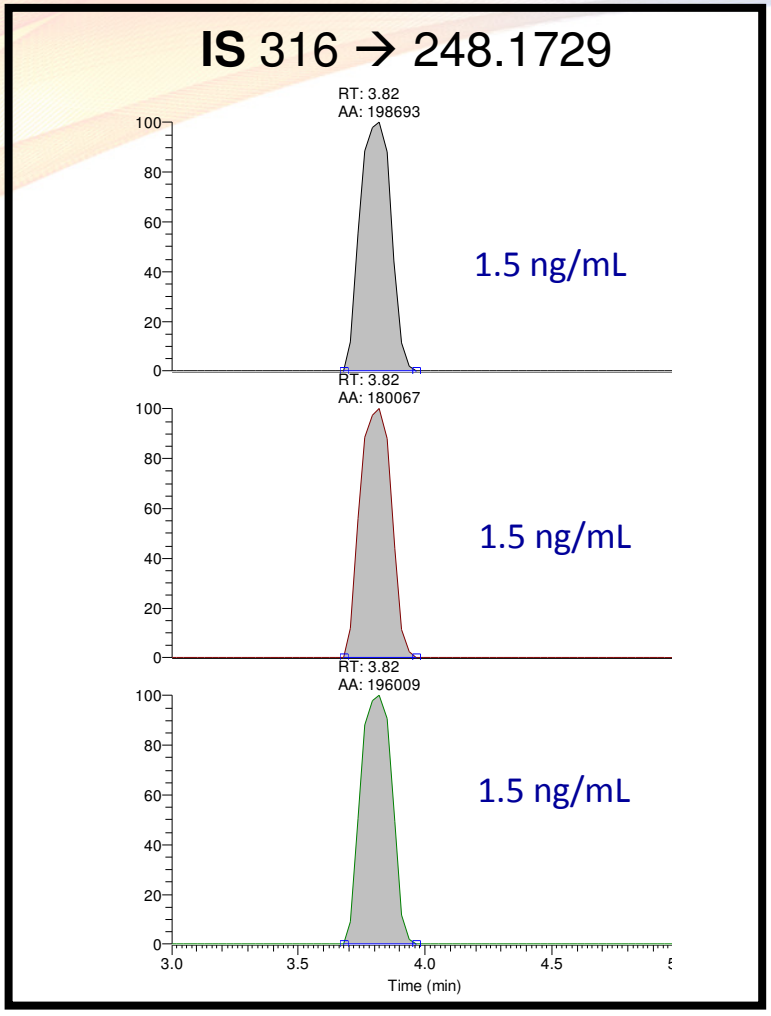
Precision

[Specified] (pg/mL)	Mean Accuracy (%)	Precision (%)
24 (Intra-batch, n=5)	101.0	12.5
24 (Inter-batch, n=15)	96.3	10.8
120 (Intra-batch, n=5)	102.7	5.7
120 (Inter-batch, n=15)	100.1	5.3

