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Differentiating Cannabis and Hemp:

An Evaluation of Some HPLC Methodologies

Craig S. Young
HPLC Product Manager

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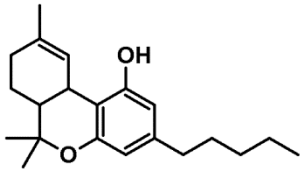
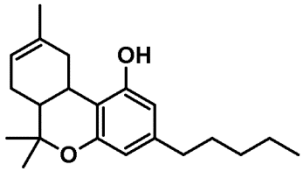
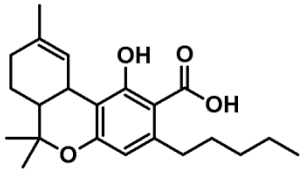
Cannabinoids of current interest in cannabis and hemp



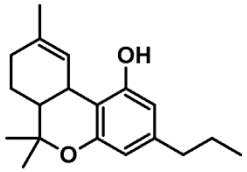
d9-Tetrahydrocannabinol
(d9-THC)



d8-Tetrahydrocannabinol
(d8-THC)



d9-Tetrahydrocannabinolic acid
(THCA)

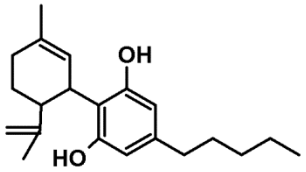


Tetrahydrocannabivarin
(THCV)

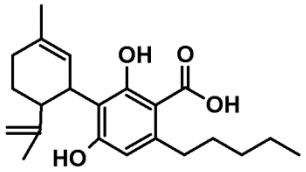
Cannabichromenic acid
(CBCA)

Cannabicyclol
(CBL)

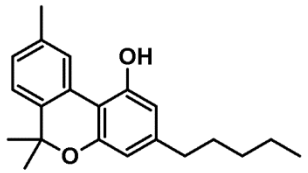
Cannabichromevarin
(CBCV)



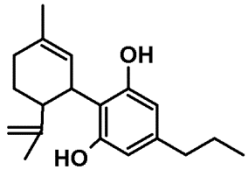
Cannabidiol
(CBD)



Cannabidiolic acid
(CBDA)



Cannabinol
(CBN)

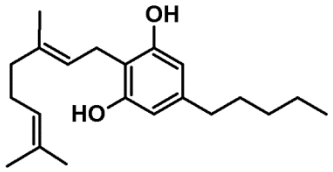


Cannabidivarin
(CBDV)

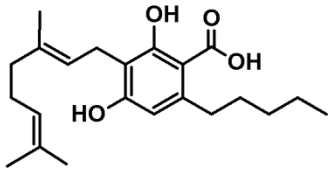
Tetrahydrocannabivarinic acid
(THCVA)

Cannabidivarinic acid
(CBDVA)

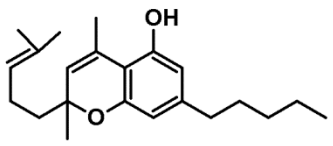
Cannabidiphorol
(CBDP)



Cannabigerol
(CBG)



Cannabigerolic acid
(CBGA)



Cannabichromin
(CBC)

Cannabichromeorcin
(CBCO)

Cannabicitran
(CBT)

d8-Tetrahydrocannabinolic acid
(d8-THCA)

d9-Tetrahydrocannabiphorol
(d9-THCP)

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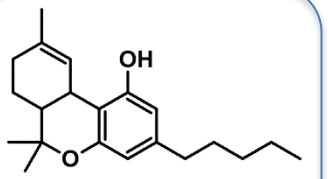
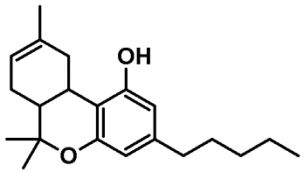
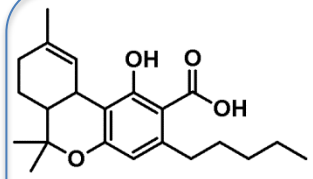
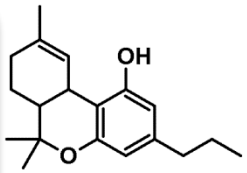
Cannabinoids of current interest in cannabis and hemp


d9-Tetrahydrocannabinol
(d9-THC)


d8-Tetrahydrocannabinol
(d8-THC)

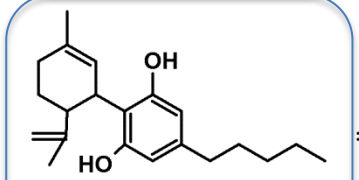

d9-Tetrahydrocannabinolic acid
(THCA)

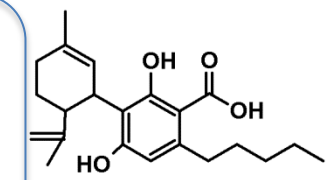

Tetrahydrocannabivarin
(THCV)

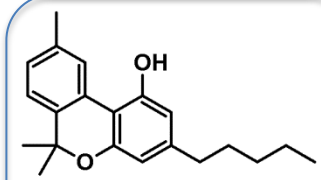
Cannabichromenic acid
(CBCA)

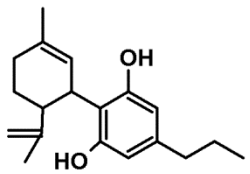
Cannabicyclol
(CBL)

Cannabichromevarin
(CBCV)


Cannabidiol
(CBD)


Cannabidiolic acid
(CBDA)

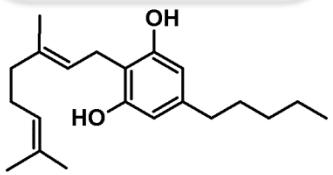

Cannabinol
(CBN)

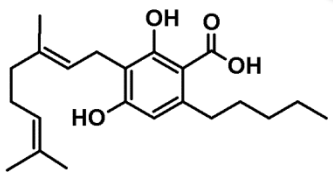

Cannabidivarin
(CBDV)

