

Agilent LC & LC-MS Cannabis Testing



Potency Analysis for Cannabinoids

Mike Adams¹, A. Roth¹, Joan Stevens²,
Karen Kaikaris¹, & Sue D'Antonio²

¹ CWC LABS, ² Agilent Technologies,

RELIABLE ANALYTICAL WORKFLOWS FOR CANNABIS TESTING

AGILENT SCIENCE & TECHNOLOGY SYMPOSIUM



UV detection with UHPLC separation.....



1220



1260 Infinity II



1290 Infinity II

RELIABLE ANALYTICAL WORKFLOWS FOR CANNABIS TESTING

AGILENT SCIENCE & TECHNOLOGY SYMPOSIUM

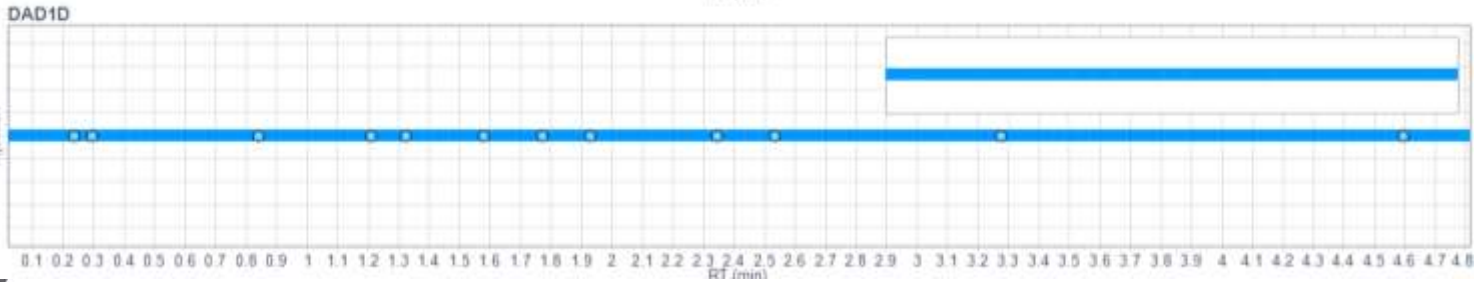


Tetrahydrocannabivarin (THCV)
(-)-trans- Δ 9-tetrahydrocannabinol (THC)
Cannabidiol (CBD)
Cannabigerol (CBG)
Tetrahydrocannabinolic acid (Δ 9-THCA)
Cannabidiolic Acid (CBDA)
Cannabinol (CBN)
Cannabigerolic acid (CBGA)
Cannabichromene (CBC)
Cannabidivarin (CBDV)

7696A SAMPLE PREP WORKBENCH

The new Agilent 1260 or 1290 Infinity II LC!

Significantly improved Workflow Solution!



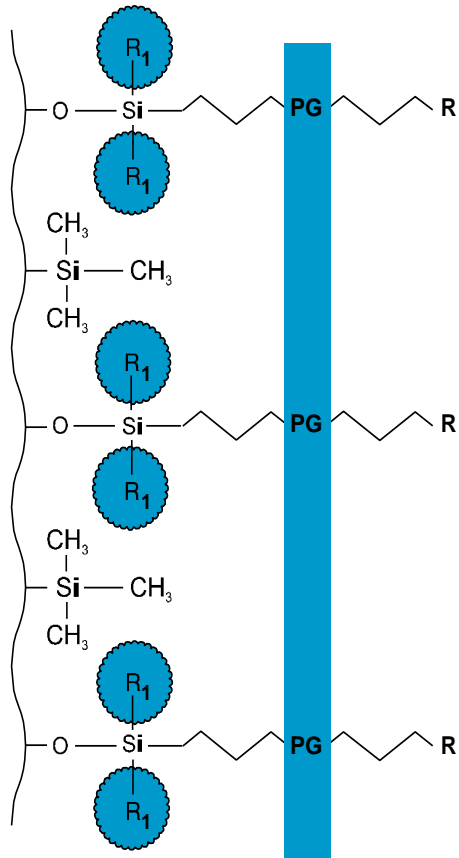
Method development system:
Up to 8 columns (used 6)
Flexible pump (quaternary)

Only limited by your imagination

Method scouting wizard (builds sequences based on your methods, tells how much solvent and sample)

Using **Peak Explorer** (OpenLab II) you can review chromatograms in a sequences quickly

Zorbax Bonus-RP Polar-linked alkyl phase



Superb peak shape for basic compounds

Long column life (pH 2-8)

Unique selectivity

Patented bonding technology

- polar-linked alkyl phase for fast mass transfer giving good peak shape
- bulky side groups give low pH stability
- triple endcapped for mid-range pH stability and good peak shape



Buffer:

1. 1 vial (2.2 mL) of Ammonium Formate (G1946-85021)
2. 1 mL of Formic Acid (G2452-85060)
3. QS to 1 Liter of LC-MS grade H₂O

Caveat: make sure to flush columns (methanol) after use

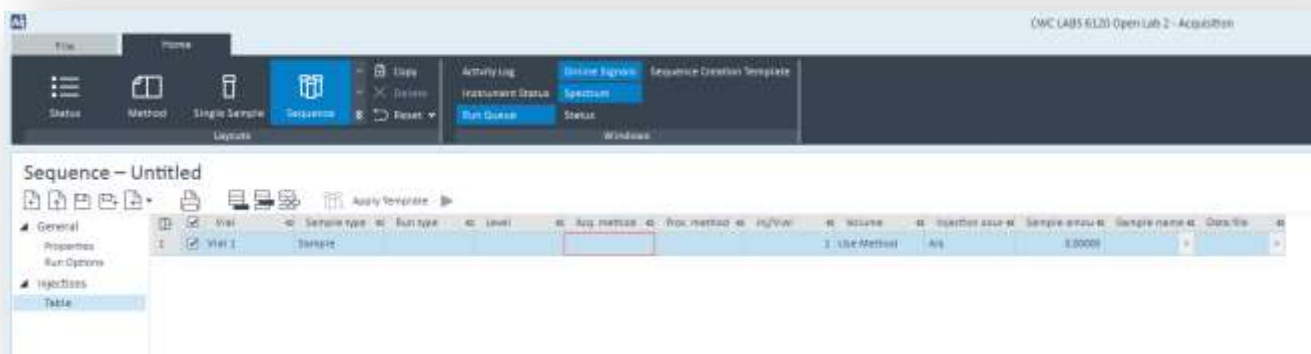
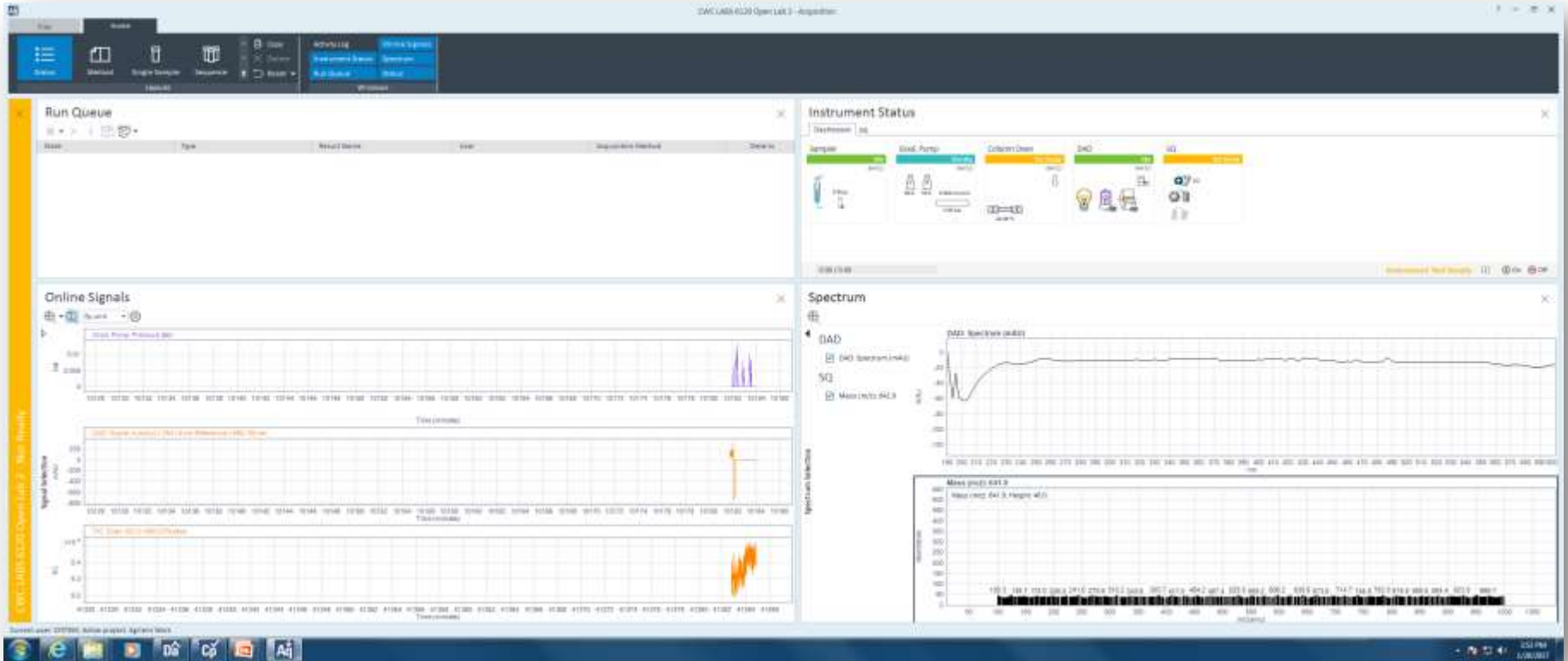
Testing this method for ruggedness with the QBD approach

QBD: Quality by design

1. We used the method development column kit. Three columns of the same type from 3 different lots.
2. We ran the system of three different types of instruments
3. We ran the method with 3 different operators making the mobile phase



The Open Lab 2.1 data acquisition



Just drop an MS-Excel® file into your sequence screen

Ease of use from ALS systems including workbench

HPLC Conditions for short method (5 minutes)

Agilent 1290 Infinity II UHPLC series Quaternary Pump, Multisampler with wash , Multi Column Thermostat, DAD

Column: Zorbax Bonus RP 2.1 x 50 mm, 1.8 μ m or Poroshell 3.0 x50 2.7 μ m

Column temperature: 50°C

Injection volume: 0.05 μ L

Autosampler temp: 23 °C

Needle wash: 3.5 s Flush Port (25:25:50)

(H₂O:IPA:MeOH)

DAD-UV 254 nm

Mobile phase: A = Water **Binary pump - A is buffer**

B= Methanol

C= 0.1% CH₂O₂ + 2.2 ml 5M NH₄formate in H₂O

Flow rate: 0.5 mL/min

Gradient:

