# A novel LC/MS/MS method with dual ion source for analysis of 102 pesticides and 5 mycotoxins in hemp

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# Outline

- **Pesticides and their action limits in USA states and Canada for hemp/cannabis**
- **Analysis of non-polar pesticides using novel LC/MS/MS method with dual ESI and APCI source**
- **Mitigation of matrix effects with LC and MS method optimization to improve method sensitivity**
- **Sample preparation method – simple solvent extraction**
- **Internal standards – method accuracy**

# Oregon, California, Canada & Colorado Action Limits for Pesticides in Hemp/Cannabis

| Analyte | OR/ppm | CA/ppm | Canada/ppm | CO/ppm |
|---|---|---|---|---|
| Acequinocyl | 2 | 0.1 | 0.03 | 0.03 |
| Chlorfenapyr | 1 | 0.1 | 0.05 | 0.05 |
| Cyhalothrin | NA | NA | NA | 0.25 |
| Endosulfan-beta | NA | NA | 0.05 | 0.05 |
| Methoprene | NA | NA | 2 | 2 |
| Quintozene | NA | 0.1 | 0.02 | 0.02 |

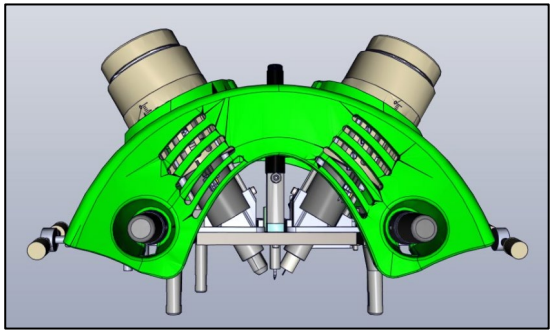
# LC/MS/MS System with Dual ESI and APCI Source

Electrospray Ion (ESI)

Atmospheric Pressure Chemical Ionization (APCI)