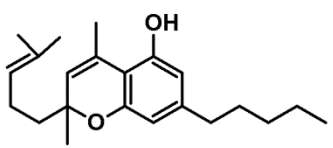
Tetrahydrocannabivarinic acid
(THCVA)

Cannabidivarinic acid
(CBDVA)

Cannabidiphorol
(CBDP)


Cannabigerol
(CBG)


Cannabigerolic acid
(CBGA)


Cannabichromin
(CBC)

Cannabichromeorcin
(CBCO)

Cannabicitran
(CBT)

d8-Tetrahydrocannabinolic acid
(d8-THCA)

d9-Tetrahydrocannabiphorol
(d9-THCP)

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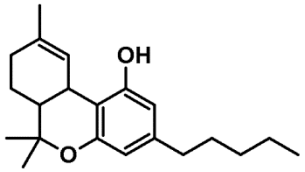
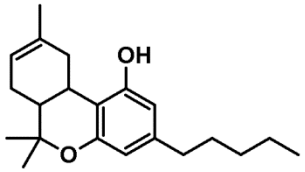
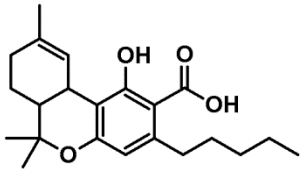
Cannabinoids of current interest in cannabis and hemp



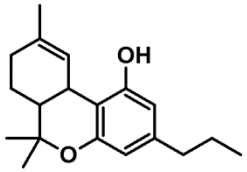
d9-Tetrahydrocannabinol
(d9-THC)



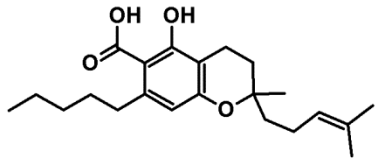
d8-Tetrahydrocannabinol
(d8-THC)



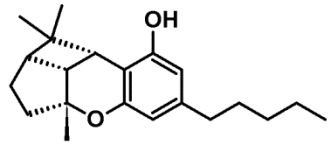
d9-Tetrahydrocannabinolic acid
(THCA)



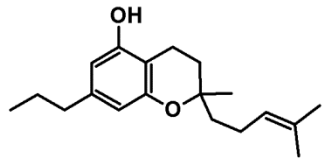
Tetrahydrocannabivarin
(THCV)



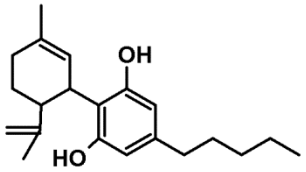
Cannabichromenic acid
(CBCA)



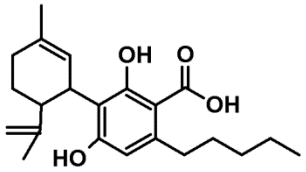
Cannabicyclol
(CBL)



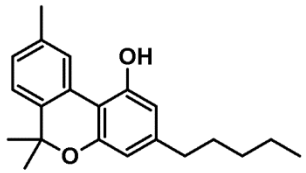
Cannabichromevarin
(CBCV)



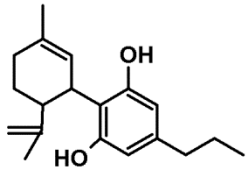
Cannabidiol
(CBD)



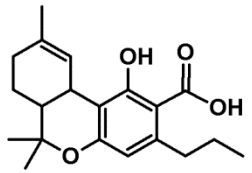
Cannabidiolic acid
(CBDA)



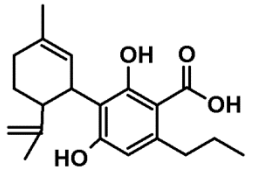
Cannabinol
(CBN)



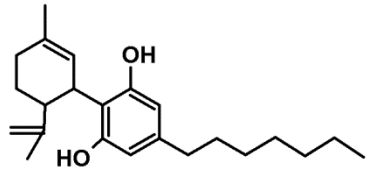
Cannabidivarin
(CBDV)



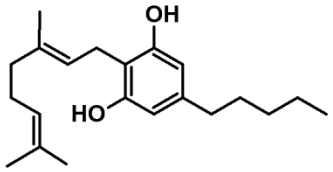
Tetrahydrocannabivarinic acid
(THCVA)



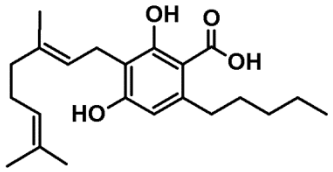
Cannabidivarinic acid
(CBDVA)



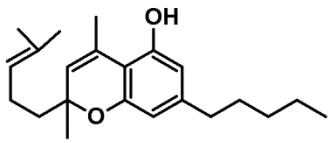
Cannabidiphorol
(CBDP)



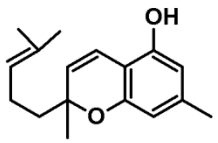
Cannabigerol
(CBG)



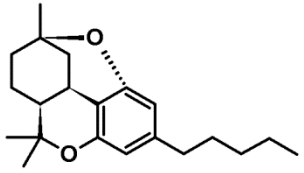
Cannabigerolic acid
(CBGA)



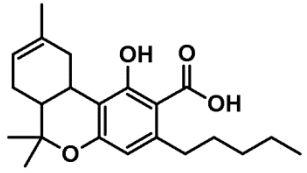
Cannabichromin
(CBC)



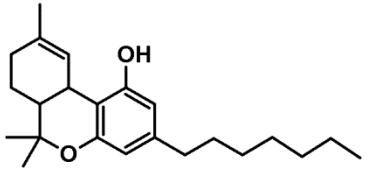
Cannabichromeorcin
(CBCO)



Cannabicitran
(CBT)



d8-Tetrahydrocannabinolic acid
(d8-THCA)



d9-Tetrahydrocannabiphorol
(d9-THCP)