Time (min)	%B	%C
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0.0	72	5
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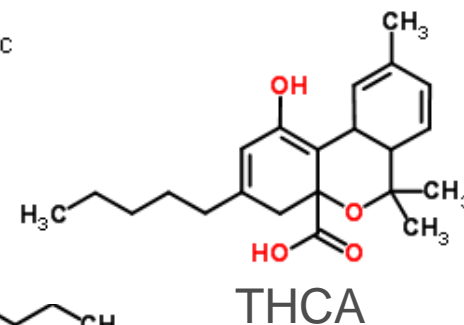
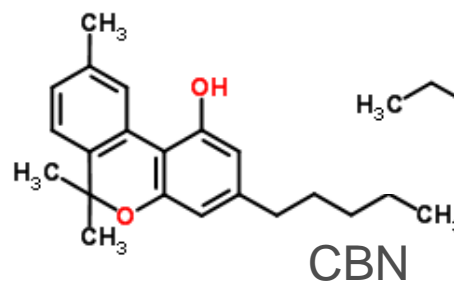
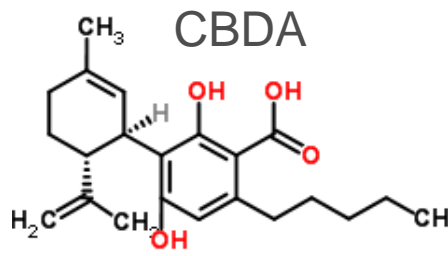
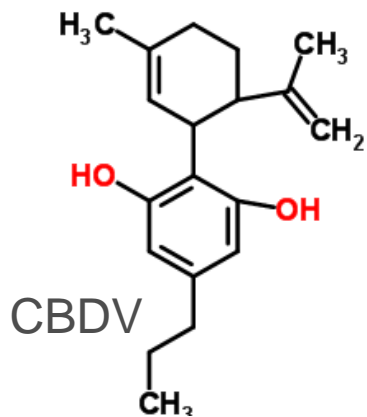
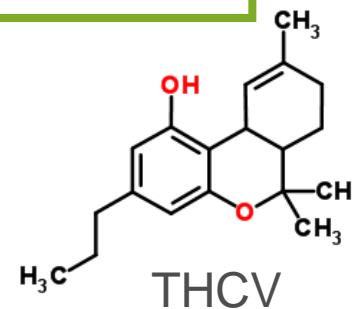
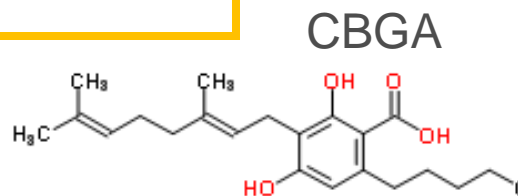
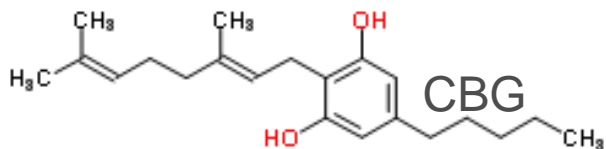
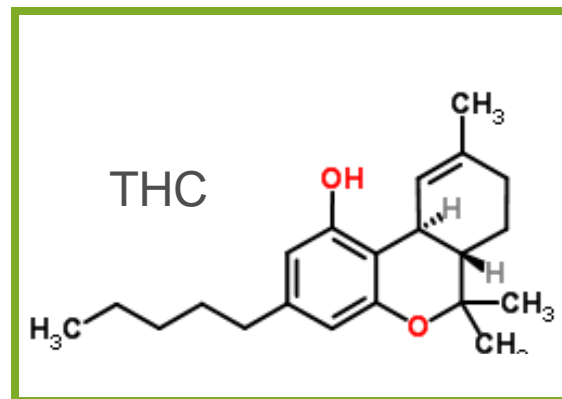
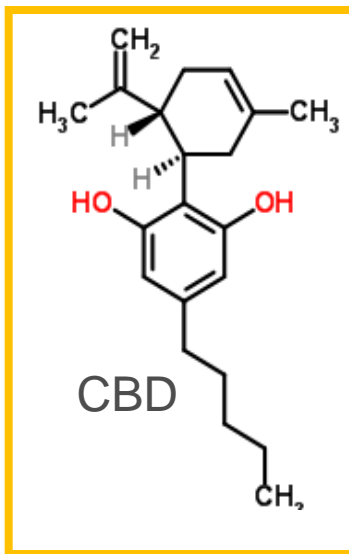
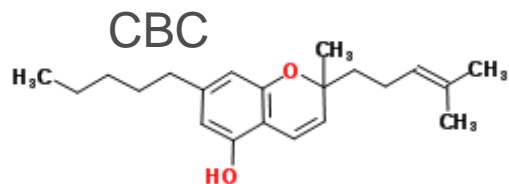
6.25	95	5
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Stop time: 5.00 min.

Post time: 1.0 min.

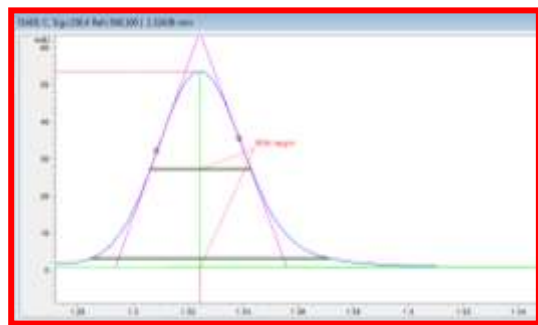
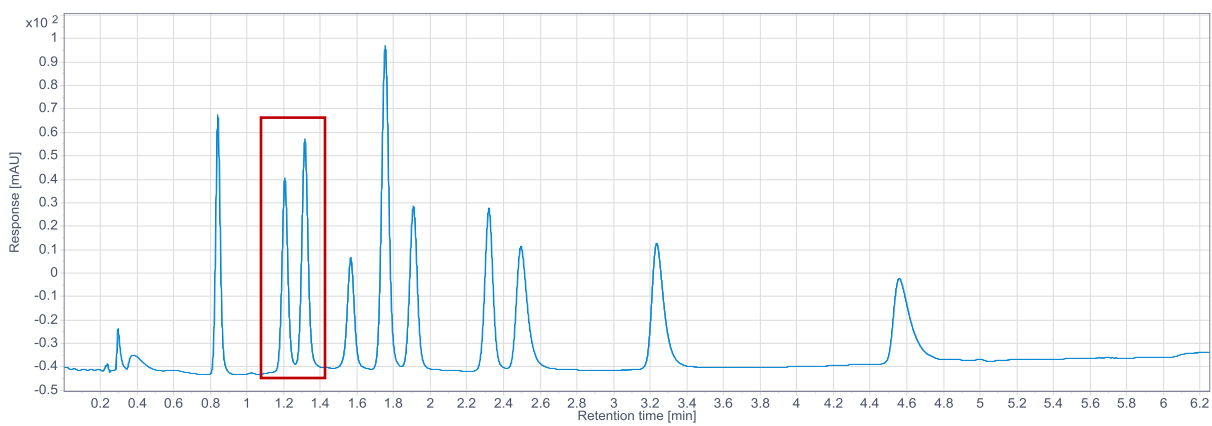
Overall run time 6.0 minutes (incl. re-equilibration)

Chemical Structures of Top 10 Cannabinoids

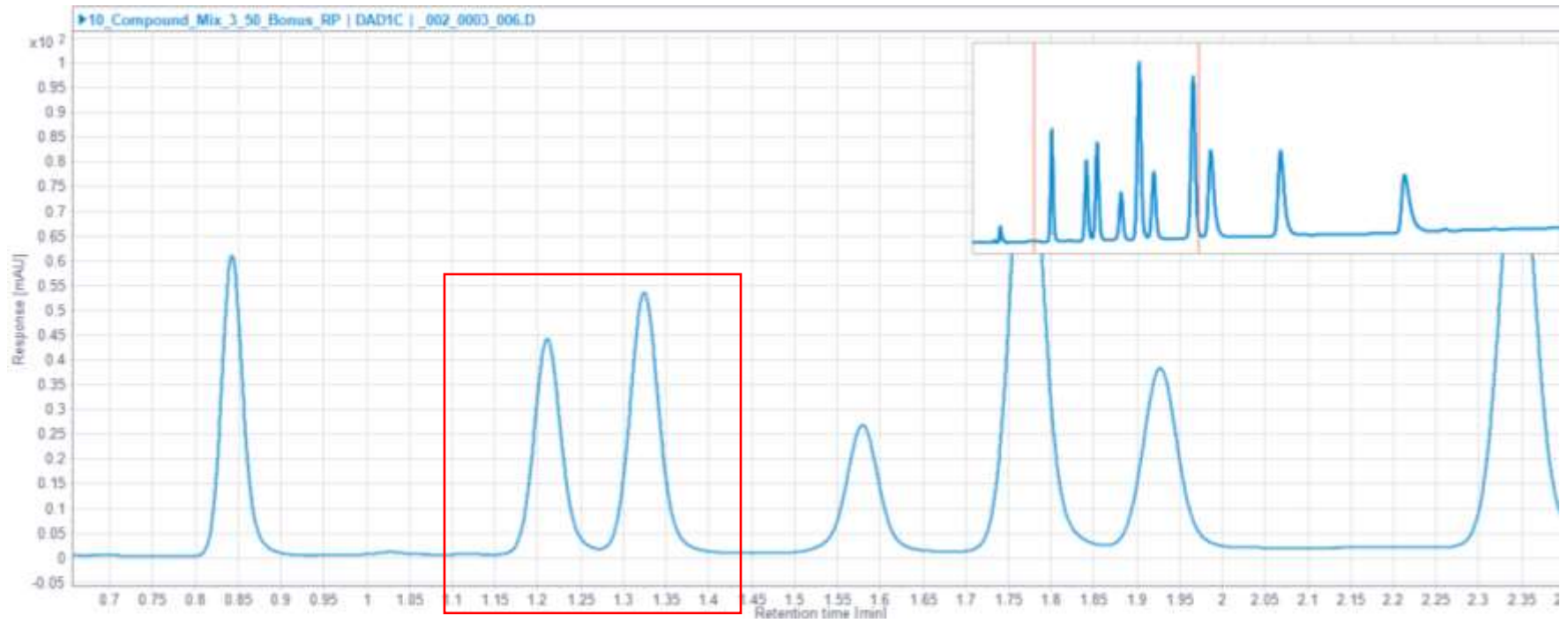


<http://herb.co/2016/02/06/top-10-cannabinoids/>

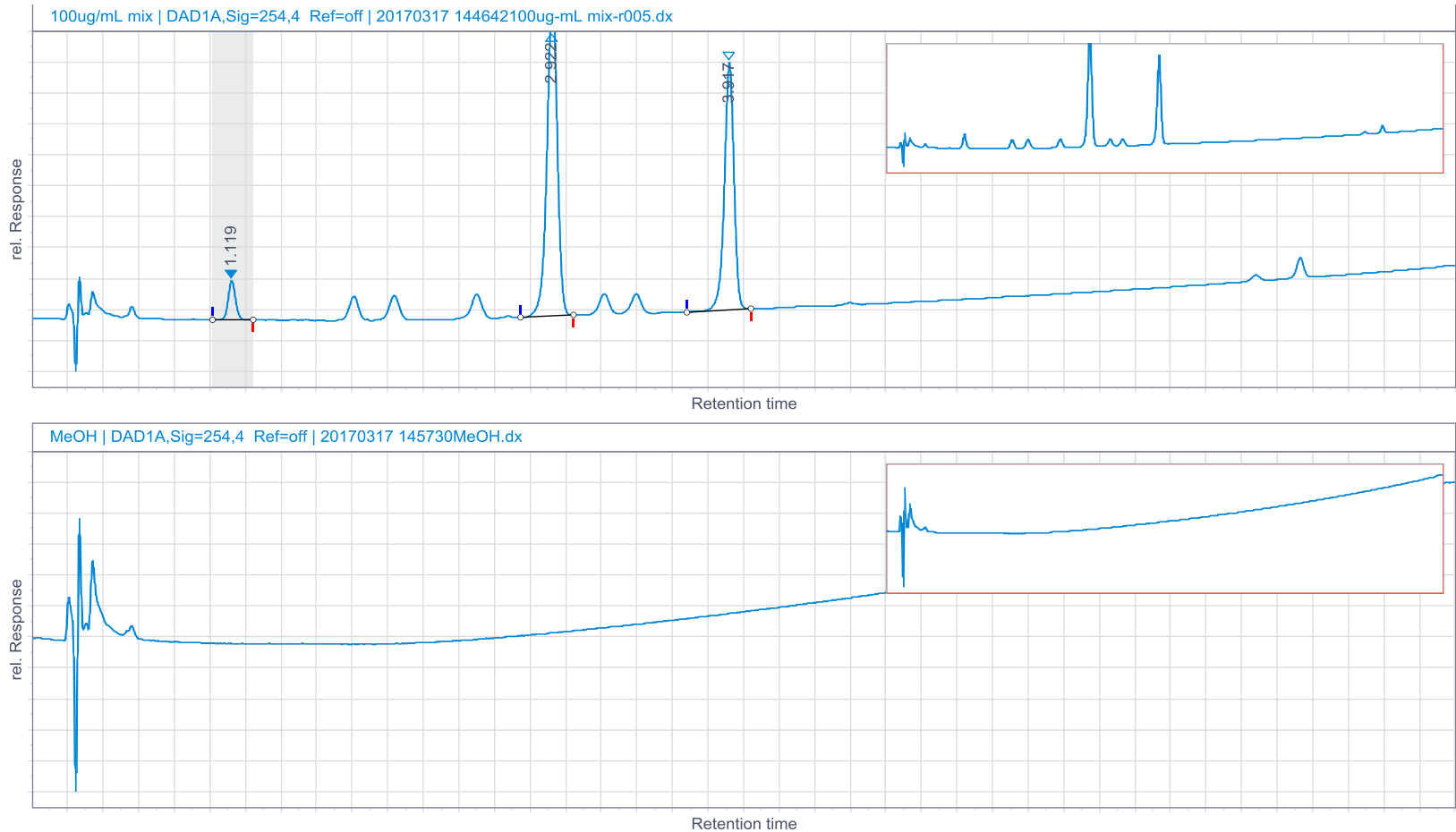
10 cannabinoids under 5 minutes with 1.86 Resolution between the critical pair



Resolution to prev peak	1.86442
Resolution to next peak	3.84507



Carryover



Carry over with the Infinity II vial sampler 1300 Bar addition (G7129A) with needle wash. Blanks after a 10 ul injection of 1 mg/ml standard.