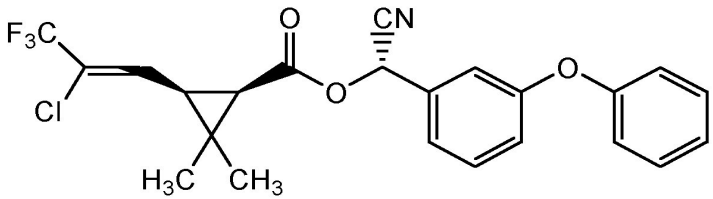
## LC/MS/MS System

## Dual ESI & APCI Source

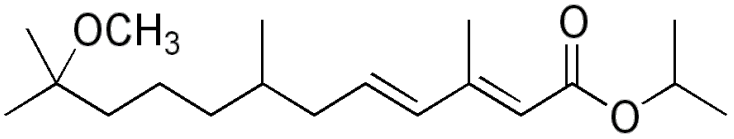


# Non-Polar Pesticides Analyzed with ESI Source

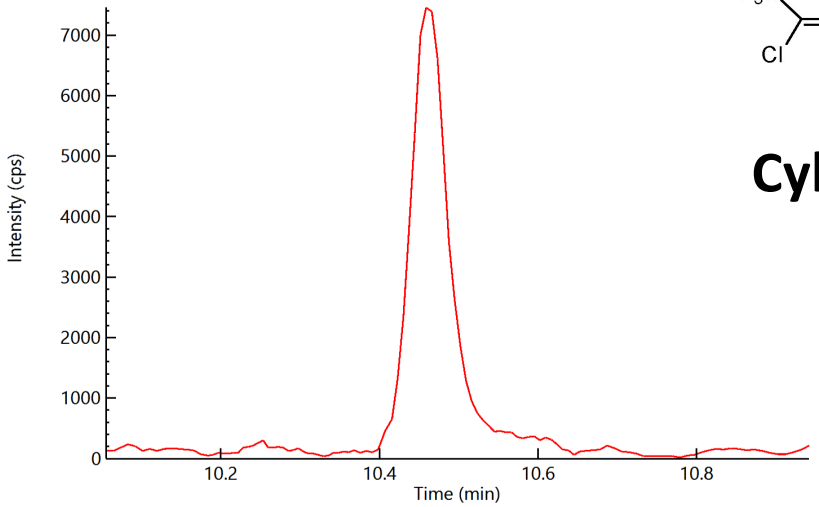
## Cyhalothrin

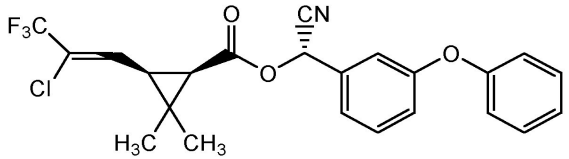



## Methoprene



# Cyhalothrin in Hemp using LC/MS/MS with ESI Source



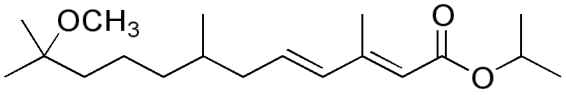

**Cyhalothrin – 0.1 ppm**

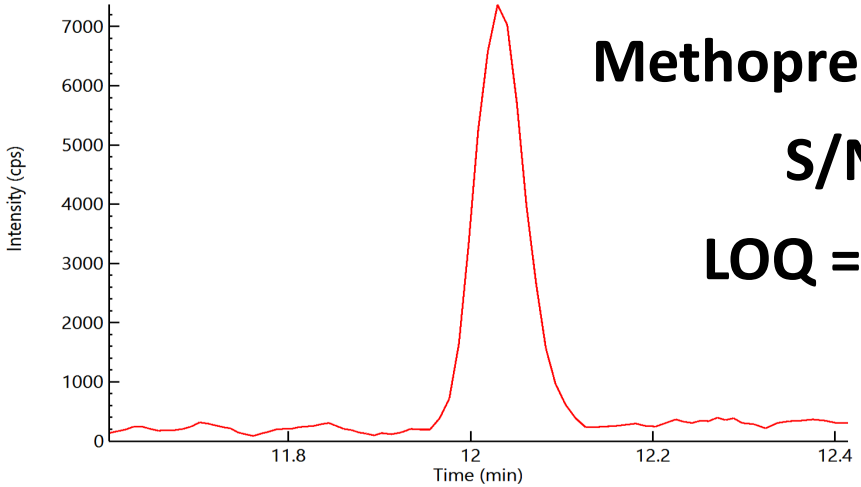
**S/N = 28**

**LOQ = 0.05 ppm**

**Method LOQ of 0.05 ppm meets action limits of 0.25 ppm in hemp**

# Methoprene in Hemp using LC/MS/MS with ESI Source




**Methoprene – 0.2 ppm**

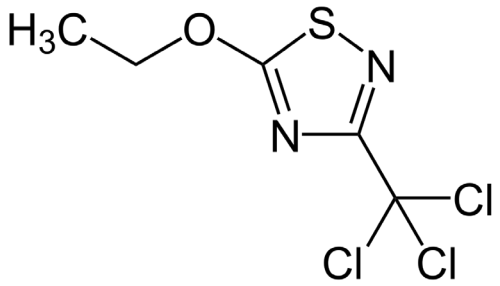
**S/N = 30**

**LOQ = 0.1 ppm**

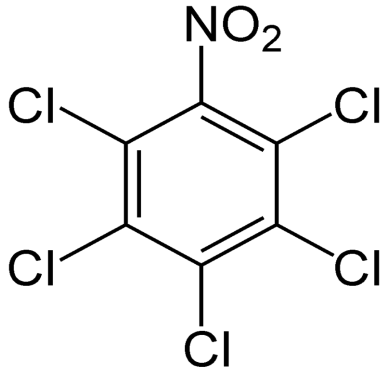
**Method LOQ of 0.1 ppm meets action limits of 2 ppm in hemp**

# Non-Polar Pesticides Analyzed with APCI Source

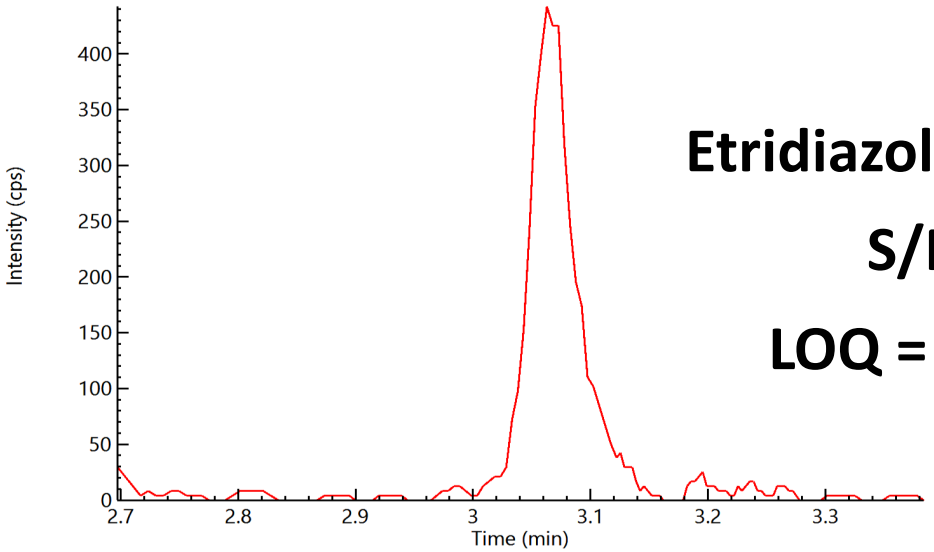
## Etridiazole

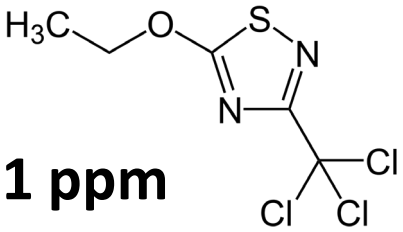



## Quintozene (PCNB)



# Etridiazole in Hemp with APCI Source



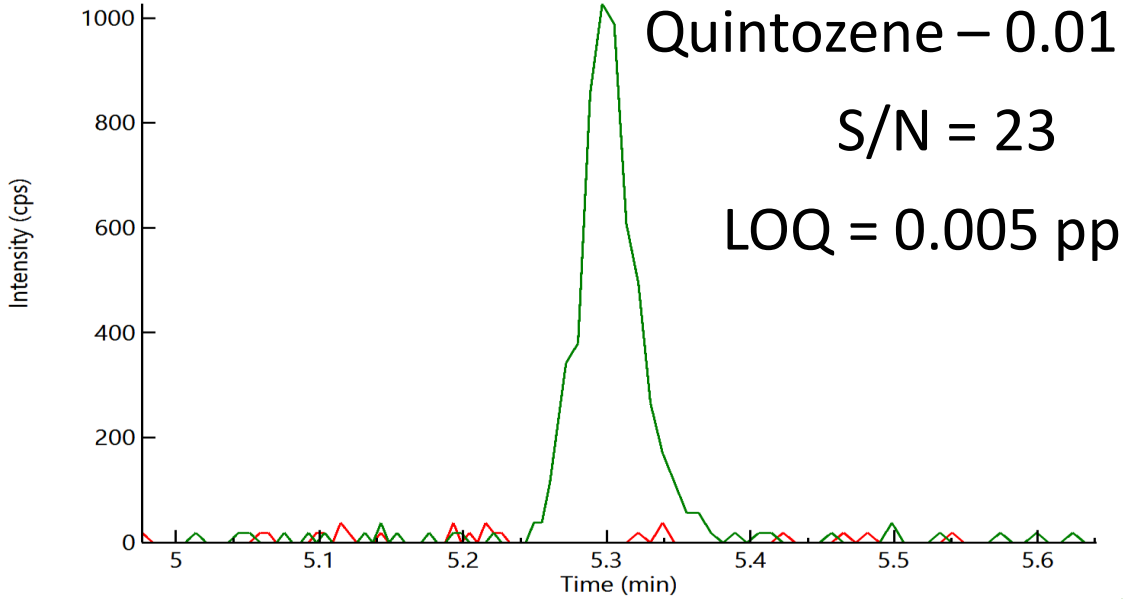

**Etridiazole – 0.01 ppm**

**S/N = 18**

**LOQ = 0.01 ppm**

**Method LOQ of 0.01 ppm meets action limits of 0.03 ppm in Hemp**

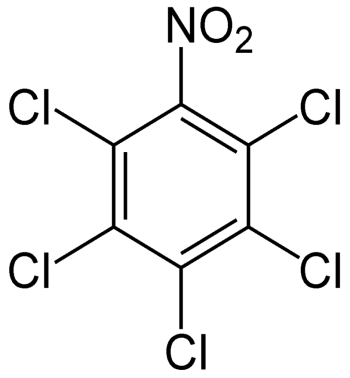
# Quintozene in Hemp using LC/MS/MS with APCI Source




