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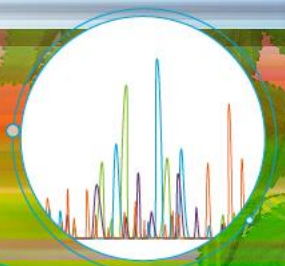
Answers for Science.
Knowledge for Life.™



It's Time to See Everything Clearly at Trace Levels

Chemovar Typing of *Cannabis* Strains with MarkerView® and SCIEX X500R QTOF System

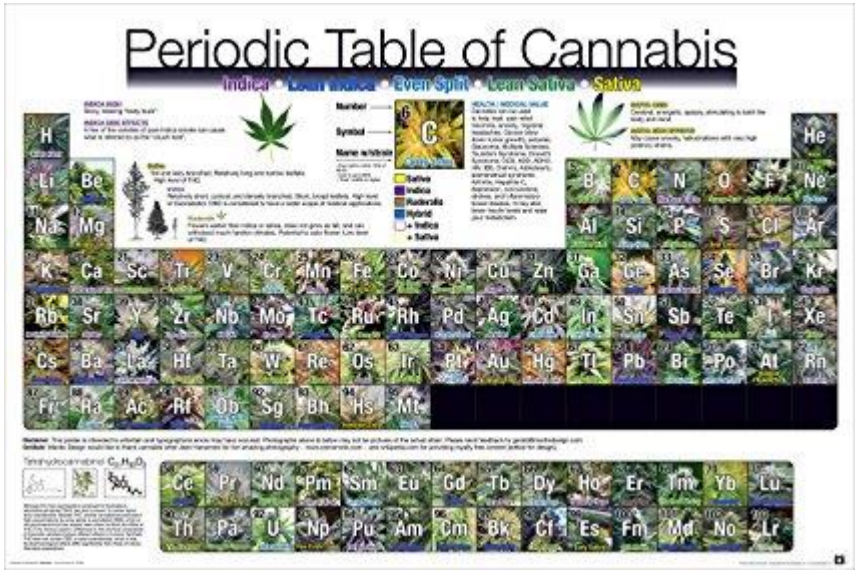
KC Hyland
Food and Environmental Technical Marketing, SCIEX

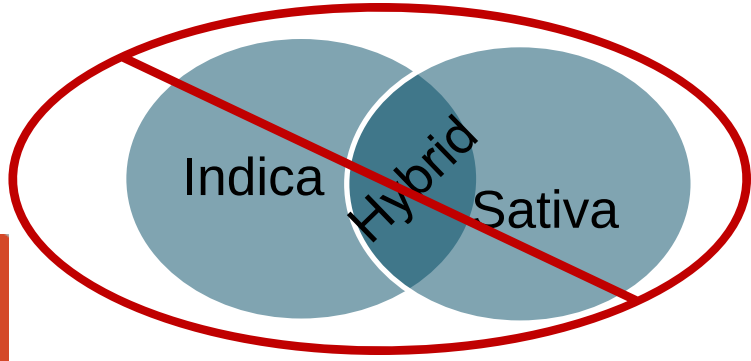
RUO-MKT-11-8252-A

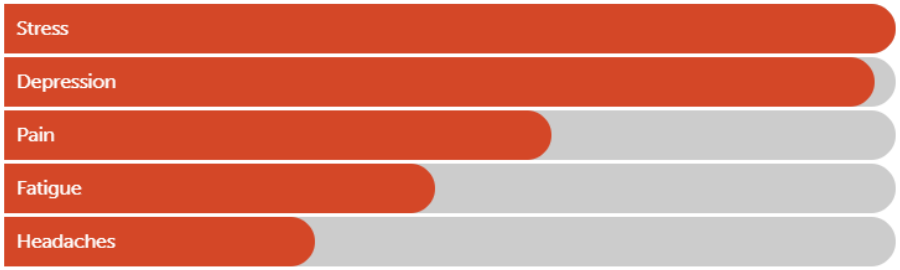
Different Strains for Different Pains

Cannabis strains are consumed for specific desired effects

- Consumers of Cannabis have long asserted the differences between cultivars, or strains, for user experience
- Historical classifications no longer explain the differences between or properties of the hundreds of commercially available Cannabis strains