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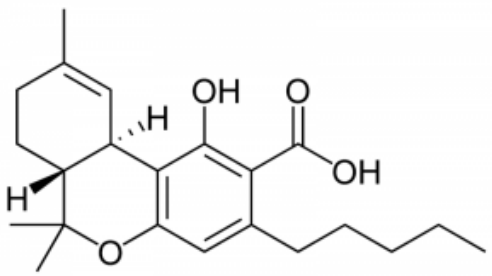
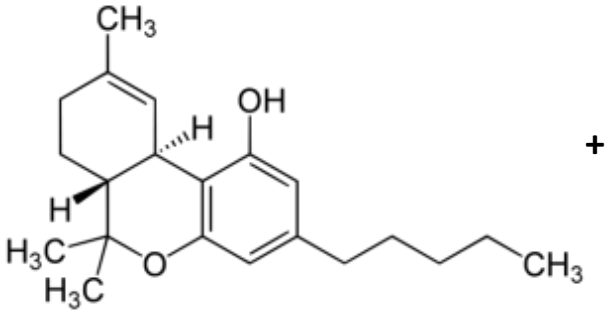
The Chemistry of Decarboxylation



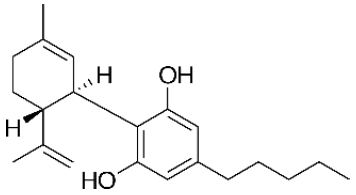
THCA

$\xrightarrow[h\gamma]{\triangle}$

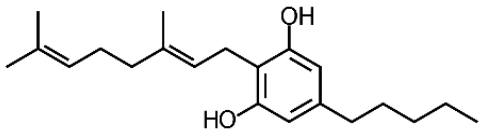


d9-THC

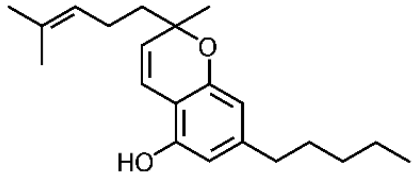
+ CO_2



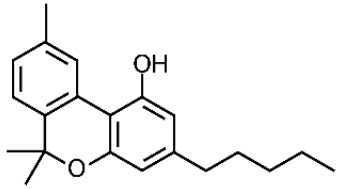
CBD



CBG



CBC



CBN

Cannabis/Hemp and Industry Background

- QC testing for cannabinoids is essential for the accurate labeling of cannabis products in both medical and recreational cannabis markets. Cannabis “potency” is normally reserved for the quantitation of the major cannabinoids, principally THCA, and d9-THC, and CBD.

- **HPLC** has emerged as the gold standard for potency determinations because separation and detection of all cannabinoids is done without causing any decomposition of the THCA.

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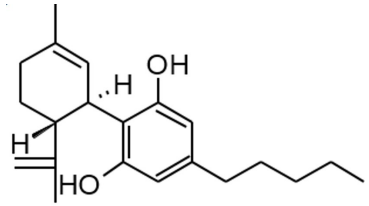
Cannabis/Hemp and Industry Background



Cannabidiol
(CBD)

CBD is non-psychoactive, but valuable for its medicinal properties:

- Pain mitigation
- Calming
- Anti-seizure
- Anti-nausea

The main source of CBD-rich oil is industrial hemp. Hemp is considered a rustic plant as it is frost resistant, adapts to poor soil, reproduces easily, and does not require chemical fertilizers, pesticides, herbicides, or fungicides to thrive. A hemp crop tends to resist mildew and requires less water than cotton. Hemp textiles are considered softer than cotton.

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Cannabis/Hemp – The Legal Definition

U.S. Hemp Farming Act, December, 2018

The law differentiates hemp from cannabis on the basis of its d9-THC content. Hemp is considered < 0.3% by dry weight.

