

# **Novel High-Sensitivity Analysis of Cannabinoids from Whole Blood by Combining a Hybrid SPE Method and LC/MS/MS**

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# Outline

- Sample Preparation Method
- Instrumental Method
- Analytical Performance

# Marijuana

- Most commonly abused drug in the United States
- Behavioral effects such as euphoria, relaxation and mood changes
- Main active ingredient: *Delta-9-tetrahydrocannabinol (THC)*  
Main metabolites in the body:  
*11-hydroxy-delta-9-THC (11-OH-THC) & 11-nor-9-carboxy-delta-9-THC (THC-COOH)*
- The blood cannabinoid concentration could be at the low level after marijuana intake

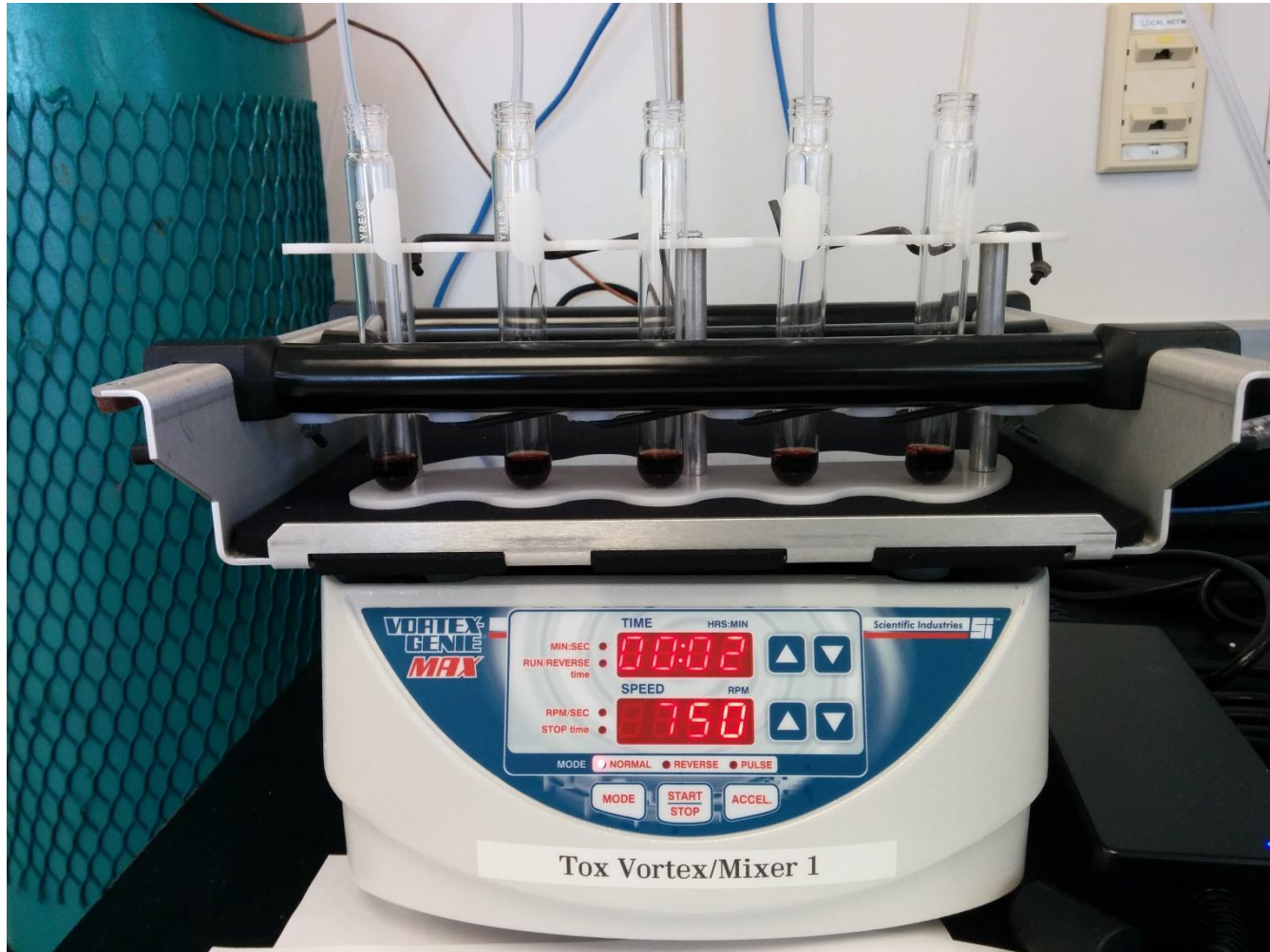


# Preparation Method for Whole Blood Samples

## First step - Protein Precipitation

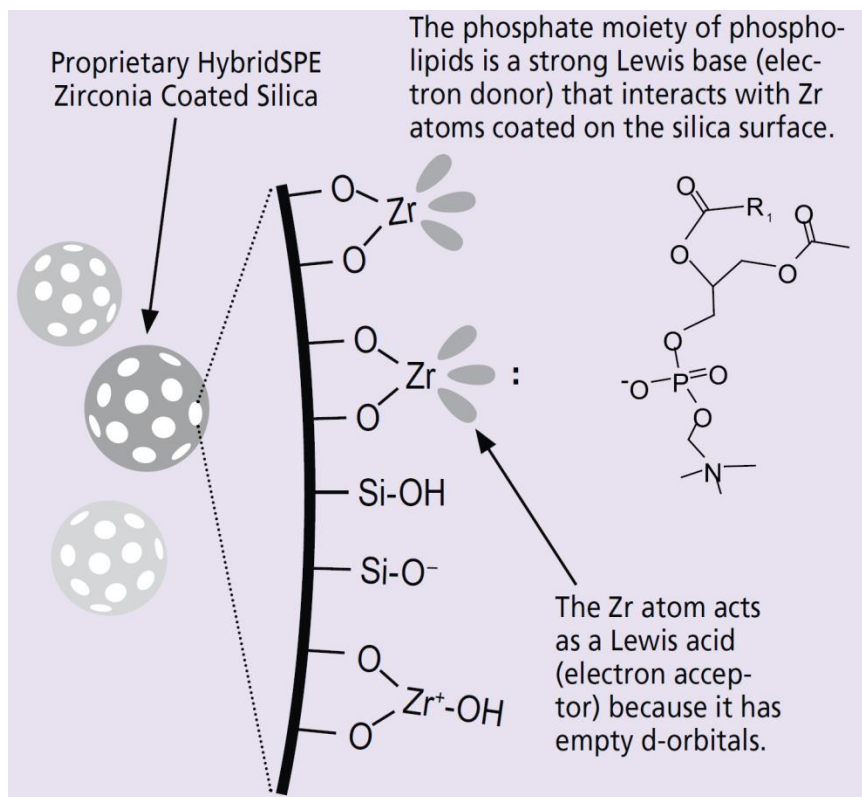
- Traditional Method
  - Manually vortex individual samples while drop-wise adding ice cold organic solvent
  - Labor-intensive and time-consuming
- Current Method using Multi-Tube Vortexer
  - Automate the vortexing process with high throughput for protein precipitation
  - Significantly reduces the manual labor and preparation time