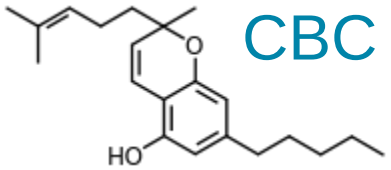




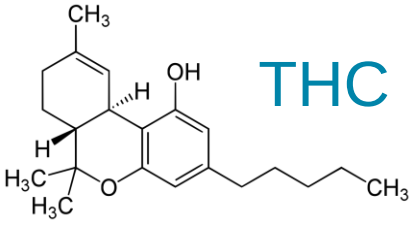


Cannabinoid Profile: Psychotropic, Therapeutic, etc.

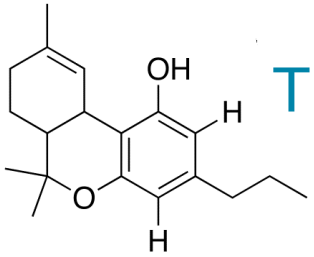
- THC, CBD
- 119 individual cannabinoids have been identified in Cannabis



CBC



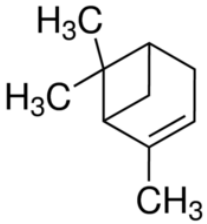
THC



THCV

Terpene Profile: Flavor and Aroma

Linalool (floral)



Pinene (pine)

- 200 terpenes have been identified in *Cannabis*
- Unique strains present varying terpene profiles
- Contribute to distinct flavor and aroma
- Both growers and consumers have interest in profiling terpene character

"The Entourage Effect"

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WHAT YOU SMELL = HOW YOU'LL FEEL