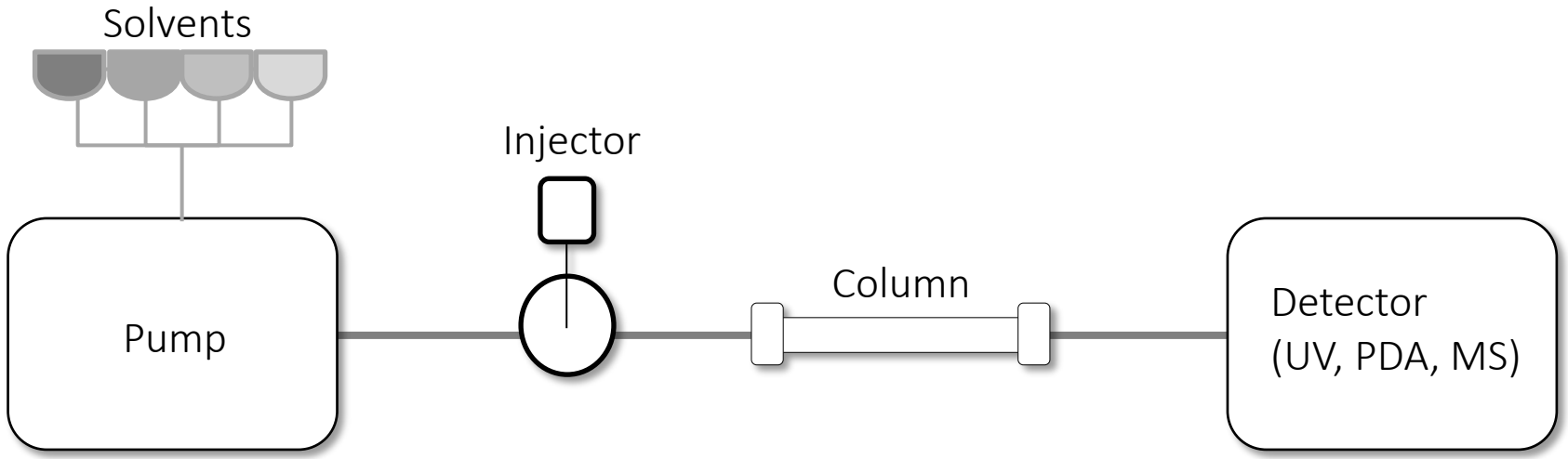
USDA Interim Final Rule, October, 2019

1. Labs conducting chemical analysis must be DEA registered to handle materials exceeding the 0.3% limit.

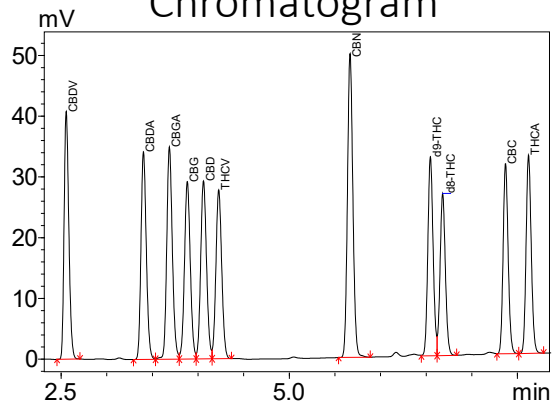
2. Redefinition of 0.3%. The "Total THC Rule." Hemp is now defined as a product with no more than 0.3% Total THC in order to take into account its potential for total psychoactive content. Testing must either be performed "post-decarboxylation" -OR- the Total THC must be taken into account, which is the sum concentration of THC-A and d9-THC.

Potency Testing by HPLC

HPLC – High Performance Liquid Chromatography



Chromatogram



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Potency Testing by HPLC – Instrumentation

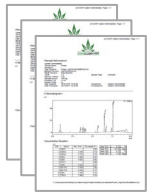
Cannabis and Hemp Analyzers

- Based on i-Series Integrated HPLC
- Built-in UV or Diode Array
- Complete, turn-key packages