# Preparation Method for Whole Blood Samples



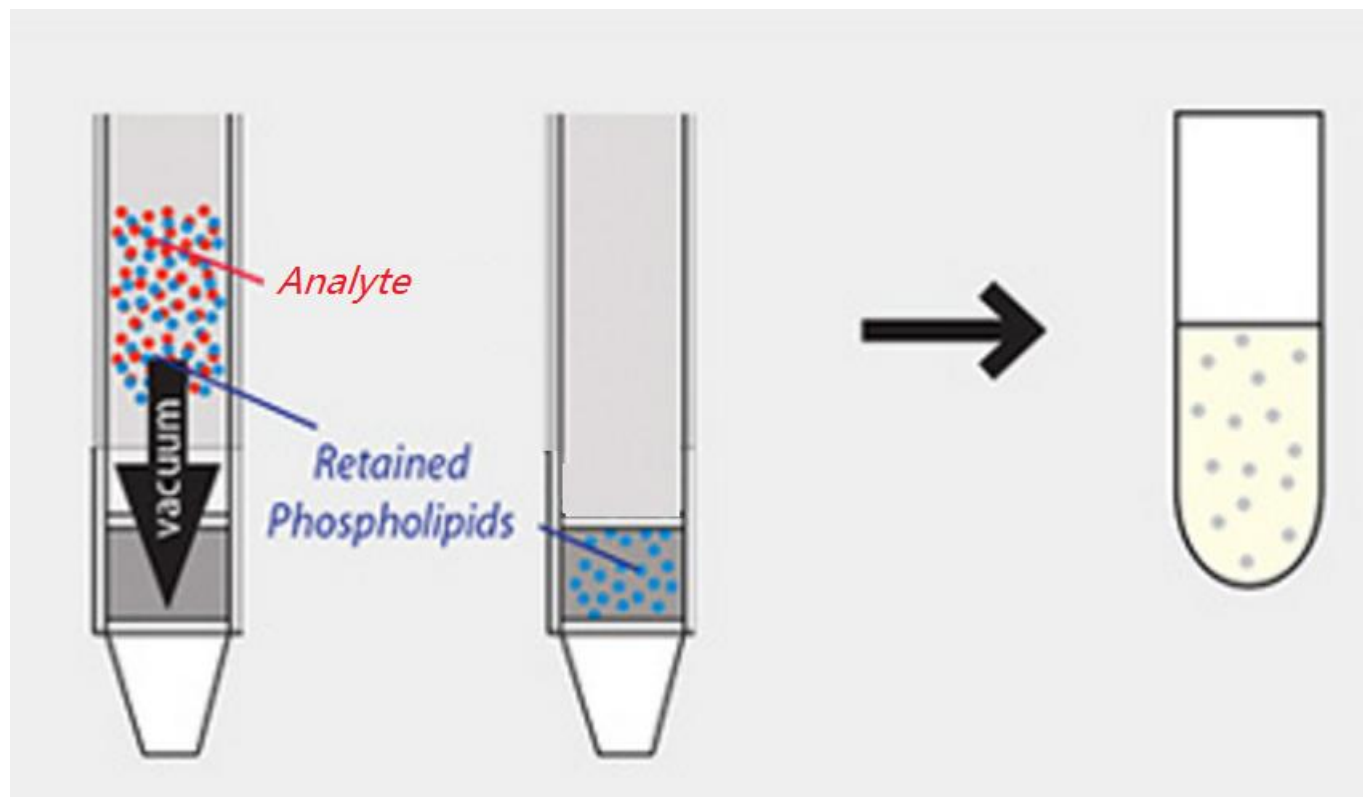
# Novel SPE Extraction

- HybridSPE-Phospholipid SPE Column
- Remove phospholipids in blood/ plasma/serum