Note the chromatography will a 10 ul injection at that concentration saturates the column

A single click adds a peak to the calibration and assigns a compound name

The screenshot displays the Agilent OpenLab software interface for data analysis. The main window is titled "THC and CBD data - Data Analysis". The interface is divided into several panels:

- Data Processing:** Shows a tree view of data files, including "10-10 mix - 3.D" and "10-10 mix - 8.D".
- Processing Method:** A table with columns for Type, Name, and Signal. The table lists 12 compounds: CBDV, TRCV, CBC, CBG, CPD_UV, CBV, CBD, CBGA_UV, CBDA, THC, CBGA-UV, and CBGA.
- Chromatograms:** A plot showing Response (mAU) versus Retention Time (min). The x-axis ranges from 0.2 to 110 minutes. Several peaks are labeled with their retention times: 11.206, 11.204, 11.828, 11.819, 27.781, 33.344, 33.344, and 33.344. A peak at 33.344 minutes is highlighted with a blue box.
- Peak Explorer:** A detailed view of the selected peak at 33.344 minutes. It shows a chromatogram and a mass spectrum. The mass spectrum is titled "DAD1 B, Sig=280.4 Ref=360.50".

A context menu is open over the peak at 33.344 minutes, listing the following actions:

- Add peak as compound to method
- Assign compound...
- Add reference spectrum to method
- Extract spectrum
- Extract MS spectrum corresponding to peak
- Extract Chromatograms...
- Copy to clipboard
- Export to file...

Assigning compounds for calibration and create reference spectra

The screenshot displays the Agilent ChemStation interface. The main window is titled 'Processing Method' and shows a '10 peak method'. A 'Compound Table' is visible with the following data:

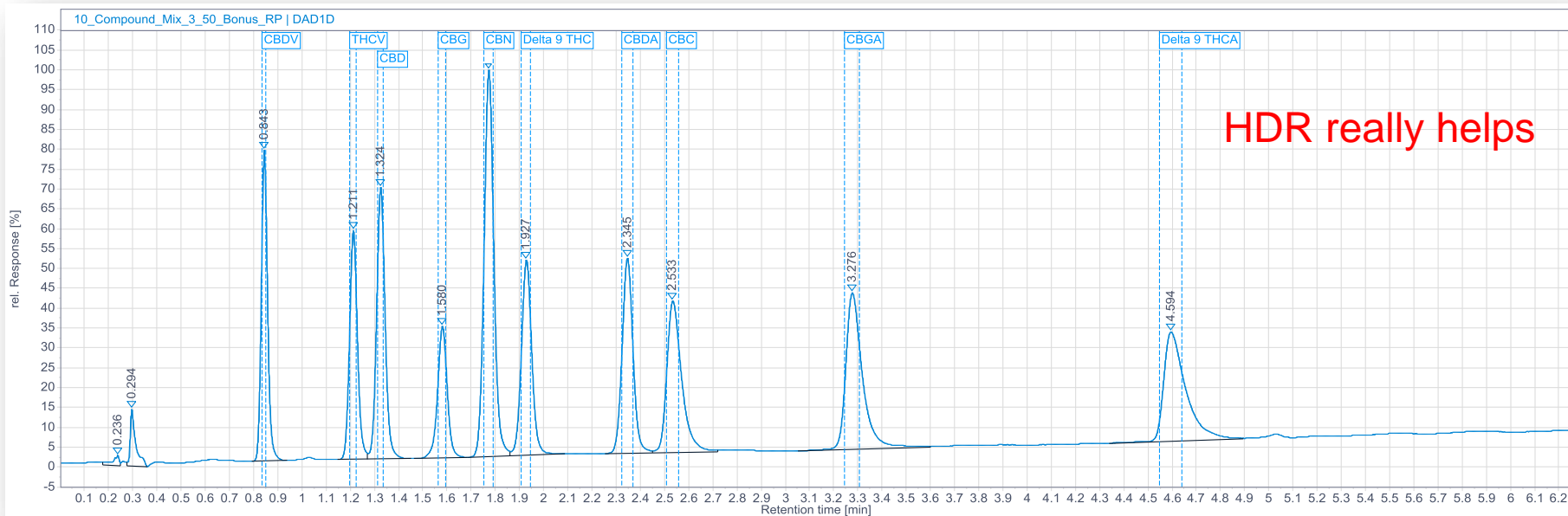
#	Type	Name	Signal
1	☺	CBDV	MSD1 +SIM(287.2) ESI Frag=70V
2	☺	THCV	MSD1 +SIM(287.2) ESI Frag=70V
3	☺	CBC	MSD1 +SIM(315.2) ESI Frag=70V
4	☺	CBG	MSD1 +SIM(317.3) ESI Frag=70V
5			
6			
7			
8			
9			
10			
11			

A context menu is open over the table, showing options such as 'Add peak as compound to method', 'Assign compound...', 'Un-assign compound', and 'Add reference spectrum to method'. Below the table, a 'Chromatograms' section shows a plot of '10 mix | DAD1 B, Sig=280,4 Ref=360,50 | 1.D'. The plot shows several peaks, with the most prominent one at approximately 3.041 minutes. A 'Peak Details' window is overlaid on the right, showing a zoomed-in view of this peak. The peak is labeled 'CBDVA_UV' and has the following parameters:

- RT: 3.041
- Area: 13.848
- Height: 2.223
- UV Purity: 274.6

The 'Peak Details' window also shows a chromatogram plot with 'Response [mAU]' on the y-axis and 'Retention time [min]' on the x-axis. The peak is highlighted with a blue line and a grey shaded area. Below the plot, there is a green and red bar chart representing the UV and MS signals respectively.

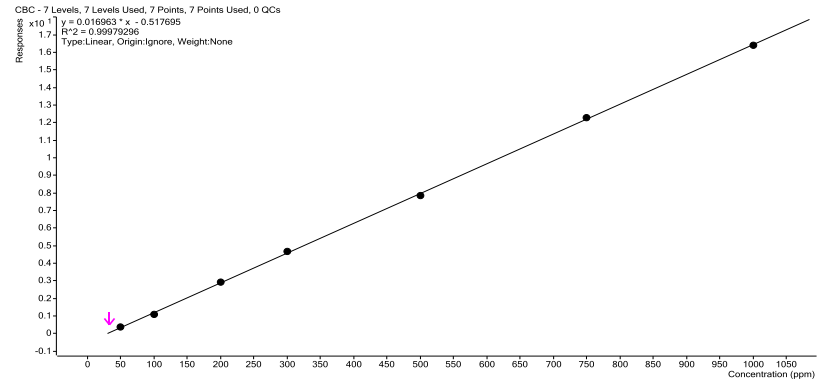
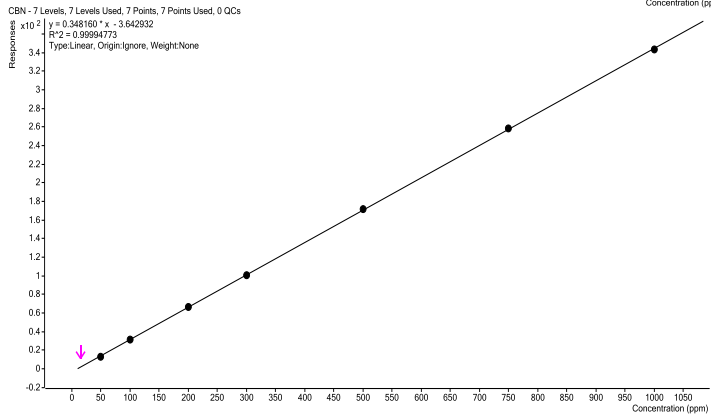
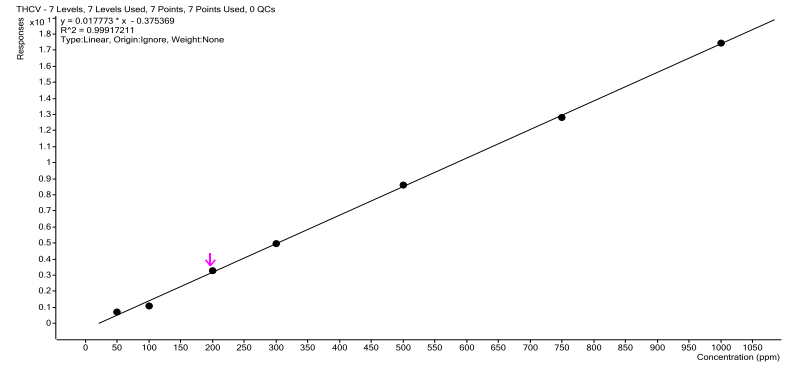
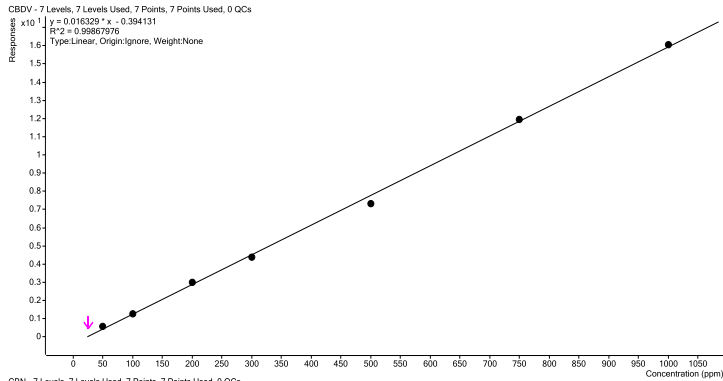
10 cannabinoids under 5 minutes @ 230 nm



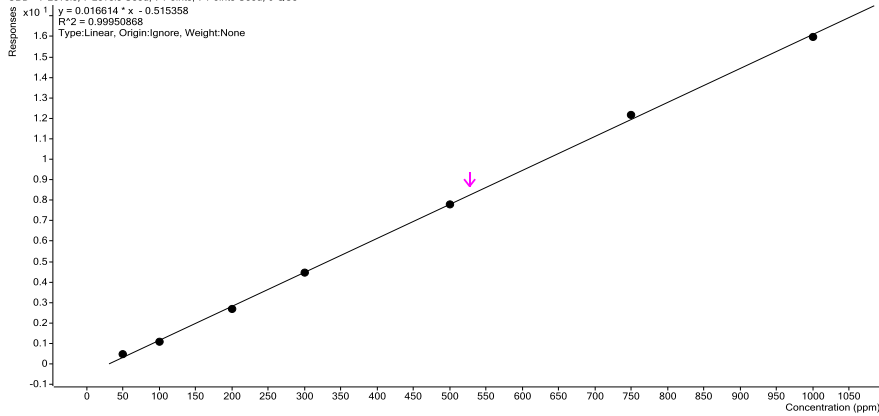
Compound Table			Qualifier Setup		General				
#	Type	Name	Signal	Exp. RT	Absolute RT window	Relative RT window	RT update	RT update	Peak match
1	☞	CBDV	DAD1D	0.843	0.000	1.000	Never	50.000	Closest
2	☞	THCV	DAD1D	1.211	0.000	1.000	Never	50.000	Closest
3	☞	CBD	DAD1D	1.324	0.000	1.000	Never	50.000	Closest
4	☞	CBG	DAD1D	1.580	0.000	1.000	Never	50.000	Closest
5	☞	CBN	DAD1D	1.772	0.000	1.000	Never	50.000	Closest
6	☞	Delta 9 THC	DAD1D	1.927	0.000	1.000	Never	50.000	Closest
7	☞	CBDA	DAD1D	2.345	0.000	1.000	Never	50.000	Closest
8	☞	CBC	DAD1D	2.533	0.000	1.000	Never	50.000	Closest
9	☞	CBGA	DAD1D	3.276	0.000	1.000	Never	50.000	Closest
10	☞	Delta 9 THCA	DAD1D	4.594	0.000	1.000	Never	50.000	Closest