Quintozene – 0.01 ppm

S/N = 23

LOQ = 0.005 ppm




**Method LOQ of 0.005 ppm meets action limits of 0.02 ppm in Hemp**

# Ionization Mechanism with APCI Source in Negative Mode

- **Anion attachment – Chlordane** $M + A^- \longrightarrow [M+A]^-$
- **Electron capture – Acequinocyl** $M + e^- \longrightarrow [M]^-$
- **Dissociative electron capture – Etridiazole**

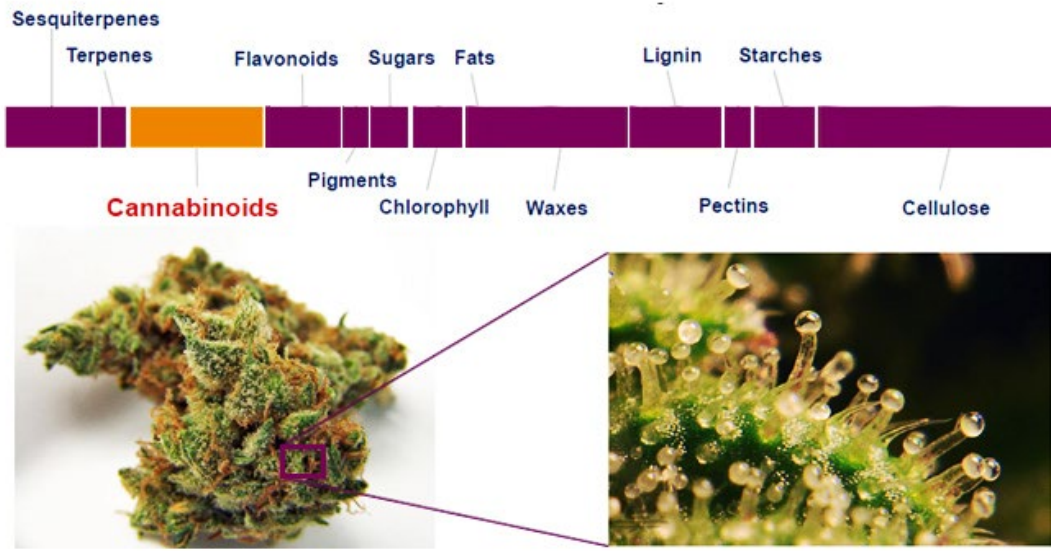
  $M + e^- \longrightarrow [F]^- + N$
- **Chemical reaction with Superoxide reagent ion – Quintozene**

  $O_2 + e^- \longrightarrow O_2^-$ **(Superoxide ion)**

  $M + O_2^- \longrightarrow [M\text{-}Cl+O]^- + ClO$

# Hemp Matrix is Challenging for Pesticides Analysis

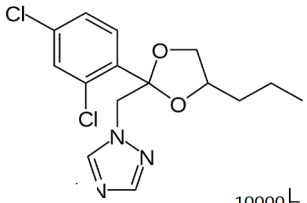
- **Complex matrix**
- **Broad Interference from cannabinoids present in 10-20% (100,000-200,000 ppm) range**
- **Terpenes and other compounds are present in high ppm range (10-5000 ppm)**