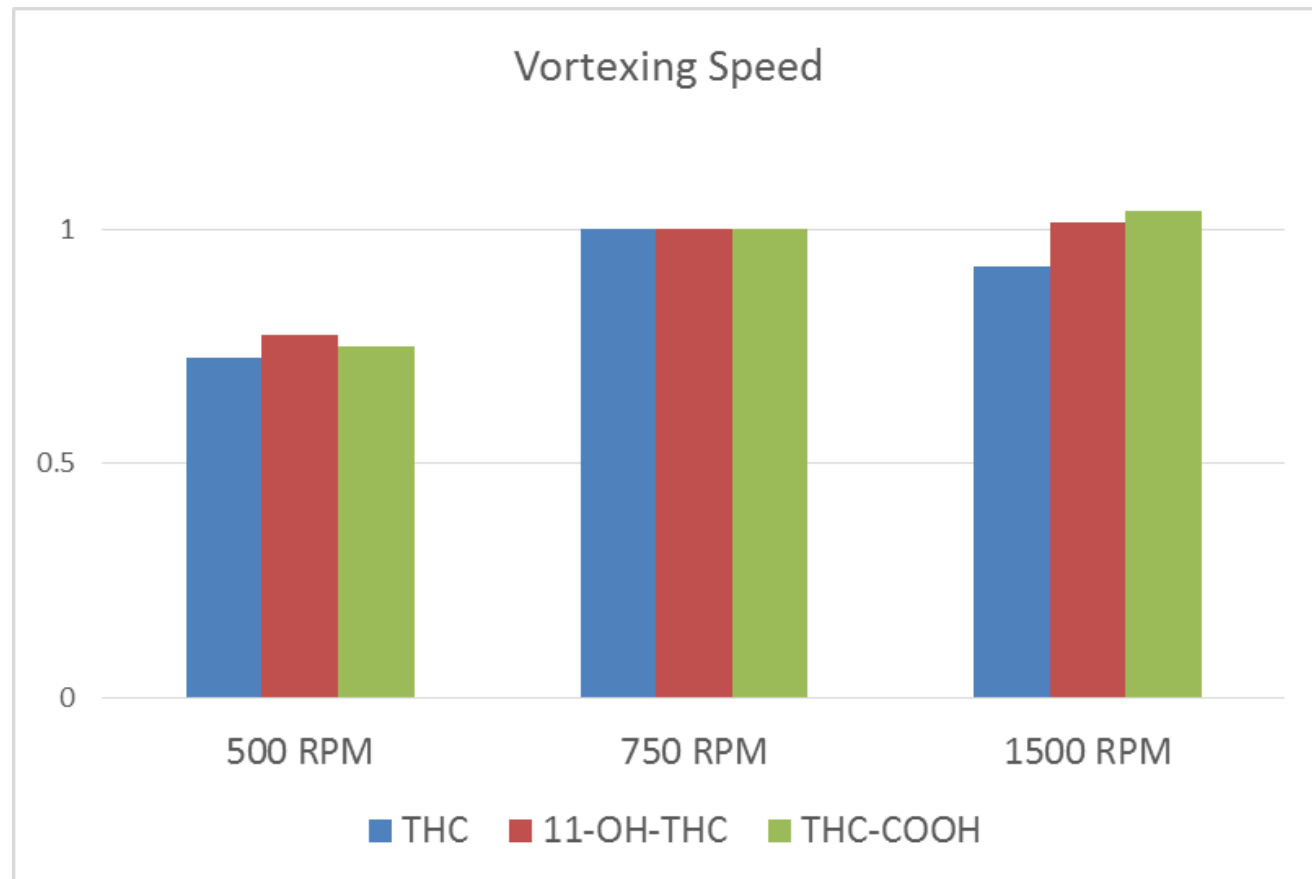
# Novel SPE Extraction

- HybridSPE-Phospholipid SPE Column



# Extraction Optimization

- Vortexing speed for protein precipitation





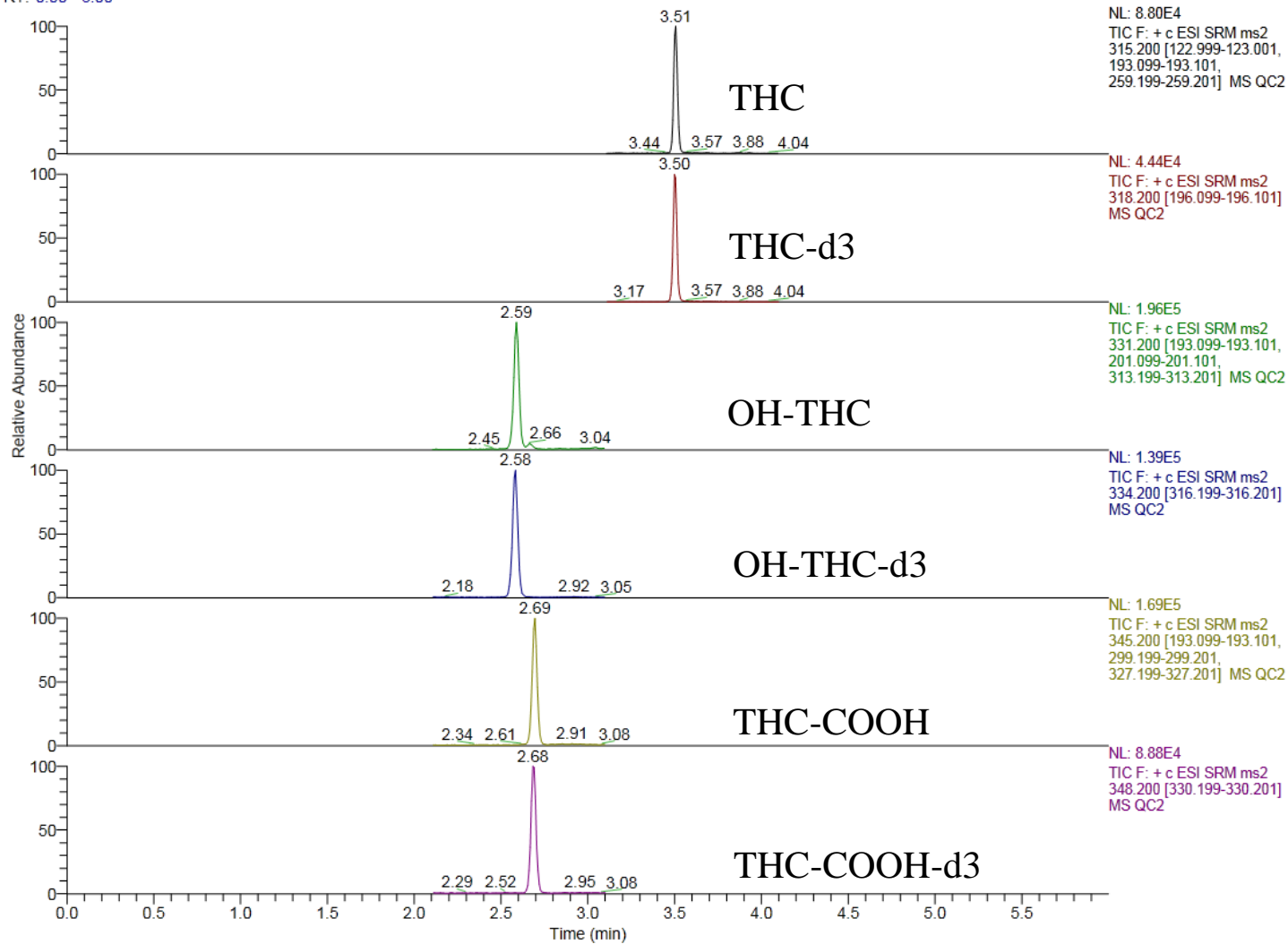
# LC/MS/MS System

Thermo Scientific TSQ Vantage triple stage quadrupole mass spectrometer



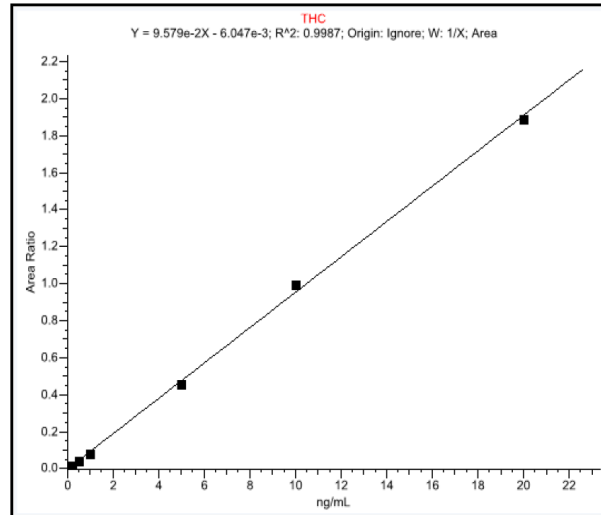
# LC/MS/MS Separation

RT: 0.00 - 6.00

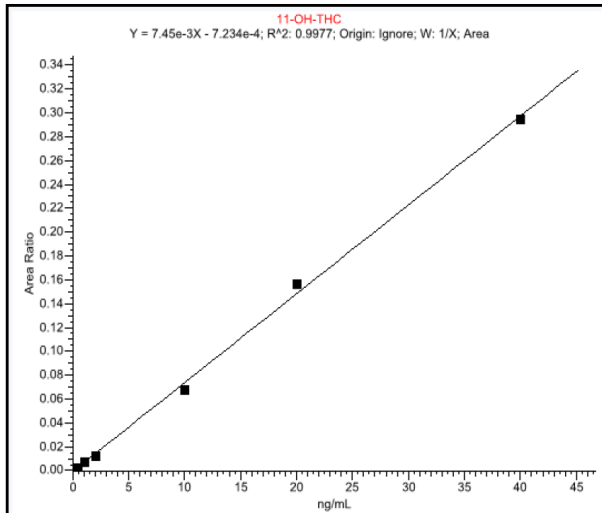


# Calibration Curves

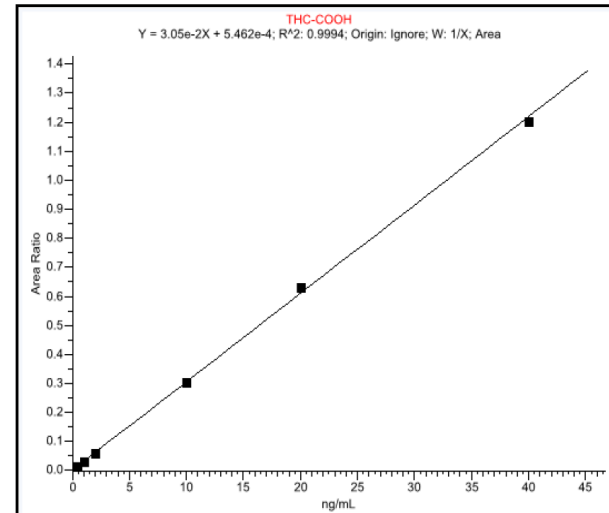
Compound Name: THC



Compound Name: 11-OH-THC



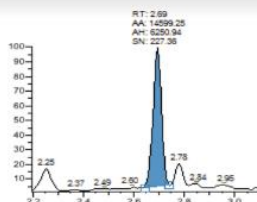
Compound Name: THC-COOH



# Analytical Performance

	<b>Cannabinoids</b>
<b>Linear range (ng/mL)</b>	0.2 – 20 or 0.4 – 40