Mobile Phase

Certified Standards

Column, Guard Columns



App Notes Reports

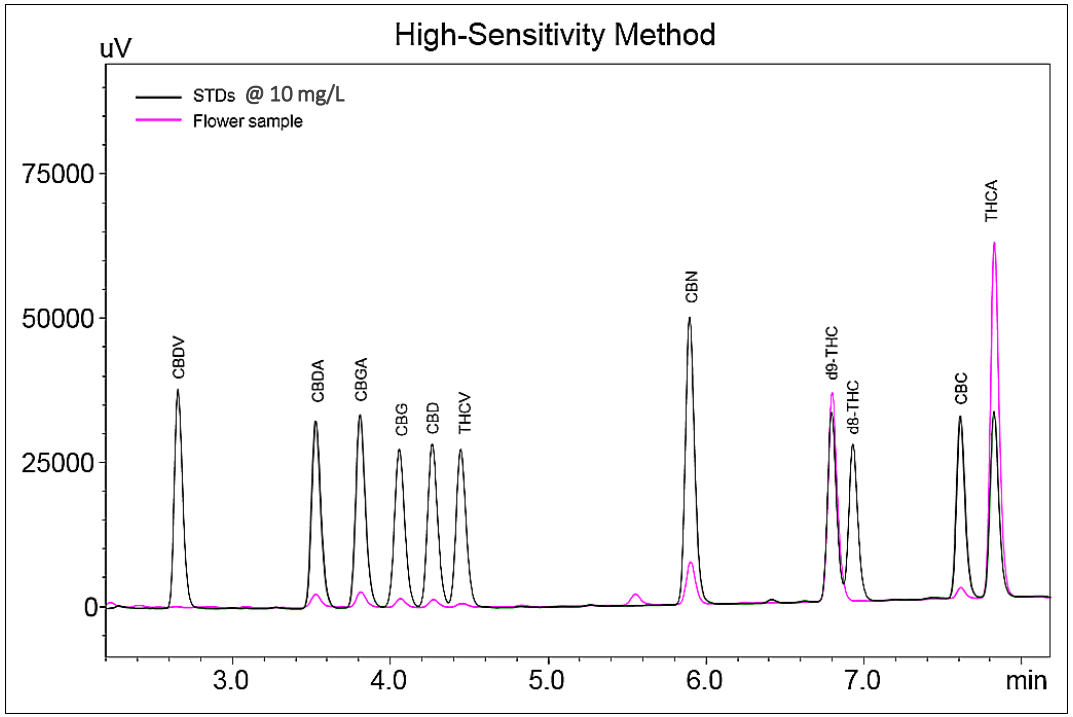
Method Package

40-Series Modular (U)HPLC

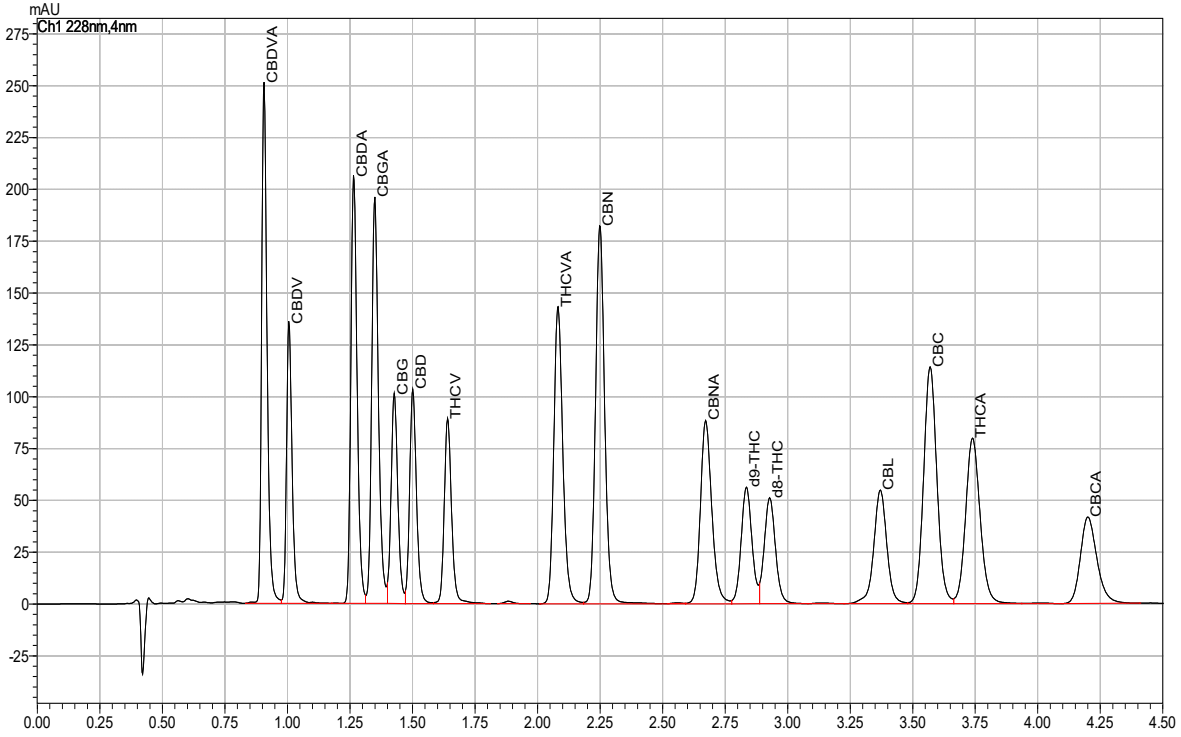
- HPLC/UHPLC
- Flexible/Expandable
- High throughput capable
- AI features
- Superb MS front end

Potency Testing by HPLC – Instrumentation

- HPLC
- Cannabinoids: 10-11
- Medium Throughput - 10 minutes



- UHPLC
- Cannabinoids: 16
- High Throughput - 5 minutes



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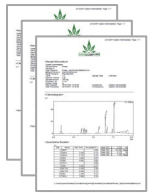
The Cannabis Analyzer for Potency

Mobile Phase

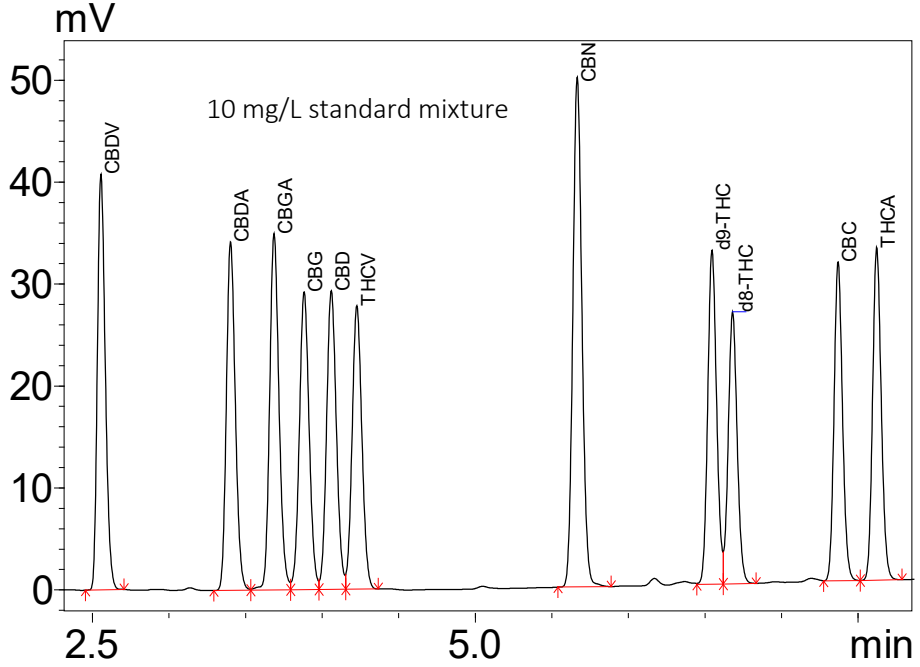
Certified Standards

Column, Guard Columns



App Notes
Reports

Method Package



High Sensitivity Method

- The most popular Analyzer method.
- Forgiving, dependable workflow, yet with reasonable sample time frame and good resolution.
- Quantitative for 11 cannabinoid targets incl. THCV – 144 samples per 24 hr. day
- Sensitivity (ug/mL; ppm; 0.1% by dry weight)