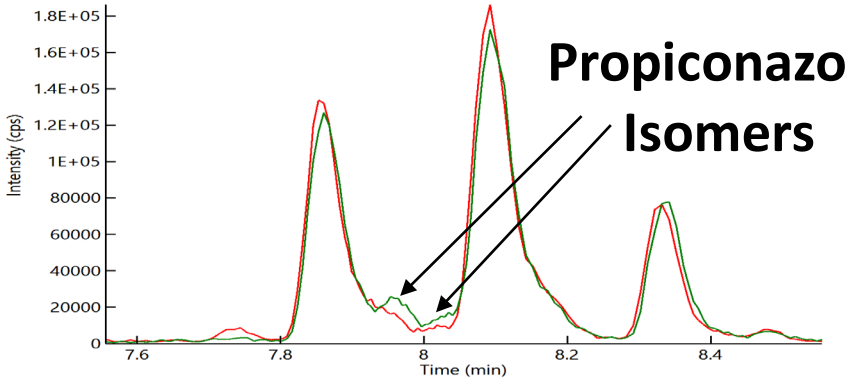


# Propiconazole (0.02 ppm) in Hemp with ESI Source

## Matrix Interference

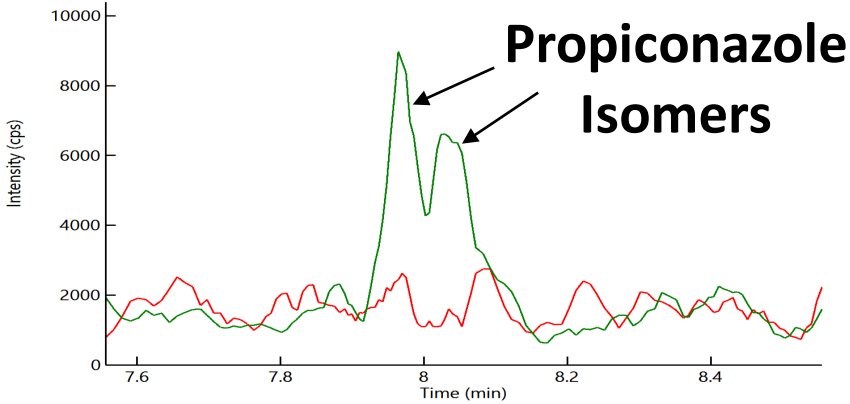
$[M+H]^+ = 342 \rightarrow 69$




## Minimal Matrix Interference

$[M+H+2]^+ = 344 \rightarrow 69$

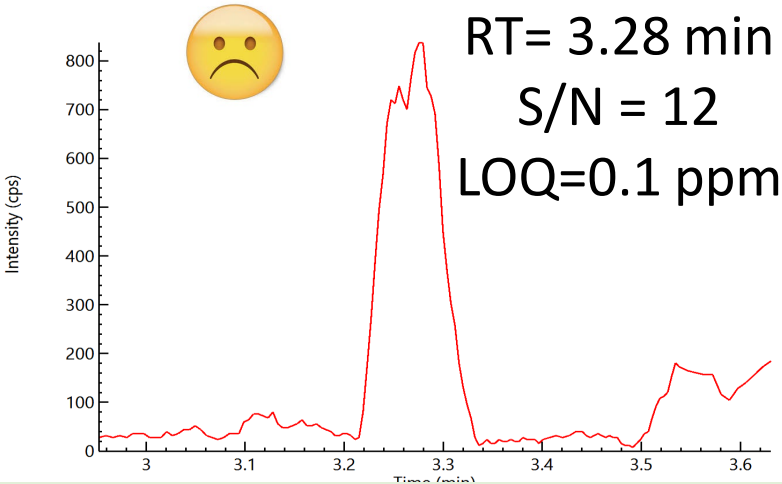



**Green** trace is signal for 20 ppb propiconazole in hemp

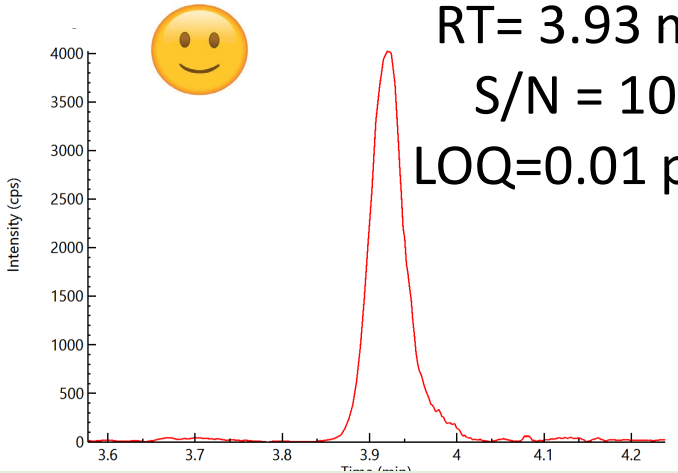
**Red** trace is signal for hemp blank

# Endosulfan-βeta (0.1 ppm) in Hemp with APCI Source

**Fast 6 minute LC Gradient**
**75 % Ion Suppression**



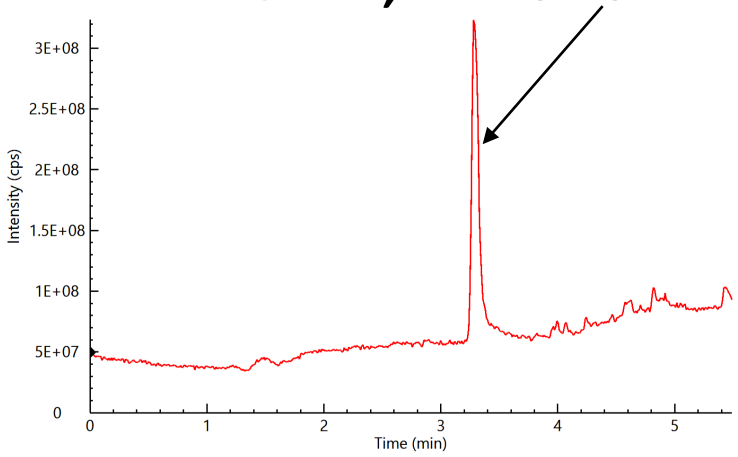

**Optimum 11 minute LC Gradient**
**Insignificant Ion Suppression**



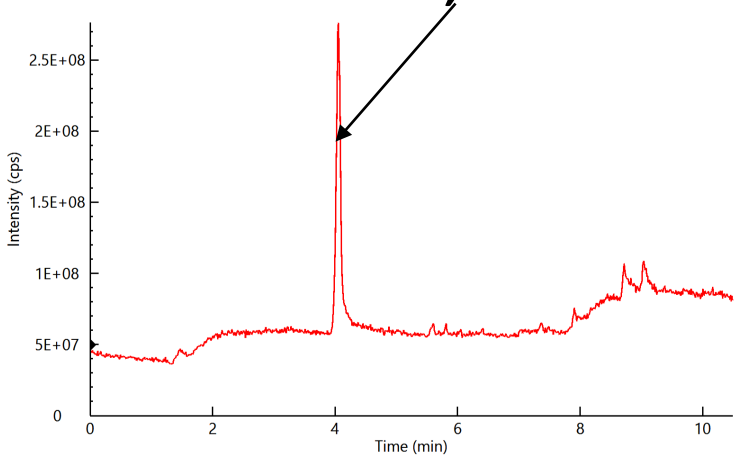

**Method LOQ of 0.01 ppm meets Action Limits of 0.05 ppm in Hemp**

# MS Scan for CBDA Retention time with 2 LC Methods

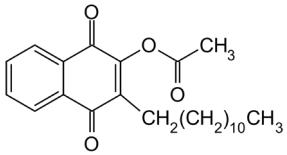
**Fast 6 min LC Gradient**
**Endosulfan-βeta, RT = 3.28 min**
**CBDA, RT = 3.29 min**