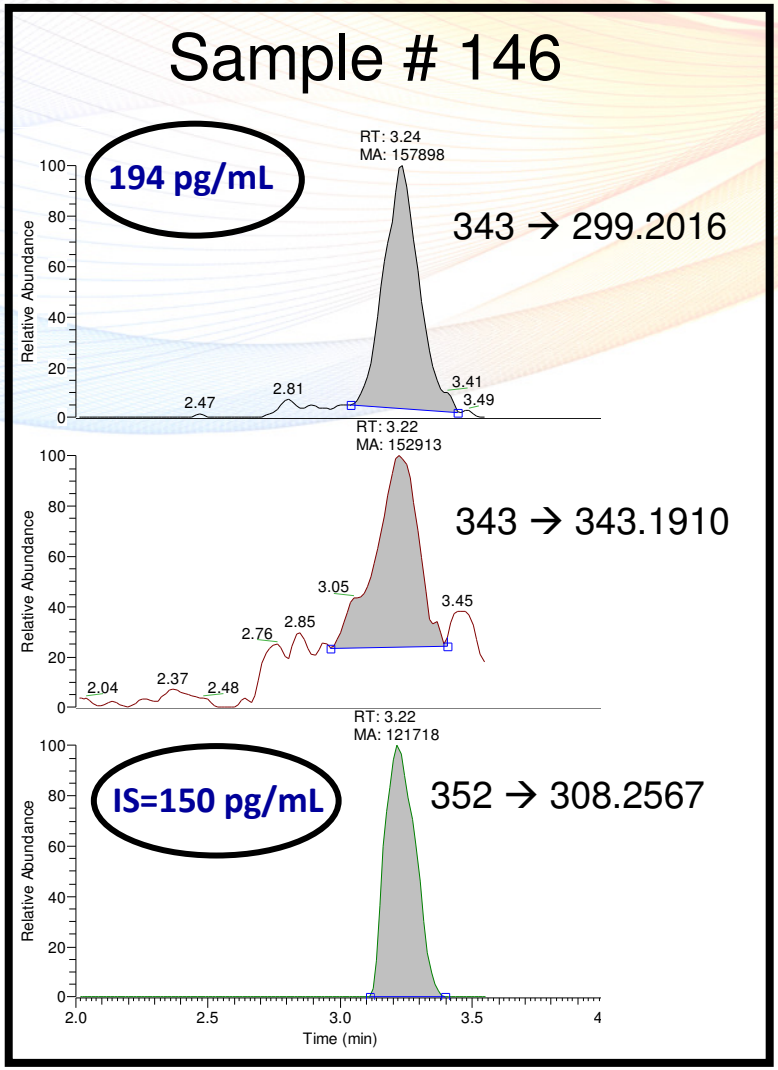
THC - Synthetic Oral Fluid

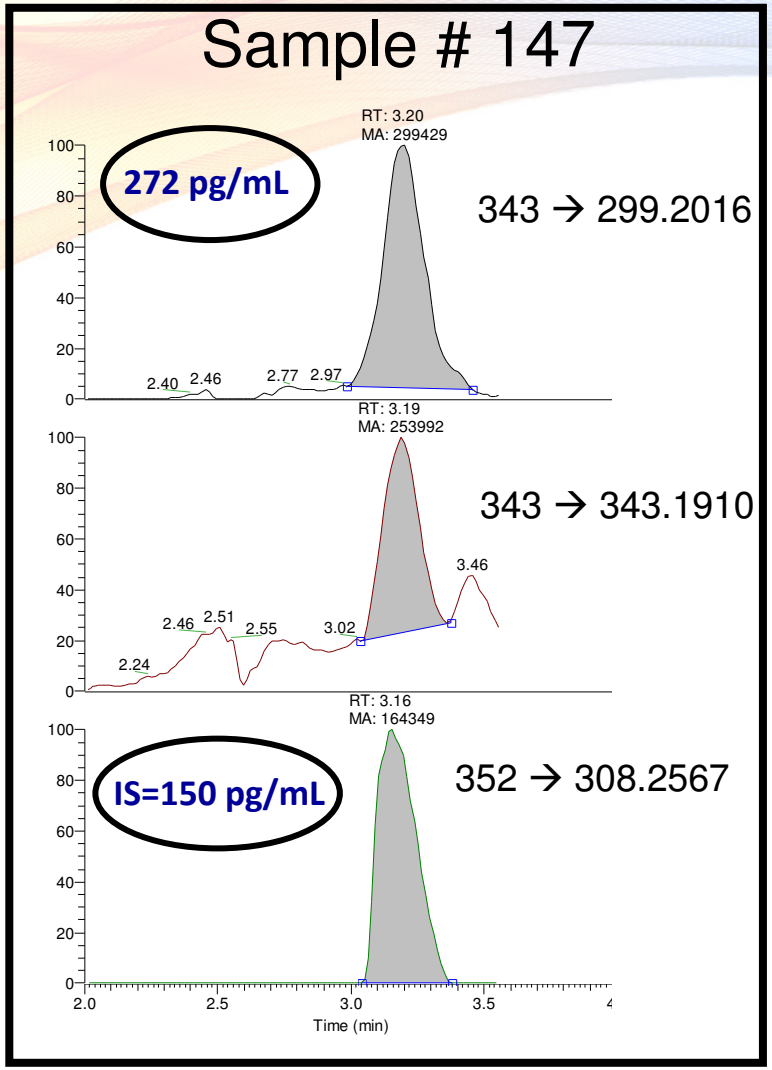
Monitor THC at high concentrations in the same run