Diode Array Quant Data

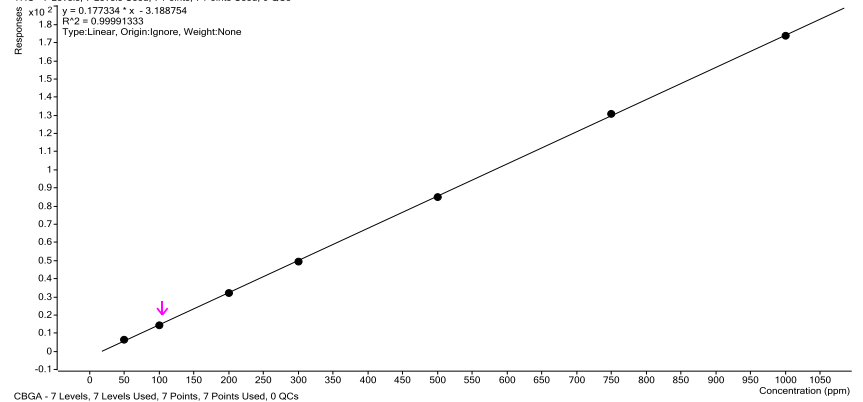
Sample		CBDV Results		THCV Results		CBC Results		CBG Results		CBN Results		CBD Results		CBDA Results		THC Results		CBGA Results		THCA Results	
Data File	Level	RT	Final Conc.	RT	Final Conc.	RT	Final Conc.	RT	Final Conc.	RT	Final Conc.	RT	Final Conc.	RT	Final Conc.	RT	Final Conc.	RT	Final Conc.	RT	Final Conc.
F1 sample.d		0.866	25.9360	1.265	196.8121	1.423	33.4284	1.716	97.2052	1.931	15.4707	2.157	527.4073	2.157	85.5359	2.472	104.8505	2.996	36.5274	3.952	261.6931
F2 sample.d		0.858	24.6873	1.267	197.1329	1.477	63.8837	1.710	95.1358	1.932	16.3020	2.157	283.4697	2.157	53.5110	2.470	57.3868	3.002	44.1850	3.955	226.5327
C_005.d	1	0.881	58.8132	1.287	60.8064	1.392	51.9682	1.683	93.6521	1.890	46.8645	2.053	59.3040	2.194	55.3583	2.496	53.5617	2.963	49.1957	3.909	48.4821
C_01.d	2	0.877	102.1864	1.287	81.3097	1.388	93.8596	1.674	132.3486	1.886	100.5738	2.046	94.9242	2.192	94.1240	2.486	99.3961	2.954	104.1515	3.897	107.3859
C_02.d	3	0.876	207.5743	1.283	206.2700	1.387	202.9129	1.675	181.4908	1.885	202.2938	2.049	192.3522	2.189	200.6918	2.489	200.3667	2.953	199.3742	3.892	199.6648
C_03.d	4	0.879	292.9750	1.287	300.3324	1.395	305.1618	1.687	245.4112	1.892	298.1676	2.053	298.4257	2.196	301.5343	2.494	295.4400	2.960	290.9286	3.901	284.6336
C0_5.d	5	0.877	473.6127	1.285	506.6916	1.392	492.9007	1.681	458.1870	1.888	502.6745	2.049	499.8125	2.190	496.7521	2.489	497.6630	2.954	507.2672	3.886	508.0259
C_075.d	6	0.877	756.8458	1.285	741.8427	1.390	755.0507	1.681	763.3711	1.887	752.2910	2.049	762.1737	2.189	750.7780	2.490	754.9083	2.953	750.9788	3.882	757.2254
C_1.d	7	0.879	1007.9926	1.288	1002.7471	1.394	998.1461	1.687	1025.5392	1.893	997.1349	2.054	993.0077	2.195	1000.7614	2.497	998.6642	2.961	998.1039	3.886	994.5823



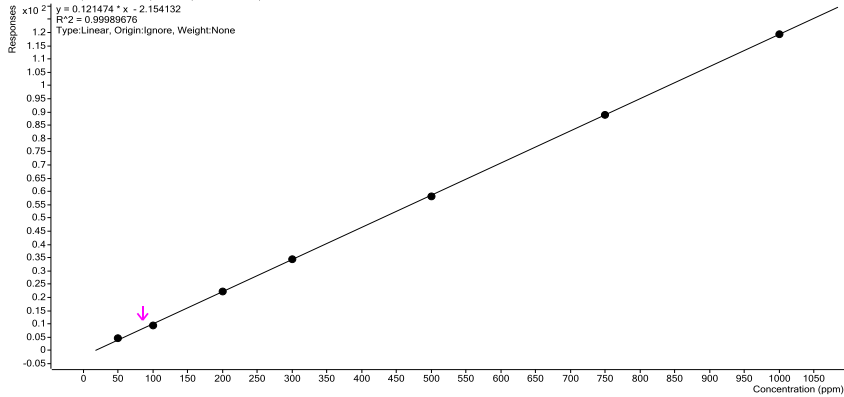
CBD - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 0 QCs



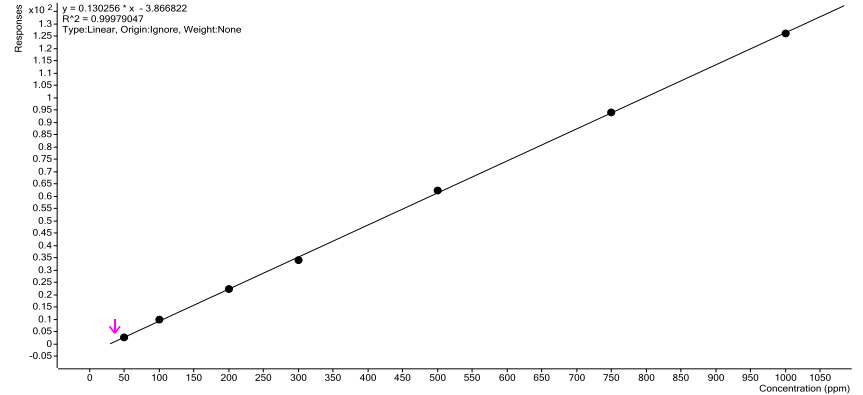
THC - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 0 QCs



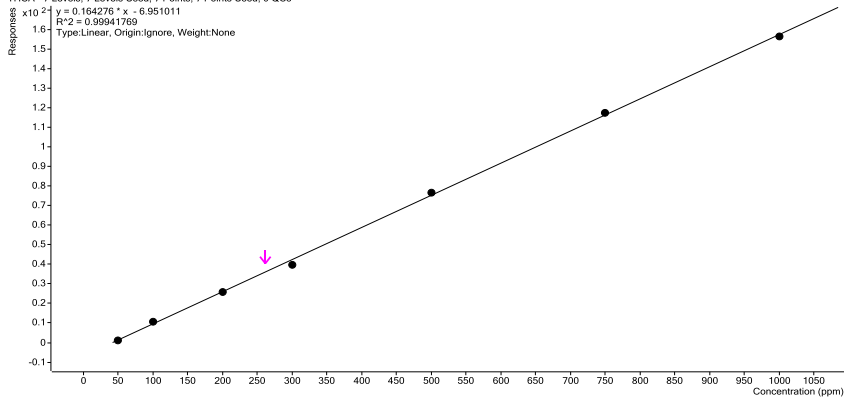
CBDA - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 0 QCs



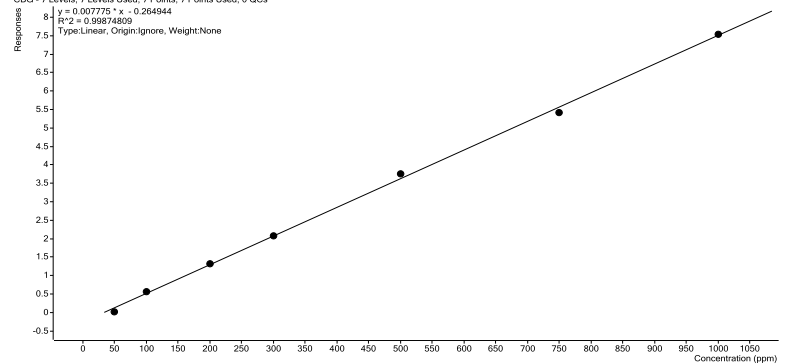
CBGA - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 0 QCs



THCA - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 0 QCs



CBG - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 0 QCs

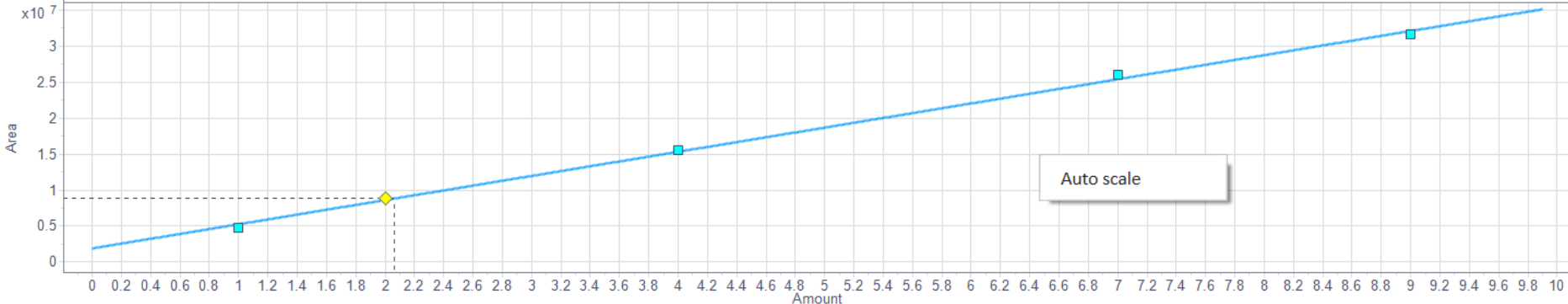


Lower end of CBG calibration curve: 1 – 9 ng/mL

Calibration Curve

CBG, 2.216 min

Formula : $y = 3357944.0541x + 1910130.7769$
 r : 0.99906
 R² : 0.99813
 Residual standard deviation : 564594.74422
 Origin : Ignore
 Weighting method : None



Calibration table

Level	Avg. response	Avg. amount	Residual per leve	Rel. residual	Rel. residual %	Response	Amount	Use	Injection
1	4781398.320	1.000	-486676.5111	-0.0924	-9.2382	4781398.320	1.000000000	<input checked="" type="checkbox"/>	C:\CDSProjects\THC and CBD\Results\cbd with quat 2016-12-1...
2	8840981.590	2.000	214962.7054	0.0249	2.4920	8840981.590	2.000000000	<input checked="" type="checkbox"/>	C:\CDSProjects\THC and CBD\Results\cbd with quat 2016-12-1...
3	15595266.613	4.000	253359.6202	0.0165	1.6514	15595266.613	4.000000000	<input checked="" type="checkbox"/>	C:\CDSProjects\THC and CBD\Results\cbd with quat 2016-12-1...
4	25976676.681	7.000	560937.5251	0.0221	2.2070	25976676.681	7.000000000	<input checked="" type="checkbox"/>	C:\CDSProjects\THC and CBD\Results\cbd with quat 2016-12-1...
5	31589043.924	9.000	-542583.3395	-0.0169	-1.6886	31589043.924	9.000000000	<input checked="" type="checkbox"/>	C:\CDSProjects\THC and CBD\Results\cbd with quat 2016-12-1...