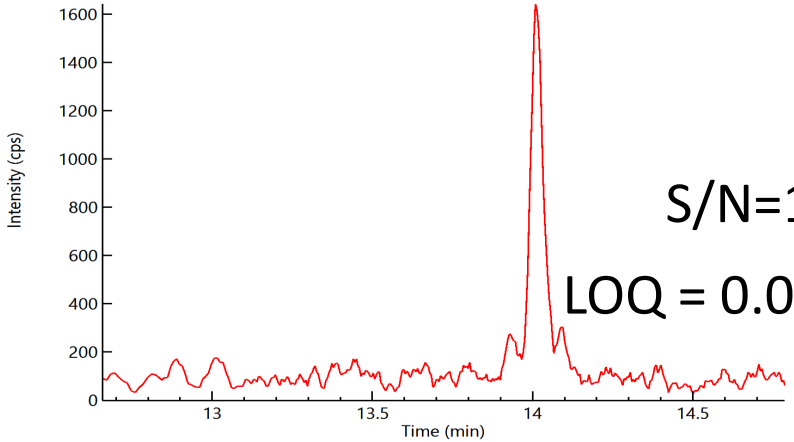

**Optimum 11 min LC Gradient**
**Endosulfan-βeta, RT = 3.93 min**
**CBDA, RT = 4.03 min**



# Acequinocyl in Hemp with ESI & APCI Source



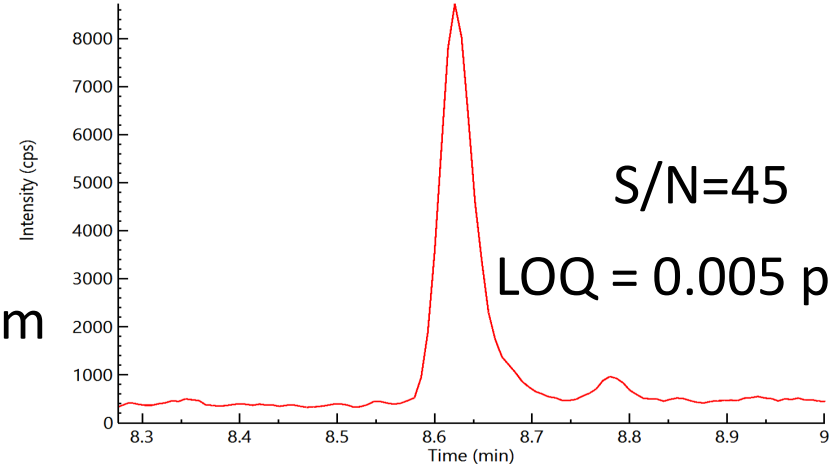

## ESI Source (0.01 ppm)




S/N=14

LOQ = 0.01 ppm

## APCI Source (0.01 ppm)




S/N=45

LOQ = 0.005 ppm

**Method LOQ of 0.005 ppm meets Action Limits of 0.03 ppm in Hemp**

# Sample Preparation Method - Solvent Extraction

**0.5 gm of Ground Hemp in 50 ml tube**

↓

**2.5 ml of ACN with 0.1 % formic acid (FA)**

↓

**Vortex 10 min**

↓

**Centrifuge 5 min at 3000 rpm**

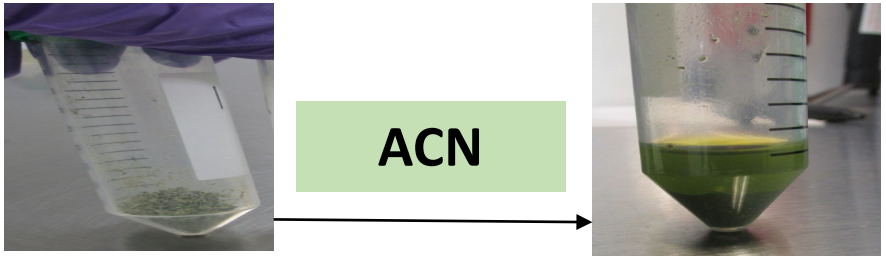
↓

**Dilute 500 µL extract with 490 µL of ACN/0.1 % FA and 10 µL of IS**

# Sample Preparation Methods

- Solvent Extraction – ( ACN extraction)
- Solvent Extraction with Evaporation
- QuEChERS
- Dispersive Solid Phase Extraction (dSPE)
- Solid Phase Extraction (SPE)
- Immunoaffinity Columns

**Simple, Speed, Cheap, Green, Automation & Extraction Efficiency**



# Automated Testing Workflow

## FULLY AUTOMATED CANNABIS & HEMP PESTICIDE-RESIDUE TESTING WORKFLOW

SimplicityLab 420™

WEIGHT & SAMPLE ID

SAMPLE ID & WEIGHT

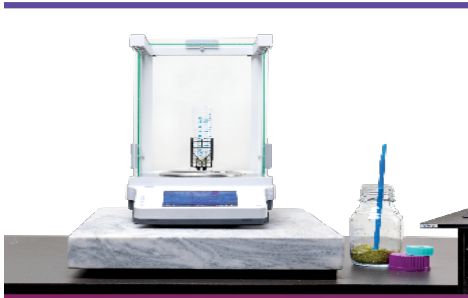
SAMPLE LIST & BATCH INFORMATION

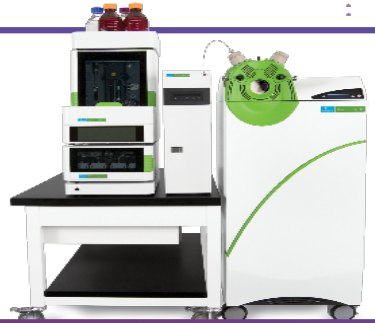
BATCH & QUALITY CONTROL INFORMATION

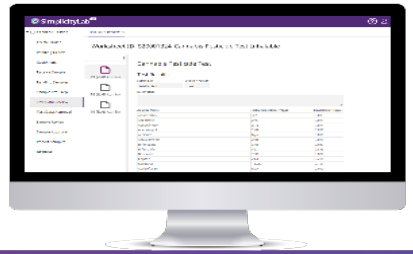
SAMPLE RESULTS & BATCH INFORMATION

SAMPLE & QC TEST DATA

METRC & COA Report







### RECEIVE & WEIGH SAMPLES

Measure out representative Cannabis or Hemp sample

### SAMPLE PREPARATION

JANUS® G3 420 Workstation with ONE Pesticide[420™] reagent kit, vortex, centrifuge & integrated barcode scanner