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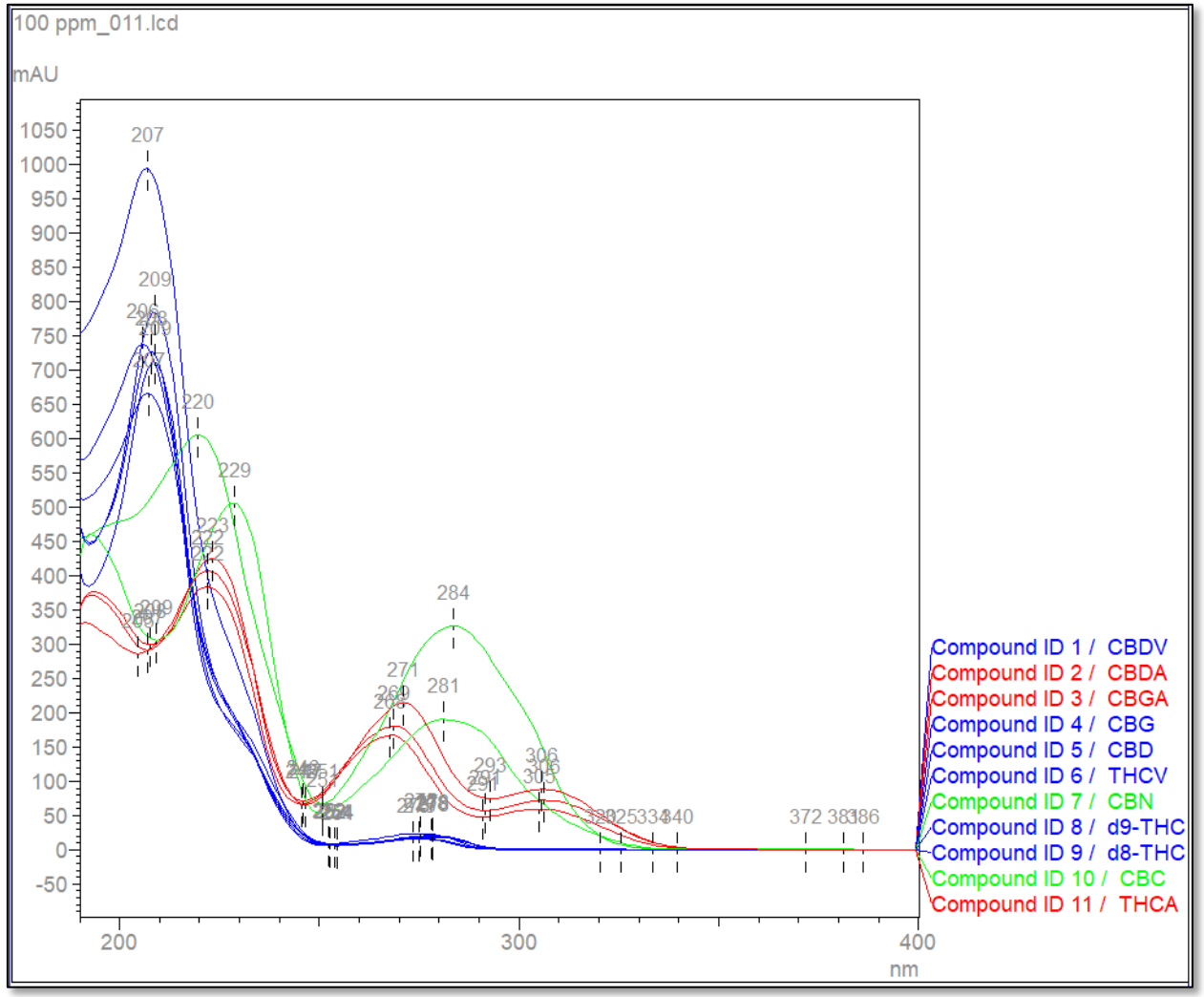
The Value of LC with Diode Array Detection



- For Cannabinoids, diode array detection may be diagnostic for cannabinoid class.
  (e.g. CBD vs CBDA; THC vs. THCA; etc.)

- It is not diagnostic for individual cannabinoids within a class.
  (e.g. CBD is indistinguishable from CBG)

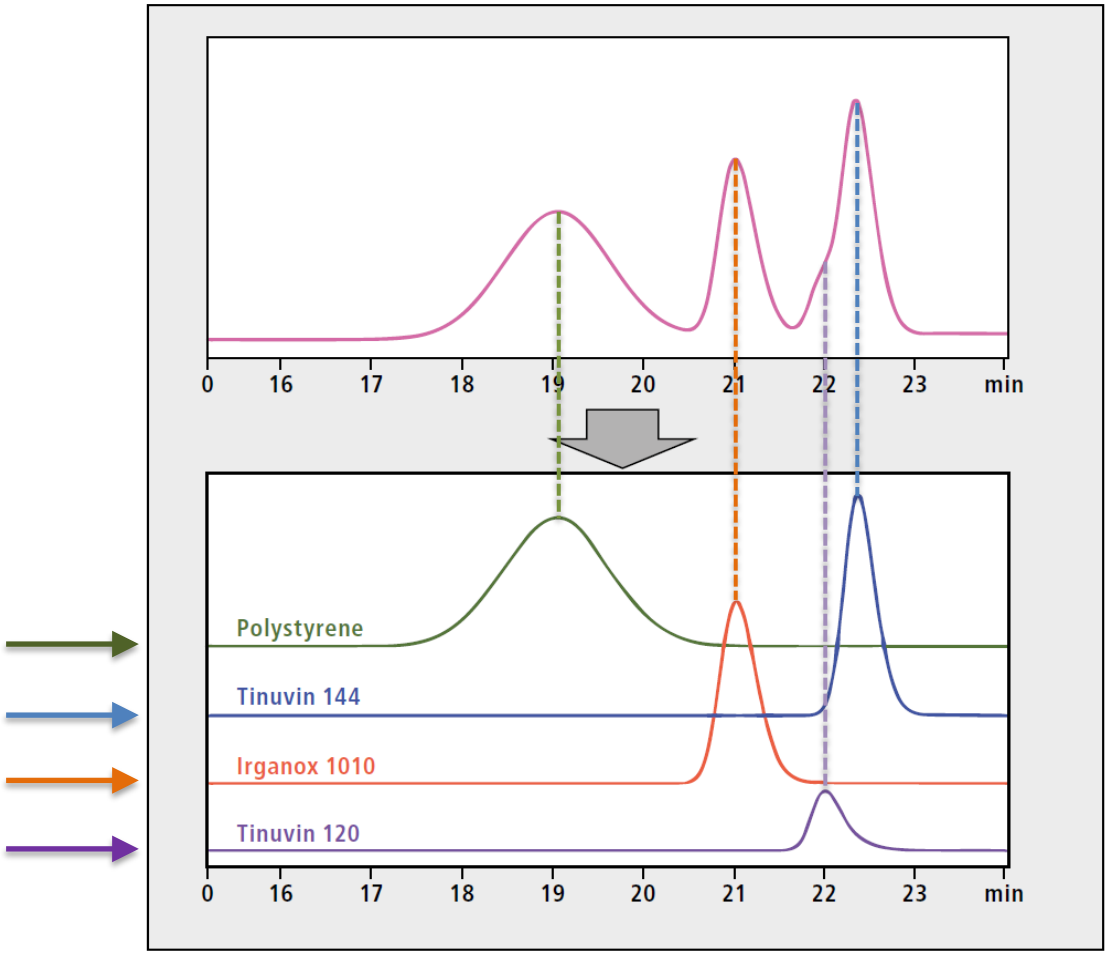
The Value of Diode Array Detection

ANALYTICAL INTELLIGENCE

i-PDeA II

Peak deconvolution made simple and automatic. The result is a virtual separation that can be used for quantitative determinations.

i-PDeA is a Shimadzu exclusive!