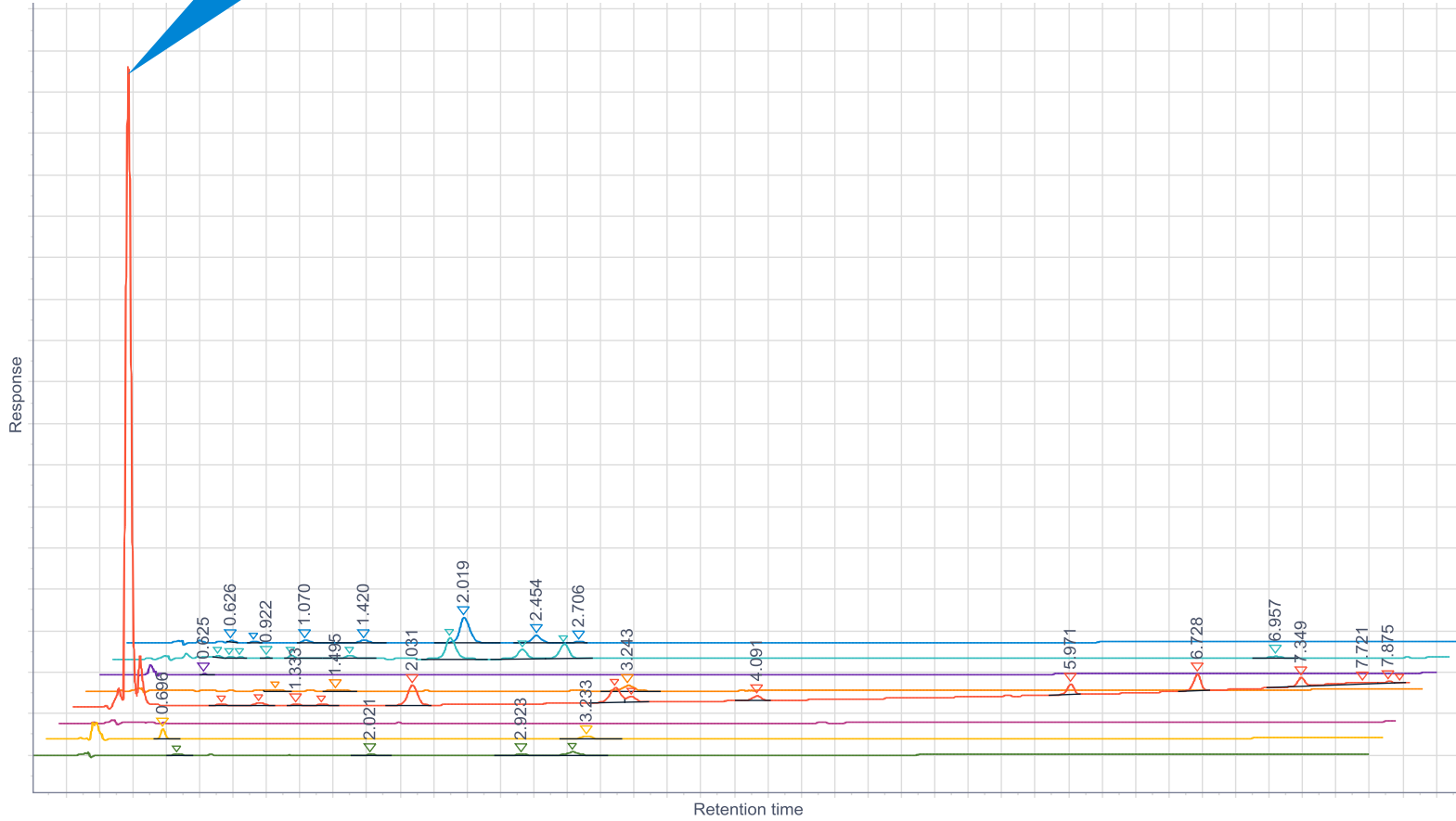
Custom Reporting

The screenshot displays the Agilent Data Analysis software interface, titled "THC and CBD data - Data Analysis". The interface is divided into several panes:

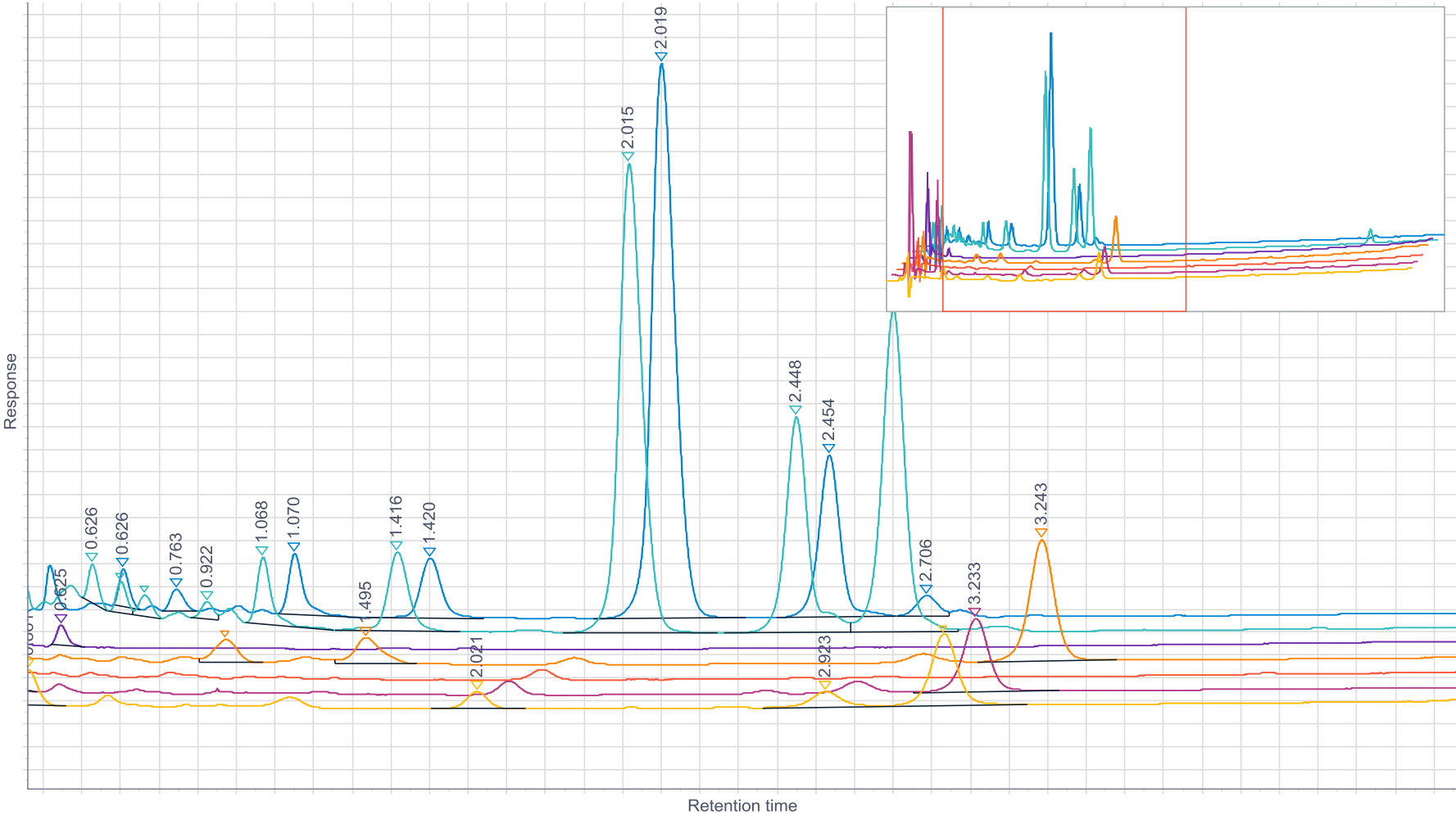
- Reporting Pane (Left):** Contains a tree view with "Injection" (cbd with quat 2016-12-16 16-47-47) and "Report Items". Under "Report Items", there are sections for "Search:" and "Report Templates". The "Report Templates" section shows a list of templates: "ExtendedPerformance.rdl", "ExtendedPerformance_Letter.rdl", and "ISTDWithGroups.rdl".
- Report Editor Pane (Center):** Shows the "Extended Performance Report" in edit mode. It includes a header with the Agilent Technologies logo and a body with various data fields. The "Data Selection" section shows "Column: ZORBAX Eclipse Plus C18" and "Diameter: 3 mm". The "Data Processing" section shows "Dilution: 1". Below the fields is a chromatogram plot with a peak at 1.194 minutes.
- Report Preview Pane (Right):** Shows the "Extended Performance Report" in preview mode. It includes a header with the Agilent Technologies logo and a body with various data fields. The "Data Selection" section shows "Column: ZORBAX Eclipse Plus C18" and "Diameter: 3 mm". The "Data Processing" section shows "Dilution: 1". Below the fields is a chromatogram plot with a peak at 1.194 minutes.

Custom reporting is wizard based. It allows you to use all your existing IR reports. It allow a simple answer with chromatographic calculations with **Pass/Fail** color flags

Added
Flavor
Component



Overview of all the samples



CBD results in commercially available hemp oil products (names masked)

Sample Identifier	Average Concentration (mg/mL)	Standard Deviation	Standard Error	99% CI
SIA-1	508.1	9.8	4.9	479.5, 536.7
SIA-2	460.7	14.7	7.4	417.8, 503.6
SIB	Not detected	N/A	N/A	N/A
SIC	2.3	0.17	0.085	1.7, 2.9
SID	9.3	0.86	0.43	6.8, 11.8
SIE	11.6	0.85	0.43	9.1, 14.1
SIF	12.3	0.78	0.39	9.9, 14.5

SIA-1 and SIA-2: Same manufacturer

SIB: Non CBD product

SIC: Filtered product about 1/3 of labeled concentration

SID: 15% higher than labeled concentration

SIE and SIF: 50% higher than labeled concentration

* Calibrators and samples were note matrix matched

RELIABLE ANALYTICAL WORKFLOWS FOR CANNABIS TESTING

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Optional detectors and what they add... High Dynamic Range (HDR)

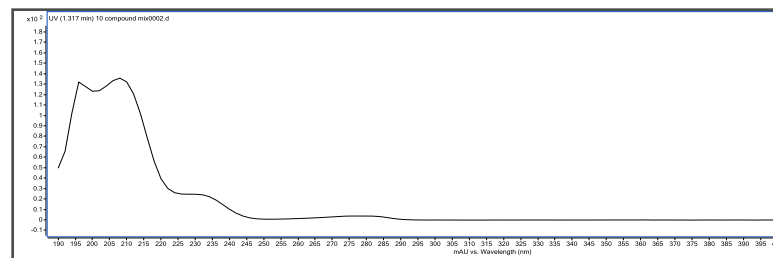
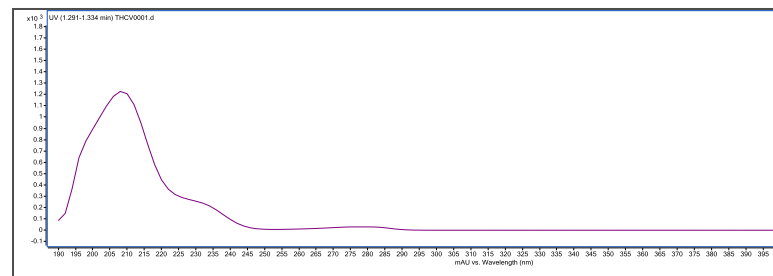
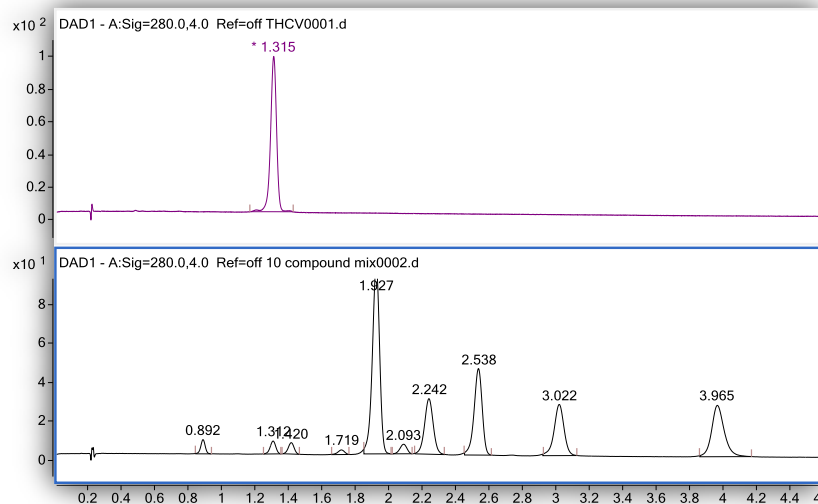


1260 Infinity II DAD-HS



1260 Infinity II DAD-WR

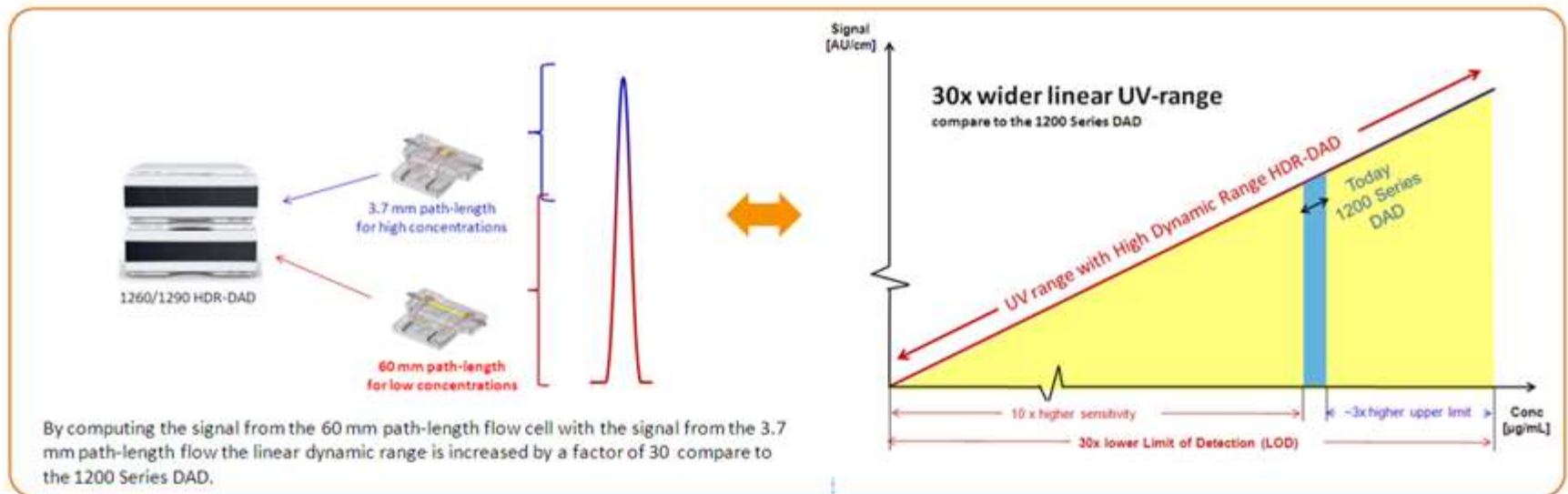
BIO
INERT



Expanding the linear range

Expanding the linear dynamic range is achieved by making use of the technology in *Agilent's Max-Light flow cells*. Optofluidic waveguides in these flow cells facilitate total internal reflection for superior light transmission.