### INSTRUMENT ANALYSIS

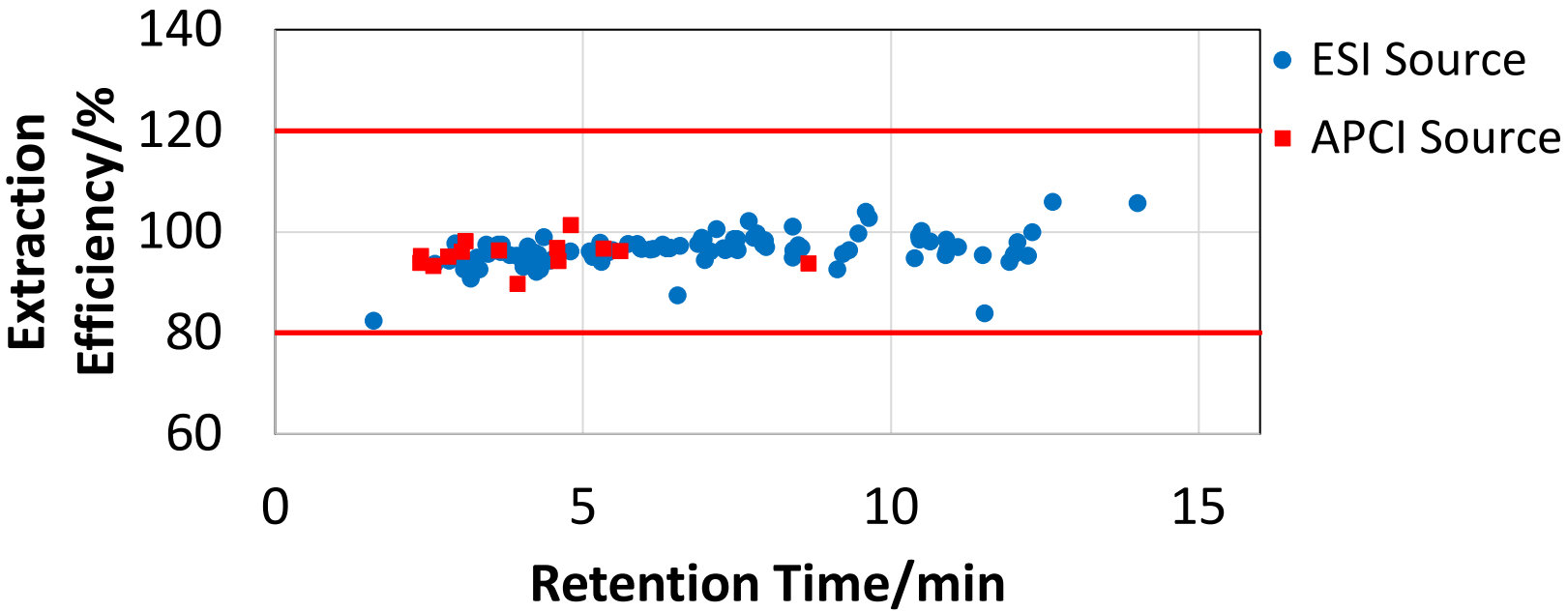
QSight® 420 LC/MSMS System - Completes the entire pesticide method

### DATA PROCESSING

Full Regulatory Compliance & Client COA Report

$$\text{Extraction Efficiency} = \frac{\text{Prespiked extract} * 100}{\text{Postspiked Extract}}$$

# Extraction Efficiency with Solvent Extraction



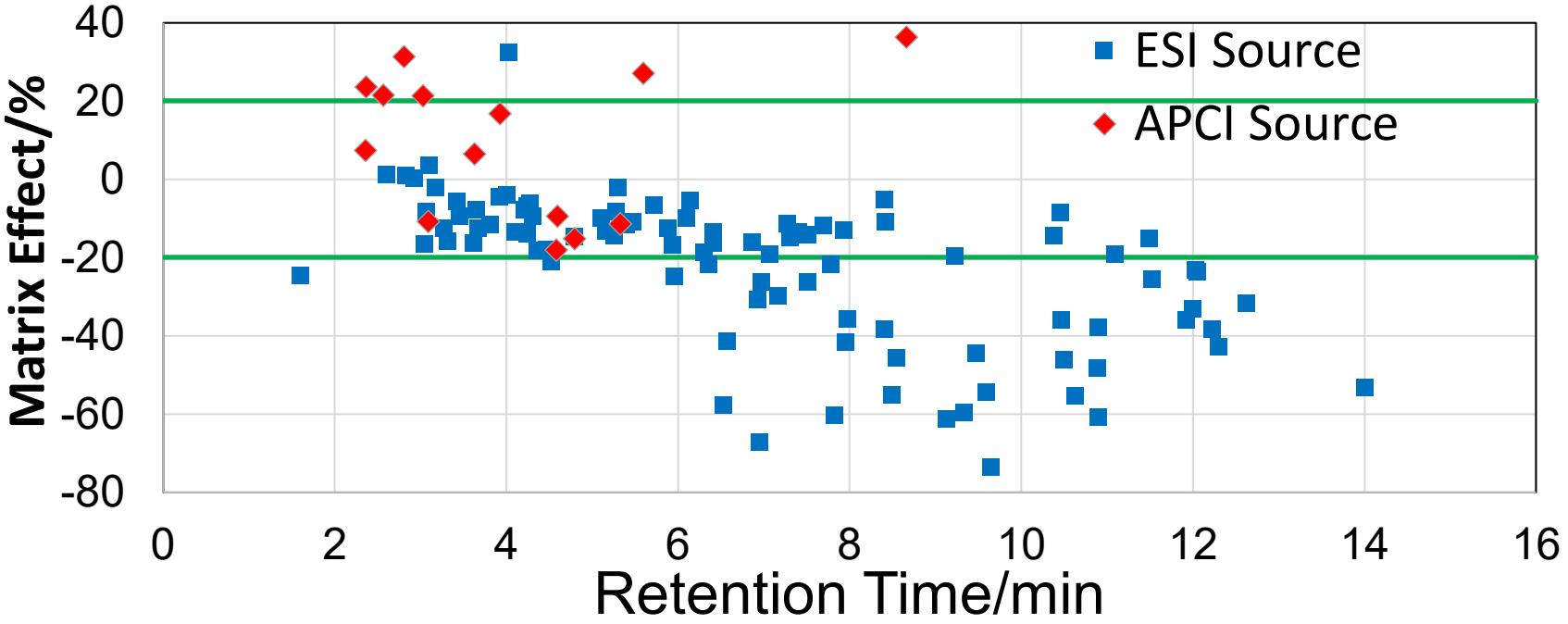
# Extraction Efficiency – Different Sample Preparation