THCA - Donor OF Samples





Forensic Toxicology Use Only

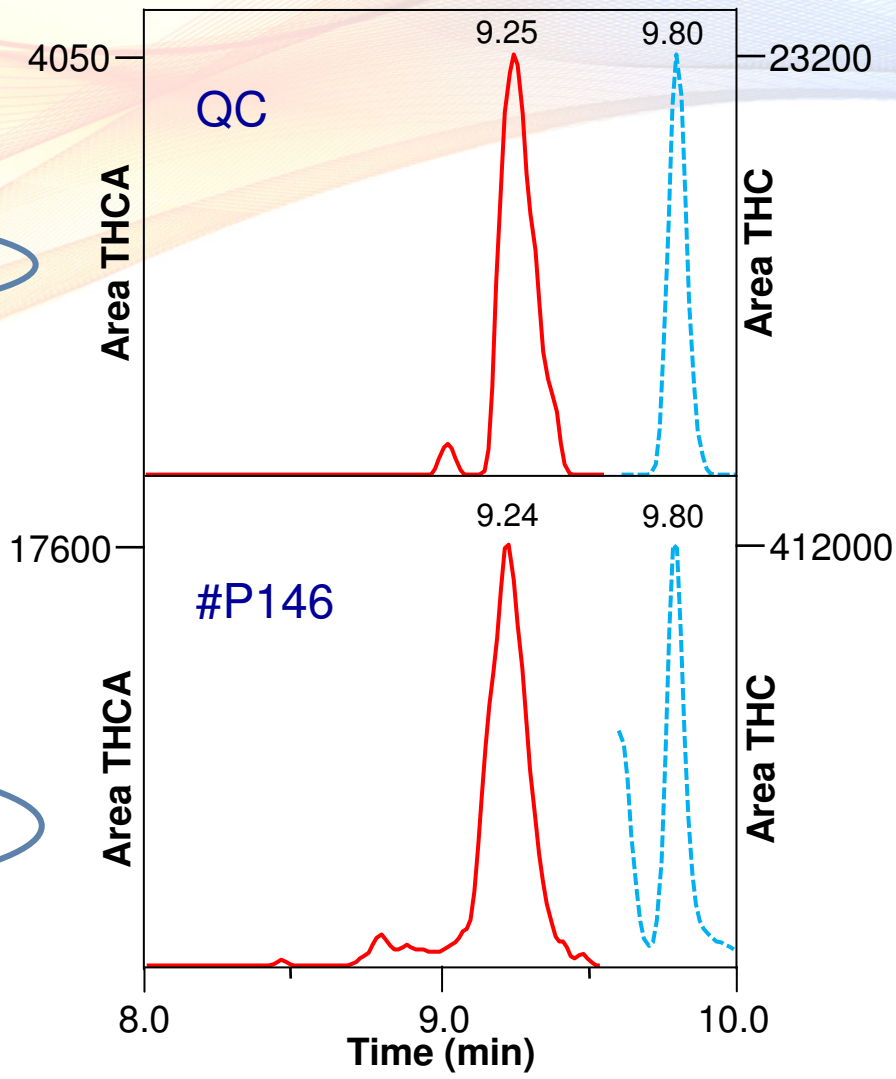
Simultaneous THCA and THC Analysis

THCA

THC

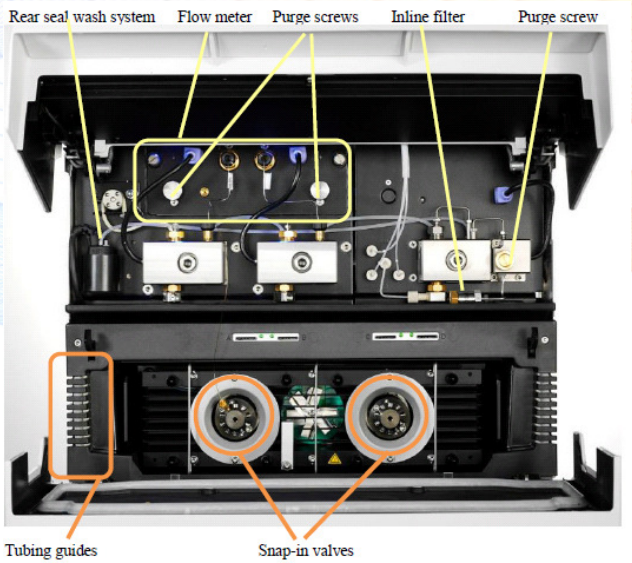
QC = OFNC + 24 pg/mL THCA + 800 pg/mL THC

#P146 = OF sample with 194 pg/mL THCA & 42,000 pg/mL of THC

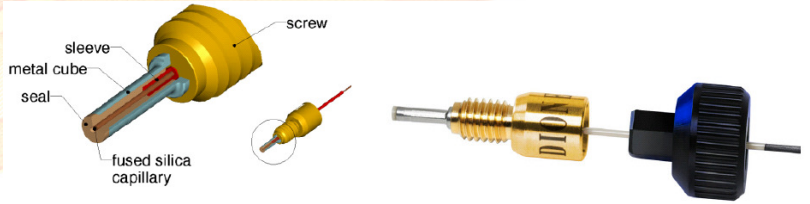


OFNC: Oral Fluid Negative Control

Why Micro LC Works Now?



- **Reliable high pressure mixing pumps**
- **Robust Trap-Elute setup**
- **Snap-On valves**
- **Separate uHPLC load and eluting pumps**
- **Upgrade to two-plex setup by adding a pump**



"The little things that make a huge difference"

Nano Viper tubing and fitting
Zero dead volume and hand tight

Range of high efficiency capillary and microbore columns

Next Steps

- Complete on-going validation study
- External validation
- Explore SPE for even better LOQ
- Confirm performance on hair samples (don’t expect issues)
- Confirm performance for Immunalysis and OraSure kit

Conclusion

We have a robust combined THCA and THC OF method that demonstrates necessary quantitation performance and is ready for validation