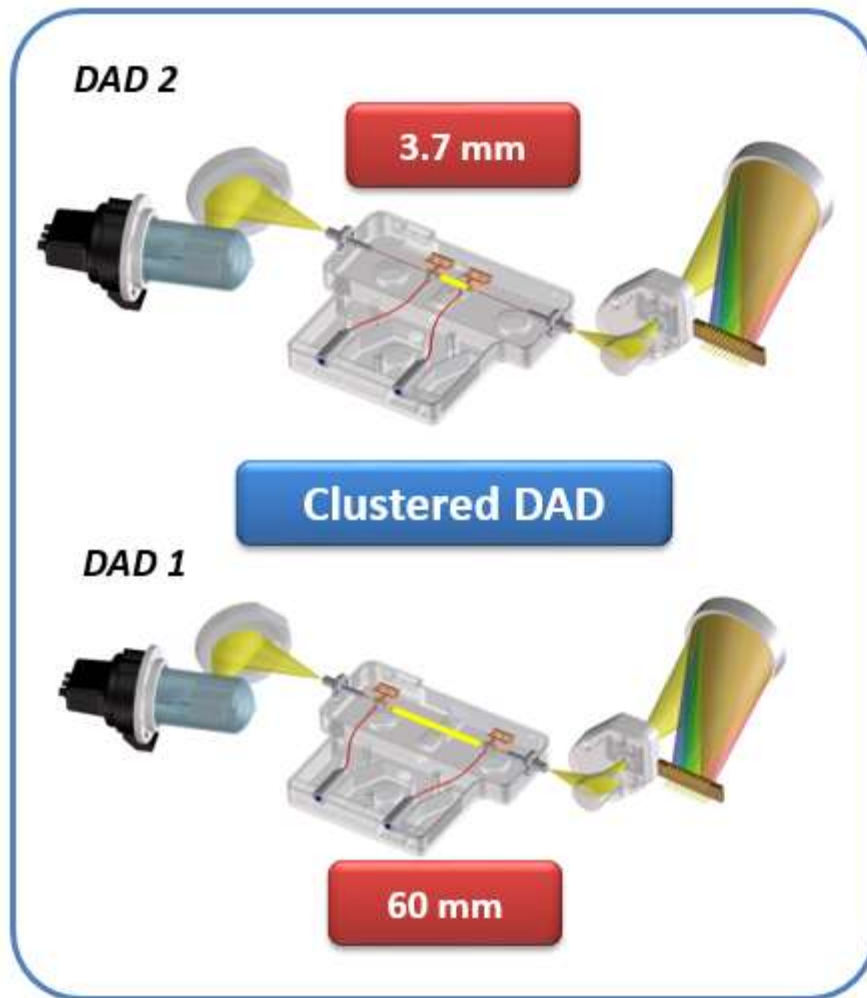
This gives you extremely low detector noise regardless of the length of the optical path.



The Agilent **high dynamic range** detection solution now takes the signal from the **60** mm path length flow cell to quantify the low level concentrations and the signal from the **3.7** mm cell for the high level concentrations.

Specially developed algorithms compute these signals to expand the linear dynamic range by a factor of 30

High Dynamic Range Diode Array



Cluster of two DADs

- **DAD 2:**
3.7 mm Cell – for high concentrations
- **DAD 1:**
60 mm Cell – for low concentrations
- **Output:** One, combined signal,
normalized to 10 mm path length
(HDR range: 0.6×10^{-6} to 6.7 AU/cm)
vs for 1200 Series: 7×10^{-6} to 2 AU/cm)

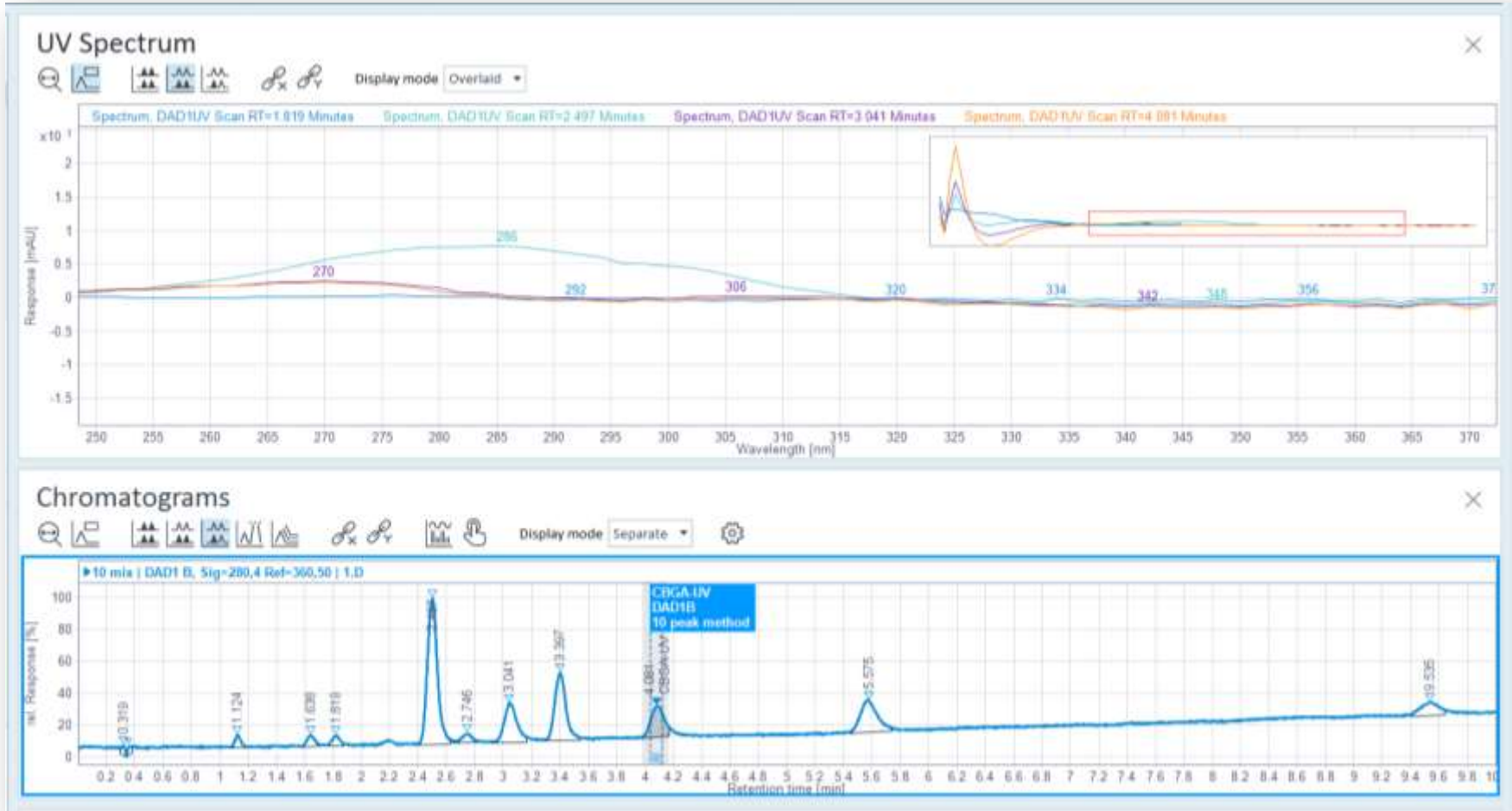
Control / Usability

- Like standard 1290/1260 DAD

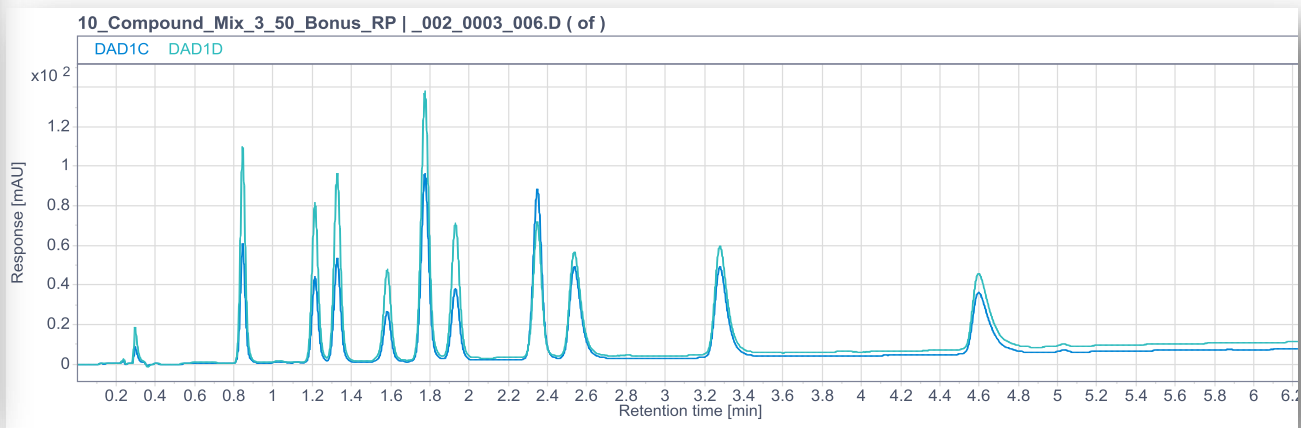
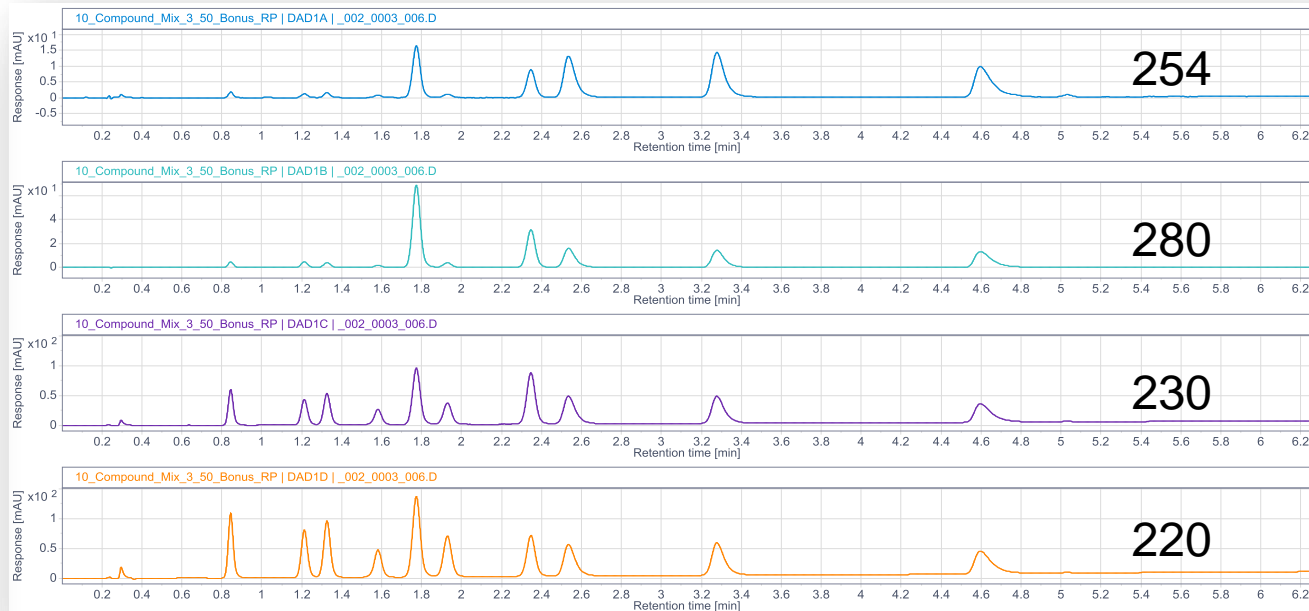
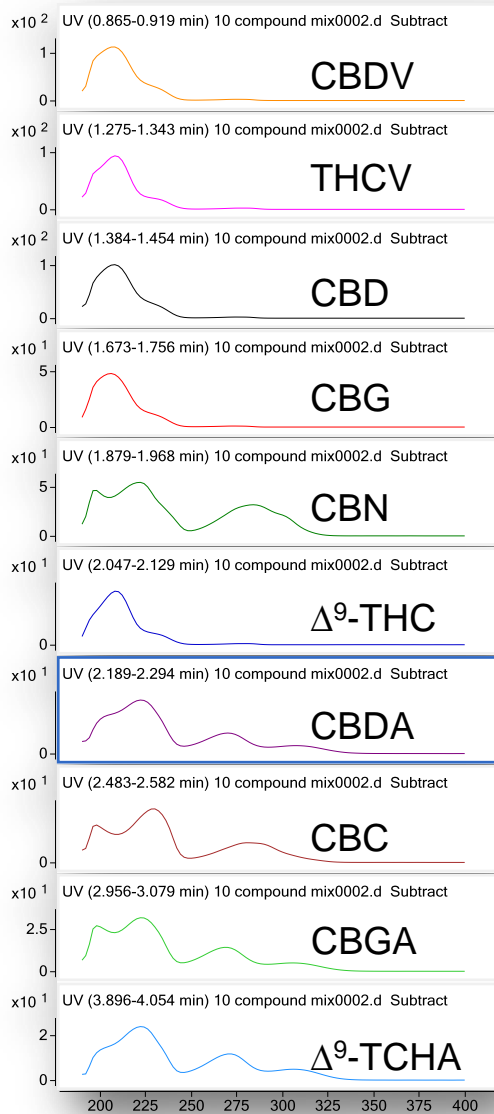
Investment Protection