| Analyte | QuEChERS with dSPE(1) | Solvent Extraction/SPE/ dSPE(2) | Solvent Extraction/ Evaporation(3) | Solvent Extraction |
|---|---|---|---|---|
| Abamectin | ND | 18% | ND | 94% |
| Fenpyroximate | 74% | 61% | 44% | 97% |
| Imazalil | 49% | 21% | 114% | 93% |
| Pyridaben | 68% | 58% | ND | 95% |
| Spirotetramat | 76% | 63% | 132% | 98% |


1. www.sigmaaldrich.com
2. Moulins, et al., J. AOAC International, (2021) vol. 101, 1948-1960.
3. Michlig, et al., J. Chrom. A (2021), doi:https://doi.org/10.1016/j.chroma.2021.462097

$$\text{Matrix Effect} = \frac{(\text{Postspiked extract} - \text{solvent standard}) * 100}{\text{Solvent standard}}$$

# Hemp Matrix Effect



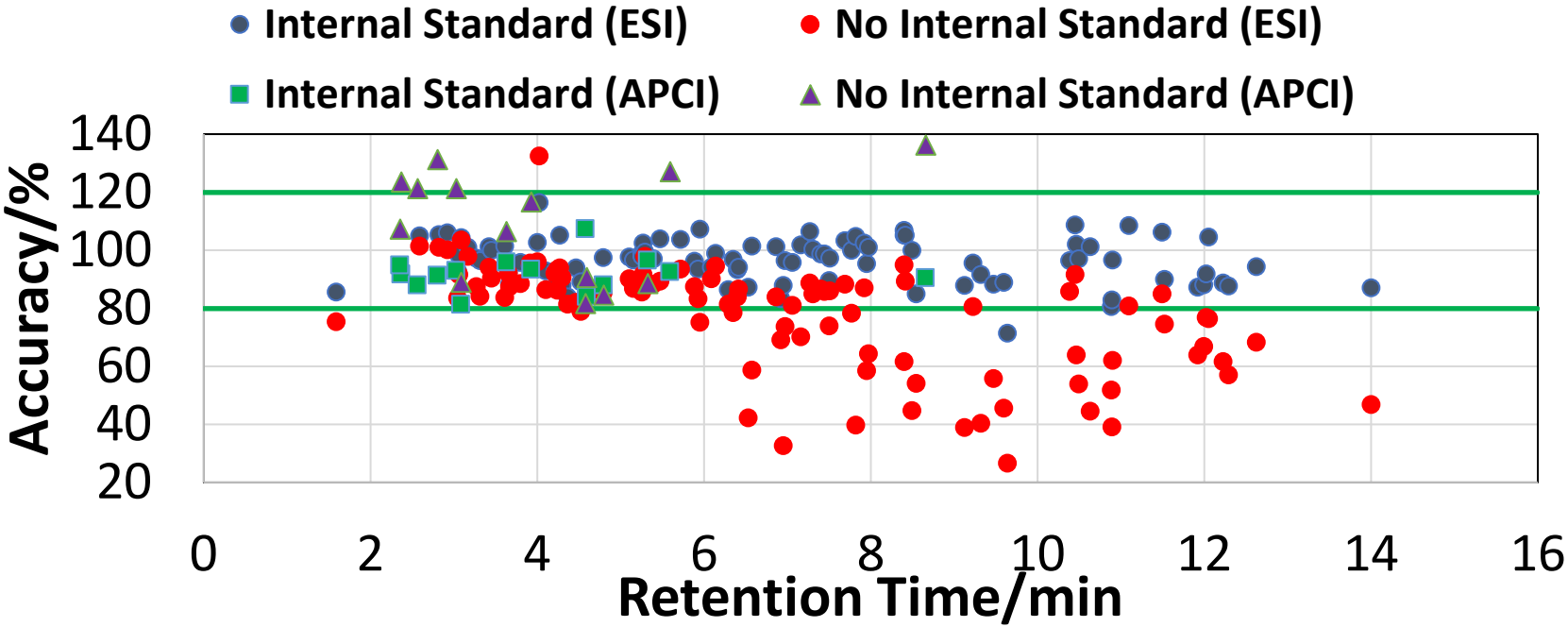
# Internal Standards – Accounting Matrix Effects

| Internal Standard |
| --- |
| Acequinocyl-d25 |
| Atrazine-d5 |
| Bifernthrin- d5 |
| Boscalid-d4 |
| Captan-d6 |
| Carbaryl-d7 |
| Chlorpyrifos (Diethyl-d10) |
| Coumaphos-d10 |
| Daminozide-d4 |
| Diazinon-d10 |
| Dichlorvos-d6 |
| Dimethoate-d6 |
| Doramectin |
| Fenoxycarb-d3 |
| Fipronil-$^{13}$C2,$^{15}$N2 |

| Internal Standard |
| --- |
| Fludioxonil-$^{13}$C3 |
| Imidacloprid-d4 |
| Malathion-d6 |
| Methylparathion-d6 |
| Myclobutanil-d9 |
| Naled-d6 |
| Ochratoxin-A-d5 |
| Pentachloronitrobenzene-$^{13}$C6 |
| Permethrin-d5 |
| Phosmet-d6 |
| Piperonylbutoxide-d9 |
| Pyridaben-d13 |
| Spirotetramat-d5 |
| Thiamethoxam-d3 |
| Trifloxystrobin-d6 |

$$\text{Accuracy} = \frac{\text{Measured Concentration} * 100}{\text{Spiked Concentration}}$$