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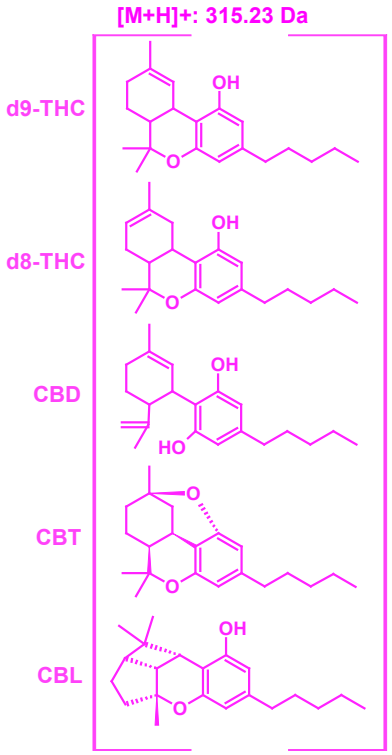
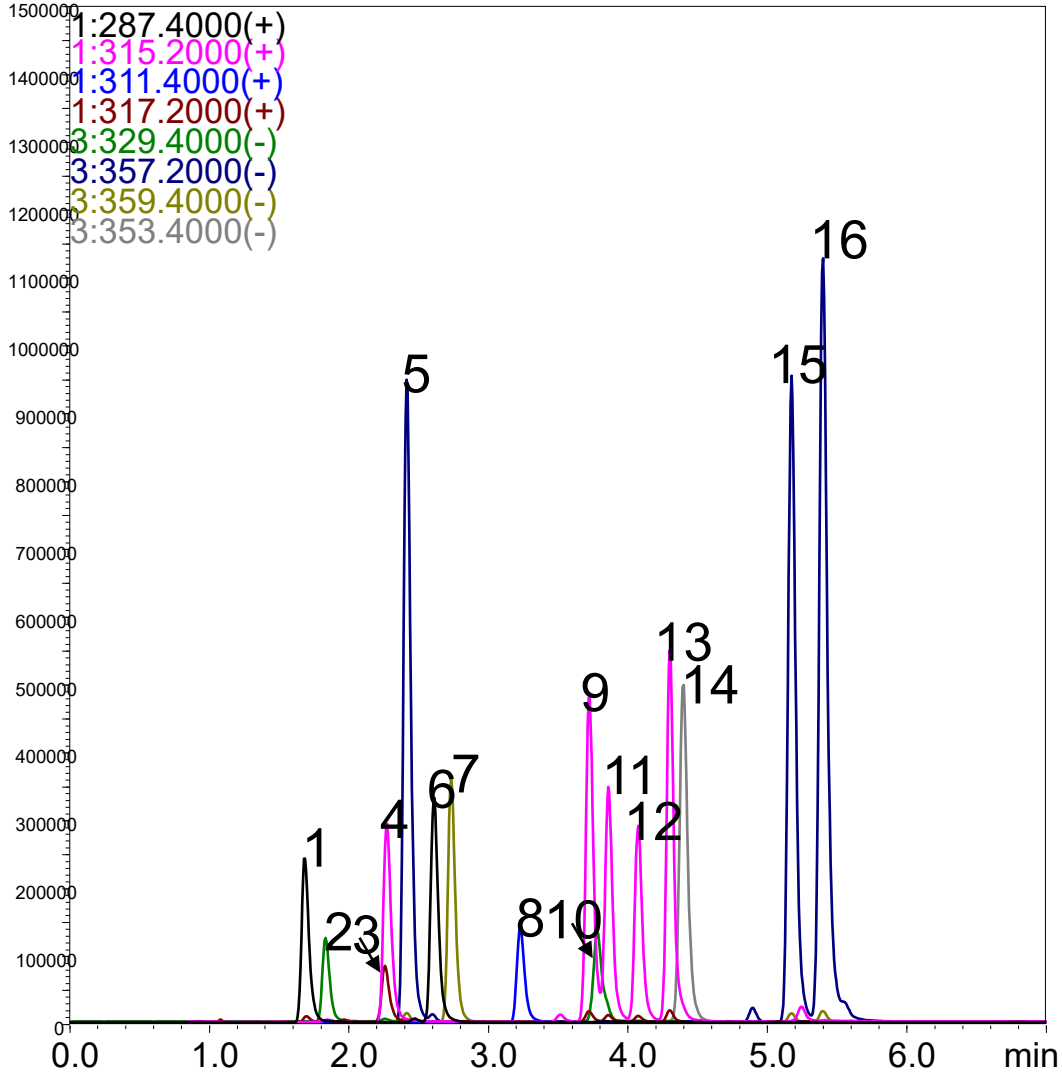
Using Single Quadrupole MS

i-Series LC-2050 (U)HPLC and LCMS-2020

- Mass-specific characterization
- Minimized matrix effects
- High sensitivity – ng/mL (ppb)
- Isobars are un-differentiated by MS alone





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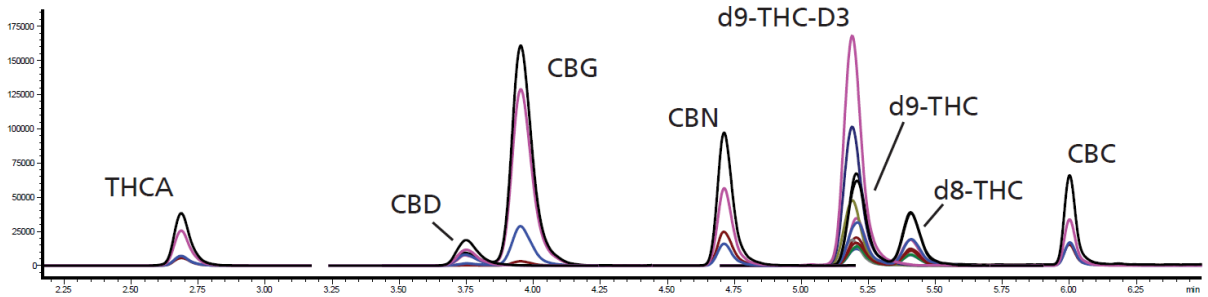
Using Triple Quadrupole MS