- Existing 1290/1260 DAD can be upgraded
(by 2nd DAD)
- Existing 1100/1200 Systems can be
upgraded (by two 1290 or 1260 DAD)

UV Spectrum and Chromatogram

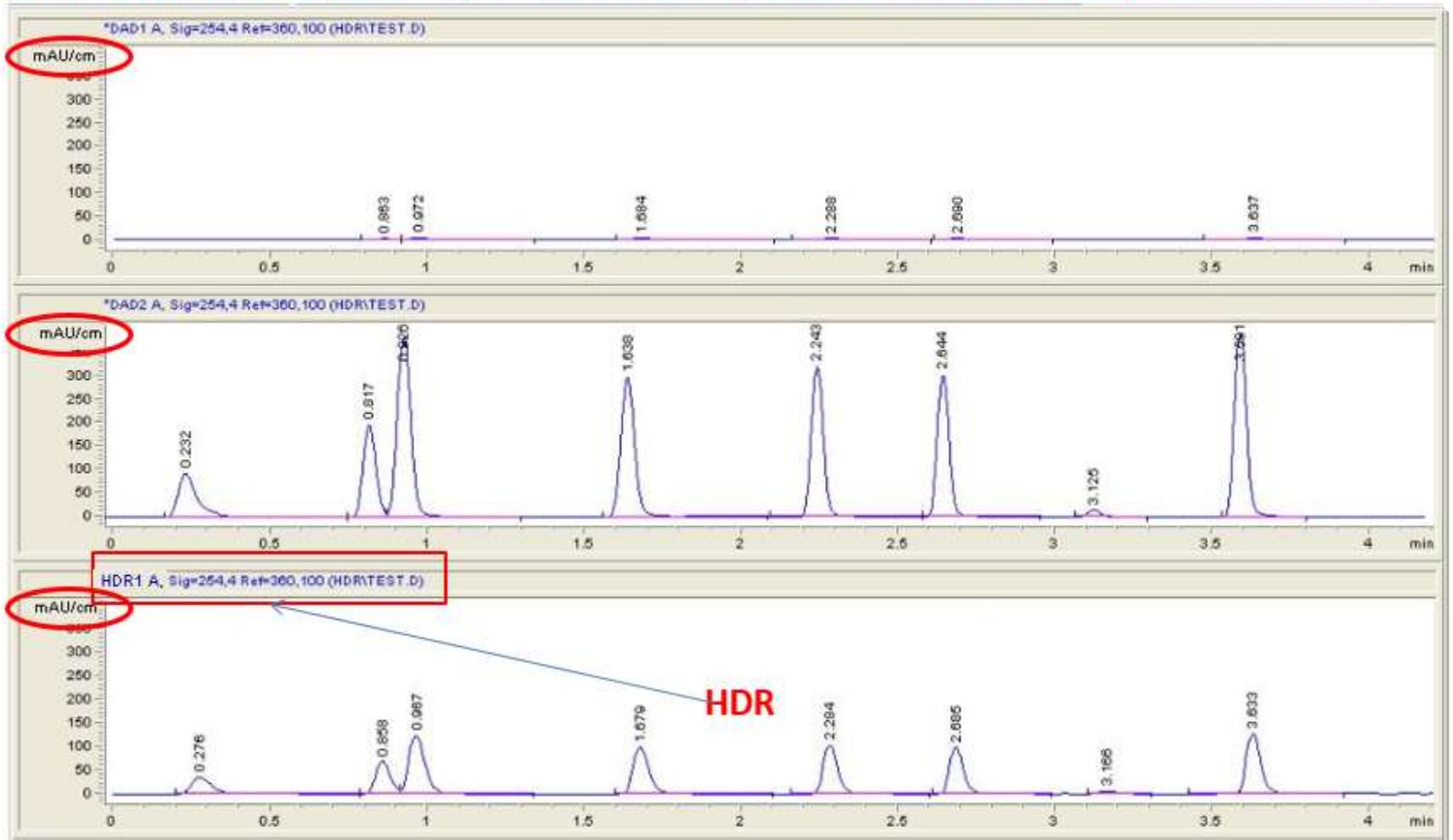


The addition of spectra and multiple signals



This is an example of the HDR chromatograms.

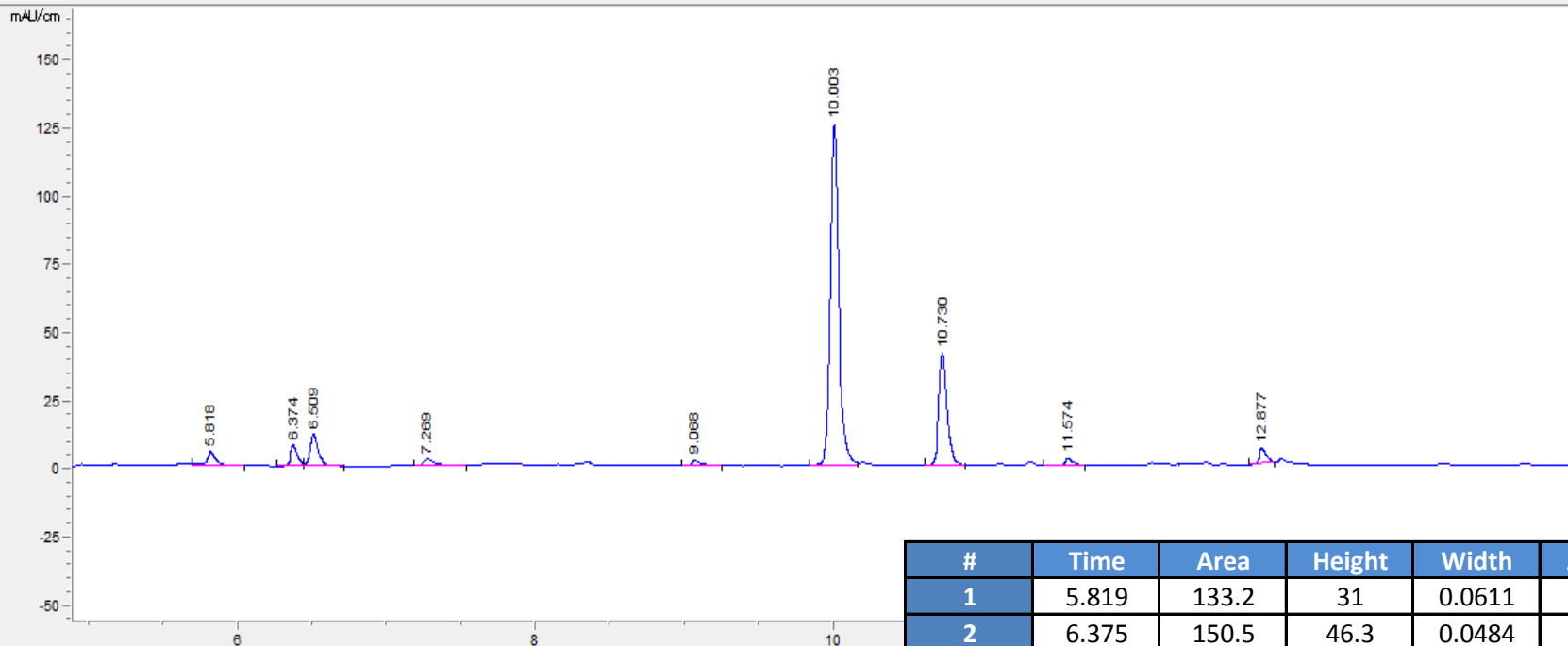
All signal are Normalized (considering the Flow cell Path length and the Delay volume between Dad 1 and DAD 2)



Note the Y axis is mAu/cm typically Y axis are mAu/ mm

HDR Chromatogram Output

HDR1 A, Sig=244.4 Ref=off (HDR\LEWER_SPINB0005.D)



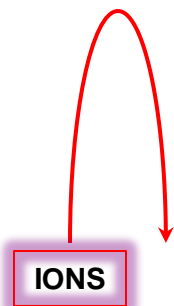
#	Time	Area	Height	Width	Area%	Symmetry
1	5.819	133.2	31	0.0611	2.802	0.872
2	6.375	150.5	46.3	0.0484	3.167	0.705
3	6.51	252.8	69.8	0.0538	5.319	0.658
4	7.27	78.2	15	0.0724	1.645	0.45
5	9.069	59.6	12	0.071	1.254	0.5
6	10.003	2954.8	752.1	0.0583	62.157	0.768
7	10.731	937.9	247	0.0563	19.73	0.755
8	11.575	64.3	15.7	0.0593	1.353	0.911
9	12.878	122.3	34.3	0.0547	2.574	0.721

Product peaks are no longer off scale and impurity peaks are quantifiable

Adding Mass Spectrometry

Time of Flight

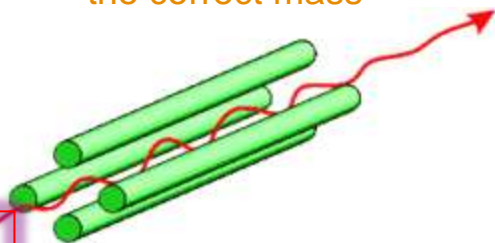
Gives ions a kick and measures their speed



IONS

Quadrupole

Passes only ions with the correct mass



IONS

Tandem Mass Spectrometry (MS-MS)

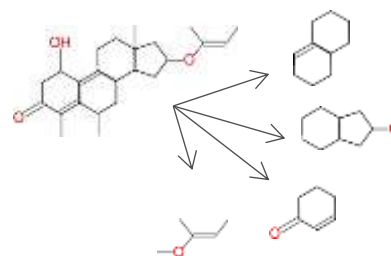
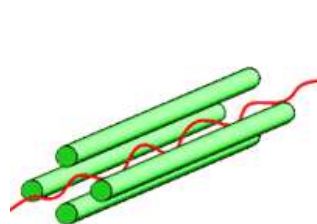
Selects a range of masses