# Method Accuracy (Internal Standard)



# Method LOQ and AL for Pesticides & Mycotoxins in Hemp

| Analyte | LOQ(AL)/ppm | Analyte | LOQ(AL)/ppm | Analyte | LOQ(AL)/ppm | Analyte | LOQ(AL)/ppm |
|---|---|---|---|---|---|---|---|
| Abamectin | 0.02(0.1) | Daminozide | 0.01(0.1) | Hexythiazox* | 0.005(0.01) | Propiconazole | 0.02(0.1) |
| Acephate | 0.005(0.02) | Deltamethrin | 0.02(0.5) | Imazalil | 0.01(0.05) | Propoxur | 0.005(0.02) |
| Acequinocyl* | 0.005(0.03) | Diazinon | 0.005(0.02) | Imidacloprid | 0.005(0.02 | Pyraclostrobin | 0.005(0.02) |
| Acetamiprid | 0.005(0.1) | Dichlorvos | 0.01(0.1) | Iprodione* | 0.01(1) | Pyrethrins | 0.02(0.05) |
| Aldicarb | 0.01(1) | Dimethoate | 0.005(0.02) | Kinoprene | 0.1(0.5) | Pyridaben | 0.005(0.05) |
| Allethrin | 0.05(0.2) | Dimethomorph | 0.01(0.05) | Kresoxim-methyl | 0.01(0.02) | Pyriproxyfen | 0.005(0.01) |
| Atrazine | 0.005(0.025) | Dinotefuran | 0.005(0.1) | (Lambda) Cyhalothrin | 0.05(0.25) | Quintozene* | 0.01(0.02) |
| Azadirachtin | 0.05(1) | Diuron | 0.005(0.125) | Malathion | 0.01(0.02) | Resmethrin | 0.02(0.1) |
| Azoxystrobin | 0.005(0.02) | Dodemorph | 0.005(0.05) | Metalaxyl | 0.005(0.02) | Spimetoram | 0.01(0.02) |
| Benzovindiflupyr | 0.005(0.02) | Endosulfan sulfate* | 0.01(0.05) | Methiocarb | 0.01(0.02) | Spinosad | 0.01(0.1) |
| Bifenazate | 0.005(0.02) | Endosulfan-alpha* | 0.1(0.2) | Methomyl | 0.005(0.05) | Spirodiclofen | 0.02(0.25) |
| Bifenthrin | 0.01(1) | Endosulfan-beta* | 0.01(0.05) | Methoprene | 0.2(2) | Spiromesifen | 0.01(3) |
| Boscalid | 0.01(0.02) | Ethoprophos | 0.005(0.02) | Mevinphos | 0.01(0.05) | Spirotetramat | 0.01(0.02) |
| Buprofezin | 0.005(0.02) | Etofenprox | 0.01(0.05) | MGK-264 | 0.01(0.05) | Spiroxamine | 0.005(0.1) |
| Carbaryl | 0.01(0.05) | Etoxazole | 0.005(0.02) | Myclobutanil | 0.005(0.02) | Tebuconazole | 0.005(0.05) |
| Carbofuran | 0.005(0.02) | Etridiazole* | 0.01(0.03) | Naled | 0.01(0.1) | Tebufenozide | 0.005(0.02) |
| Chlorantraniliprole | 0.005(0.02) | Fenhexamid | 0.01(0.125) | Novaluron | 0.005(0.05) | Teflubenzuron | 0.01(0.05) |
| Chlorfenapyr* | 0.02(0.05) | Fenoxycarb | 0.005(0.02) | Oxamyl | 0.005(3) | Tetrachlorvinphos | 0.005(0.02) |
| Chlorpyrifos* | 0.01(0.04) | Fenpyroximate | 0.005(0.02) | Paclobutrazol | 0.01(0.02) | Tetramethrin | 0.02(0.1) |
| Clofentezine | 0.005(0.02) | Fensulfothion | 0.01(0.02) | Parathion-methyl* | 0.005(0.05) | Thiabendazole | 0.01(0.02) |
| Clothianidin | 0.005(0.05) | Fenthion | 0.005(0.02) | Permethrin | 0.01(0.5) | Thiacloprid | 0.005(0.02) |
| Coumaphos | 0.005(0.02) | Fenvalerate* | 0.02(0.1) | Phenothrin | 0.02(0.05) | Thiamethoxam | 0.005(0.02) |
| Cyantraniliprole | 0.005(0.02) | Fipronil* | 0.005(0.06) | Phosmet | 0.01(0.02) | Thiophanate-methyl | 0.01(0.05) |
| Cyfluthrin | 0.2(0.2) | Flonicamid | 0.005(0.05) | Piperonyl butoxide | 0.02(0.2) | Trifloxystrobin | 0.005(0.02) |
| Cypermethrin | 0.1(0.3) | Fludioxonil* | 0.005(0.02) | Pirimicarb | 0.005(0.02) | Aflatoxin(B1+B2+G1+G2) | 0.012(0.020) |
| Cyprodonil | 0.01(0.25) | Fluopyram | 0.005(0.02) | Prallethrin | 0.01(0.05) | Ochratoxin A | 0.004(0.005) |

# Conclusions

- **LC-MS/MS method with dual APCI and ESI source analyzes all of pesticides and mycotoxins regulated by different USA states and Canada to meet their action limits in hemp**
- **Matrix interference and matrix effects were minimized by MS and LC method optimization and by changing mode of ionization to improve method sensitivity**
- **Solvent extraction can achieve high throughput with automation, less cost and excellent extraction efficiency**
- **Internal standards compensated for matrix effects such as ion suppression/enhancement to get overall accuracy of 70-120 %**

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# Thank you for your Attention!

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