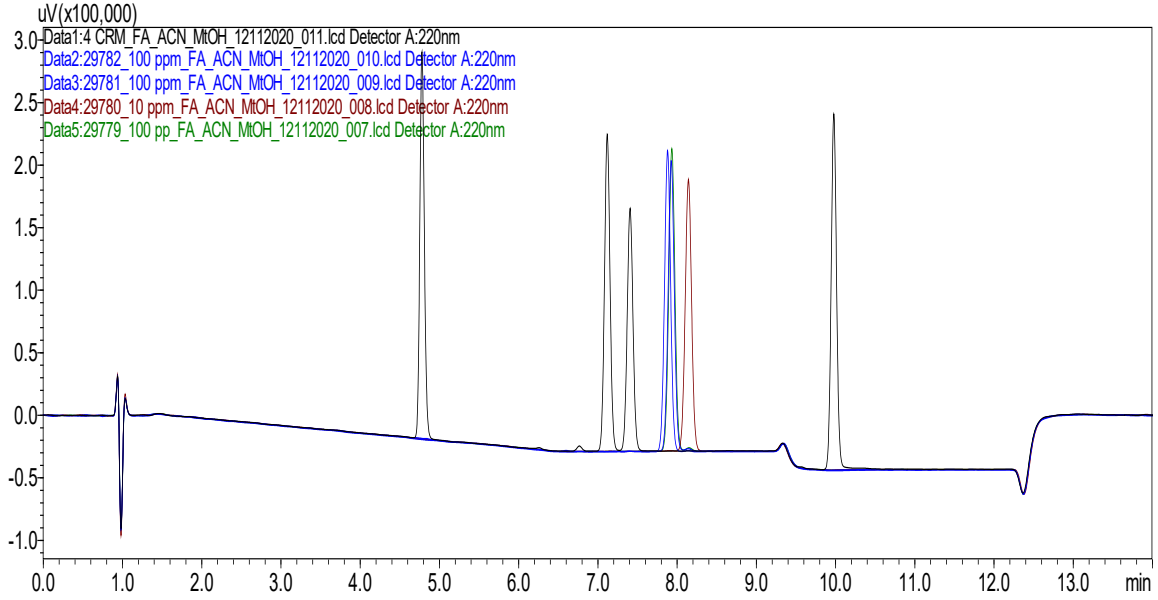
40-Series X3 (U)HPLC and LCMS-8060

- Mass-specific characterization
- Minimized matrix effects
- Highest sensitivity – fg/mL to pg/mL
- The ultimate in target specificity – mass transitions (fragment ions)
- Isobars are un-differentiated by MS alone

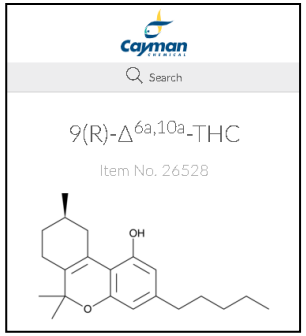
Target cannabinoids in Positive Mode
* $[M+H]^+$ with multiple transitions
* Incl. deuterium-labeled internal standards



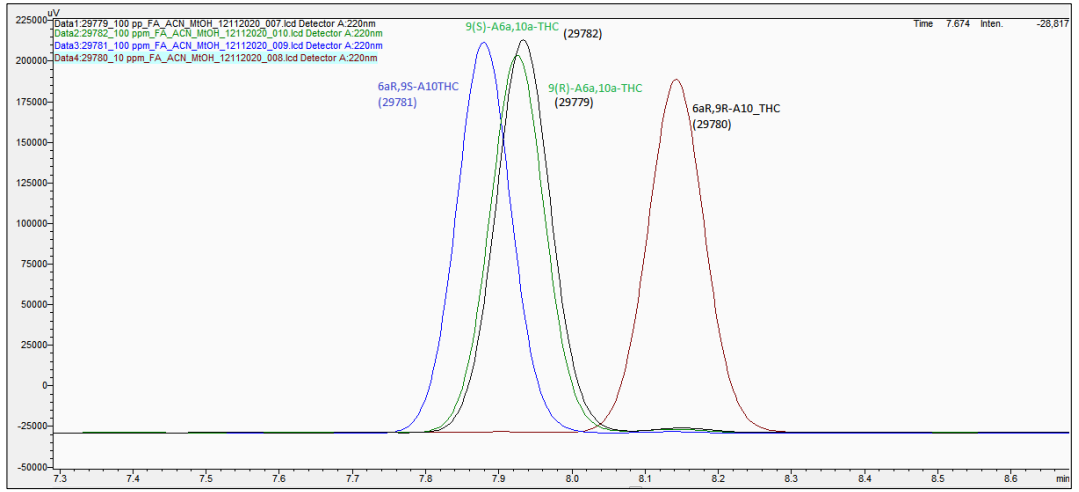
Topics of Current Interest



9(R)-$\Delta^{6a,10a}$-THC
9(S)-$\Delta^{6a,10a}$-THC
(6aR,9S)-Δ^{10}-THC
(6aR,9R)-Δ^{10}-THC



- Interest in new targets driven by academic research –AND- availability of standards
- Chiral pairs difficult to separate
- Diastereomeric pairs readily separated
- May prove impractical in reasonable time frames for real samples.



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Please see www.InvestigateYourLab.com

THANK YOU