Fragments by collision



Analyzes the fragments



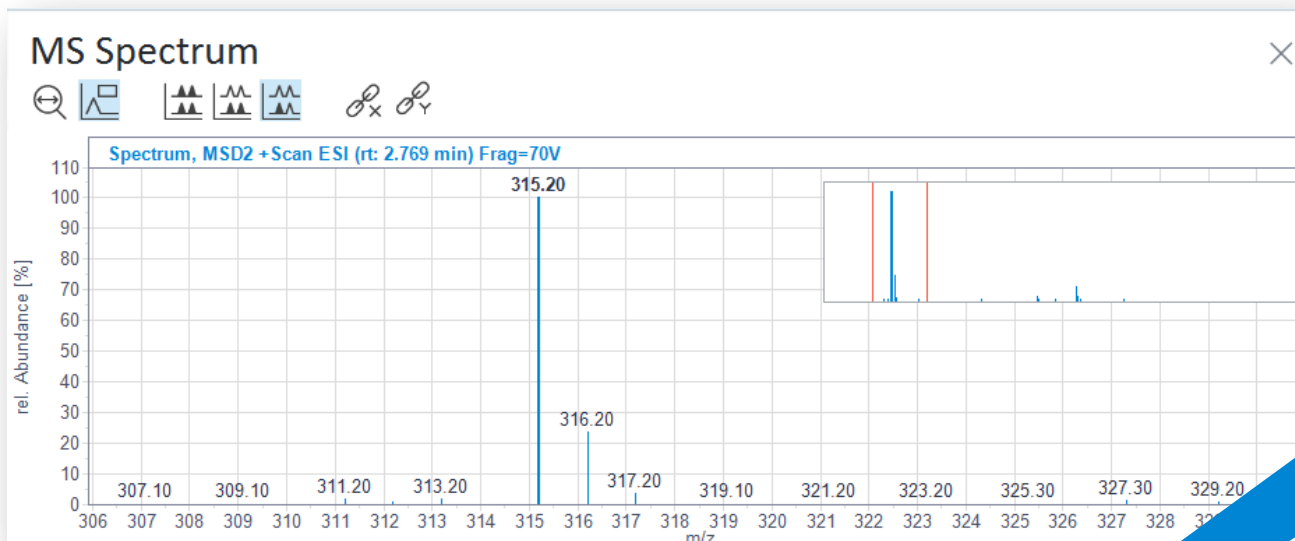
QTOF



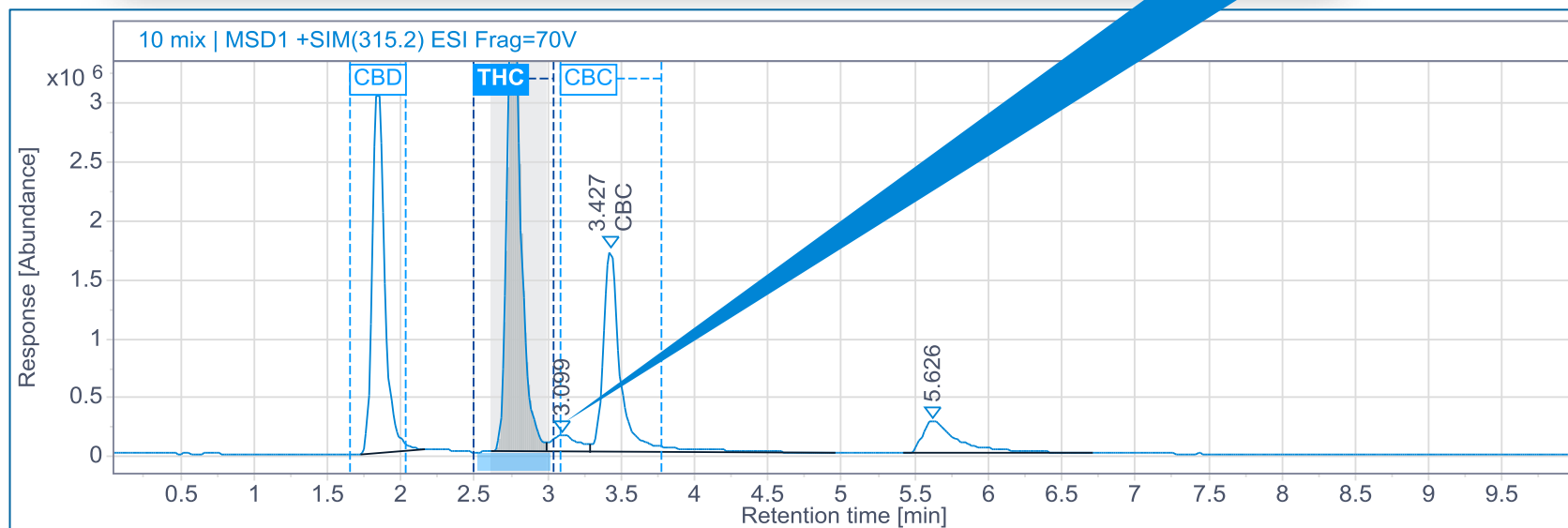
QQQ



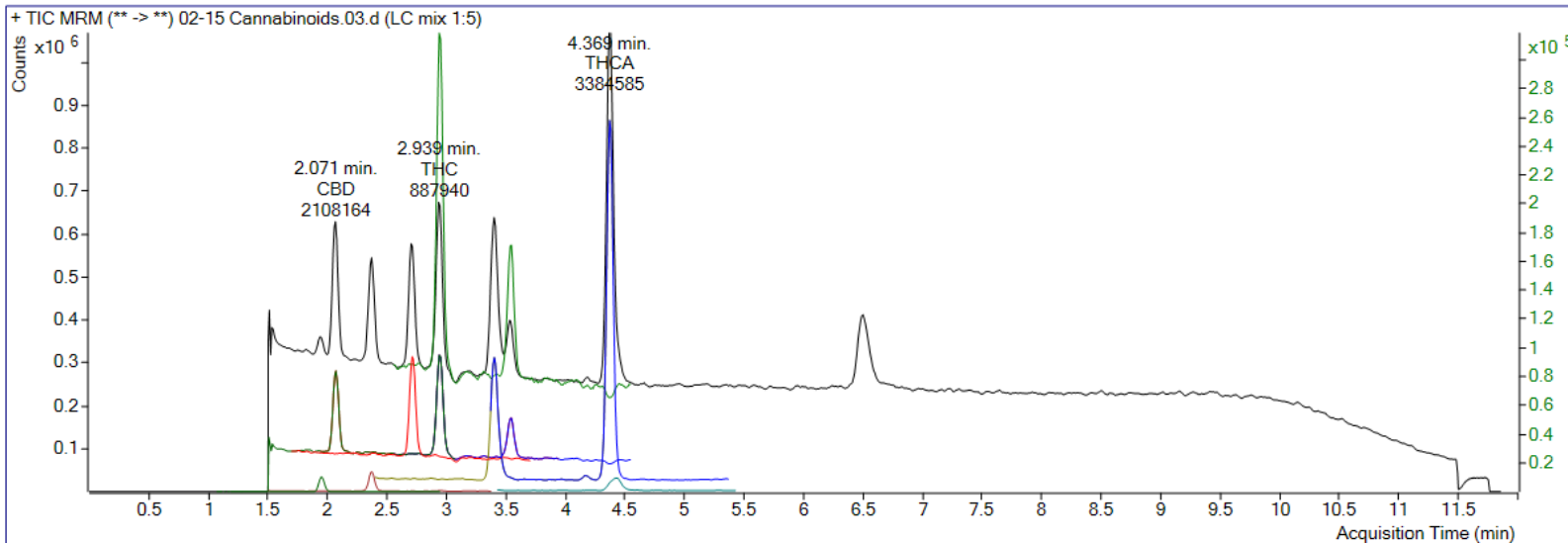
MSD Spectrum of Peaks Bonus RP column



Δ^8 -THC



Roy Turton
Adele Blackler



This is QQQ data with the Agilent G6470 using Acetonitrile as a mobile phase. They used a 2.1 x 100 poroshell Bonus RP

Can do potency and pesticides on same system (different columns)
D8 will separate

Check out www.agilent.com for more information, applications, videos, etc.



General Article

Cannabis Testing Solutions

Agilent solutions are at the forefront of research and screening efforts to ensure quality control and safety of medicinal and recreational cannabis.



Flyer

Cannabis Testing Solutions From your complete workflow partner

Agilent solutions are at the forefront of research and screening efforts to ensure quality control and safety of medicinal and recreational cannabis.



Cannabis Testing Solutions Video

Comprehensive cannabis testing with Agilent, your complete workflow partner

Playing Time: 01:02:32



Selection Guide

Agilent Cannabis Testing Informational Graphic of Agilent Offered Solutions

Information Graphic for Agilent Offered Solutions

Created: 27 Sep 2016 / File Size: 4 MB



Flyer

Cannabis Testing Solutions From your complete workflow partner, Agilent

Agilent solutions are at the forefront of research and screening efforts to ensure quality control and safety of medicinal and recreational cannabis.



Selection Guide

Agilent Cannabis Testing Informational Graphic of Agilent Offered Solutions

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Created: 26 Sep 2016 / File Size: 4 MB



eSeminar

Cannabis Analytical Testing Events

Cannabis Analytical Testing Events



eSeminar

Laboratory Workflows for Cannabis Testing Webinar Series

A free webinar series displaying Agilent's complete workflows and instrumentation to achieve unparalleled sensitivity and reliability for a broad range of cannabis-testing methods such as pesticides, potency, residual solvents, heavy metals, and terpenes.



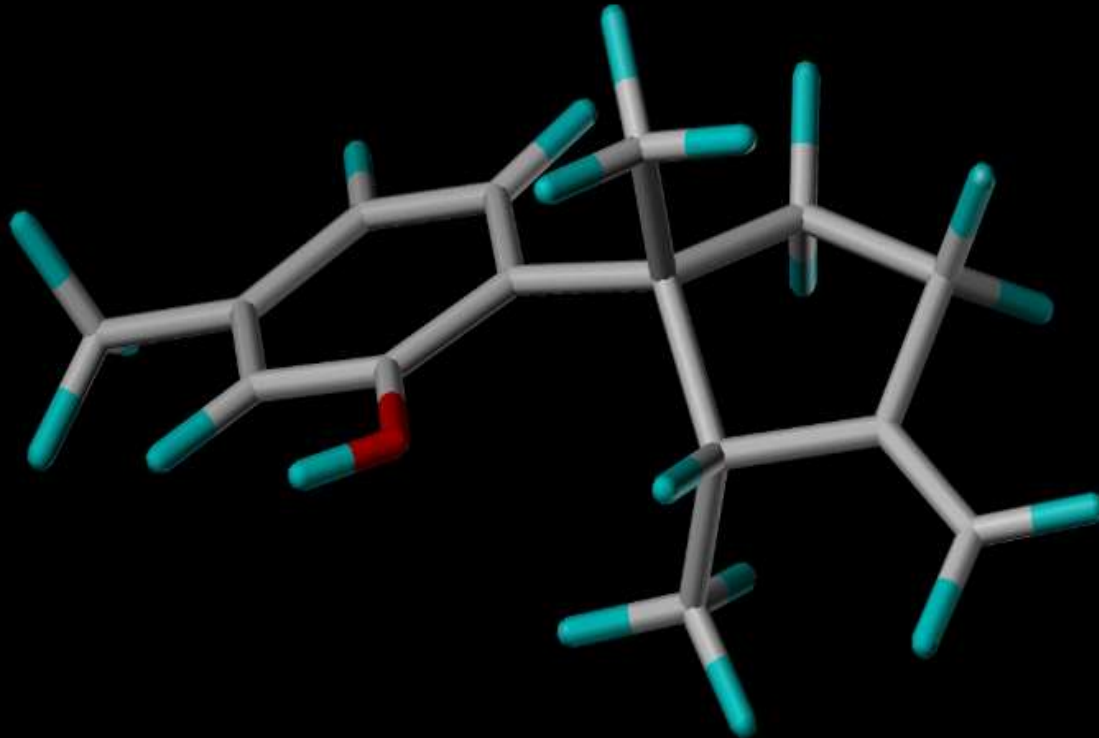
Application

Solid Phase Extraction of THC, THC-COOH and 11-OH-THC from Whole Blood

Application note for Solid Phase Extraction of THC, THC-COOH and 11-OH-THC from Whole Blood

Publication Part Number: 2622 / Created: 02 Mar 2017 / File Size: 164 KB

Questions?



Thank you!