<b>Correlation coefficient (R<sup>2</sup>)</b>	> 0.99
<b>Limit of Detection (ng/mL)</b>	0.2 or 0.4 ng/mL
<b>Recoveries (%)</b>	50 - 80
<b>Ion suppressions (%)</b>	< 15%

# Case Samples



THC-COOH

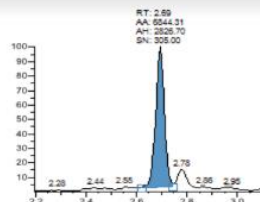
Quan Peak: 299.200 m/z

TotalArea: 14599

Peak Area: 14599

RT: 2.69min (2.70)

Amount: 3.196 ng/mL

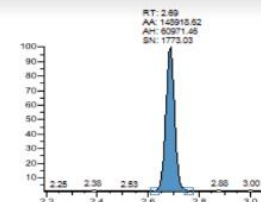


Qual Peak: 193.100 m/z

Area: 6844

Ratio: 46.88 %

Range: 36.07 % - 54.11 %



THC-COOH-D3

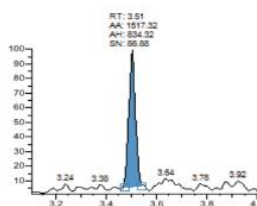
Quan Peak: 330.200 m/z

TotalArea: 148919

Peak Area: 148919

RT: 2.69min (2.70)

Amount: 20.000 ng/mL



THC

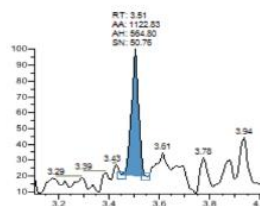
Quan Peak: 193.100 m/z

TotalArea: 1517

Peak Area: 1517

RT: 3.51min (3.51)

Amount: 0.318 ng/mL

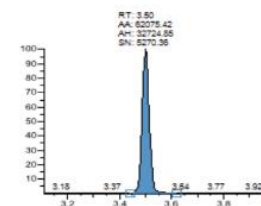


Qual Peak: 123.000 m/z

Area: 1123

Ratio: 74.00 %

Range: 50.91 % - 76.37 %



THC-D3

Quan Peak: 196.100 m/z

TotalArea: 62075

Peak Area: 62075

RT: 3.50min (3.51)

Amount: 10.000 ng/mL

Case Blood Sample	Concentration (ng/mL)
THC	0.318
THC-COOH	3.196

# Summary

- Multi-Tube Vortexer to automate the vortexing process for protein precipitation and novel SPE columns to remove phospholipid from whole blood matrix
- Sensitive and fast method for quantification of THC and its metabolites by LC/MS/MS
- Application for the forensic and clinical analyses of cannabinoids

**Thank you!**