IDENTIFYING COMMON CANNABIS TERPENES

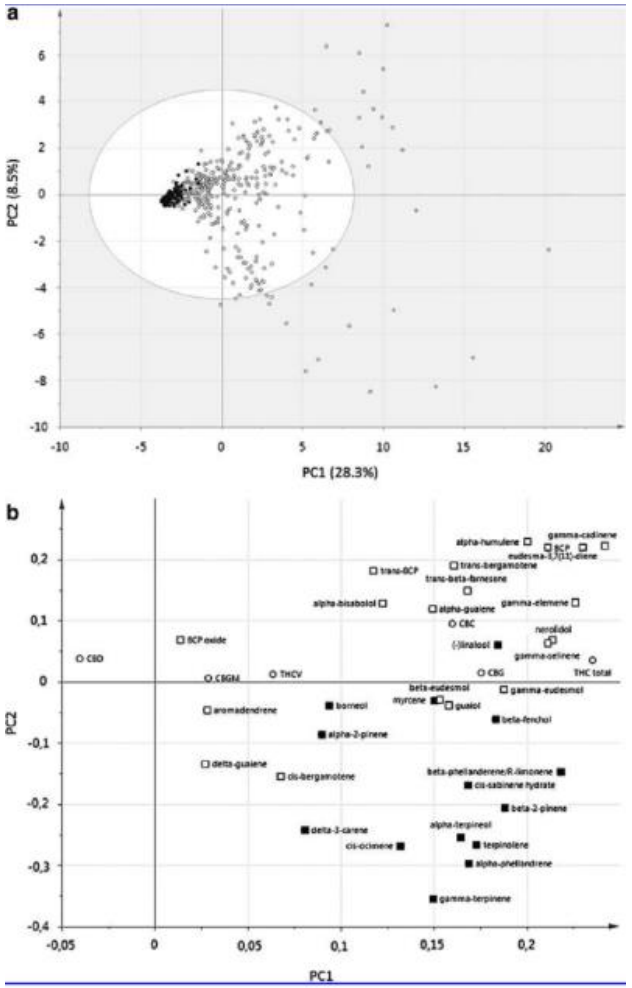
TERPENE:	ALPHA-PINENE BETA-PINENE	MYRCENE	LIMONENE	ACARYOPHY-LLENE	LINALOOL
					
	PINE	MUSKY, EARTHY, CITRUS HINTS	CITRUS	SPICY, WOODY, PEPPERY, CLOVE	FLORAL, CANDY, CITRUS
	FOUND IN: Pine, Dill, Parsley, Basil, Rosemary	FOUND IN: Mango, Thyme, Lemongrass, Hops	FOUND IN: Juniper, Rosemary, Fruit Rinds, Peppermint,	FOUND IN: Black Pepper, Clove, Cotton	FOUND IN: Lavender
	Alertness, Euphoria, Creativity, Memory Retention	"Couchlock", Sedation, Relaxation, Body High	Elevated Mood, Stress Relief	No noted effects	Anxiety Releif, Sedation
	Asthma, Antiseptic	Antioxidant, Pain, Muscle Tension, Sleeplessness, Anti-Carcinogenic	Gallstones, Gastroprotective, Heartburn, Anti-fungal, Depression	Gastroprotective, Anti-inflammitory, Arthritis, Ulcers	Anti-anxiety, Anti-convulsant, Anti-depressant, Anti-acne
	Jack Herer, Trainwreck, Bubba Kush, Chem Dawg, Super Silver Haze	Pure Kush, El Nino, Himalayan Gold, Skunk #1, White Widow	OG Kush, Super Lemon Haze, Jack the Ripper, Lemon Skunk	Big Bang, Damn Sour, Great White Shark, Ice Dream	G-13, Lavender, Amnesia Haze, LA Confidential

Made by MarijuanaPackaging.com. Information courtesy of Leafly.com

Cultivars vs Chemovars

Cannabis strains are cultivated for specific profiles and characteristics

- The concept of chemovars- **a chemistry-based, phenotypical fingerprint rather than a horticultural cultivar-** has been proposed and gained popularity in the scientific community.
- Scientific studies are yet to identify the **biochemical markers that can sufficiently explain differences between cannabis varieties.**
- Hazekamp *et al*. applied a targeted detection of 44 major terpenes and cannabinoids followed by **Multivariate Data Analysis to identify the cannabis constituents** that may act as markers for distinction between Indica and Sativa.



Overview

- High resolution mass spec workflow described for the exploration of chemovar profiling for several Cannabis strains
- A nontargeted approach which does not try to target a “short-list” of cannabinoids or terpenes
- X500R QTOF System, SWATH® Data Independent Acquisition, SCIEX OS and MarkerView™ software platforms, SCIEX All-in-One plus NIST MSMS library

Analytical Conditions

HPLC Cor

- SCIEX
- Chroma
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  Phenor
  column
  x 3 mm
- Flow ra
- Columr
  30° C
- Run tim
  full grad

system

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chemical

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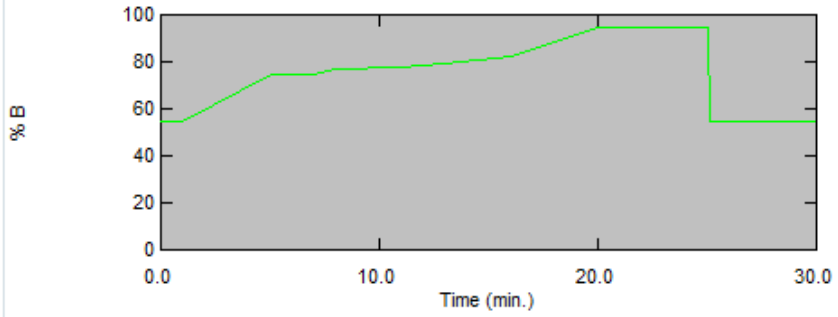
ximum MSMS

Pumps | Autosampler | Oven | Controller | Time Program

	Time	Module	Event	Parameter
1	1.00	Pumps	Pump B Conc.	
2	5.00	Pumps	Pump B Conc.	
3	7.00	Pumps	Pump B Conc.	
4	8.00	Pumps	Pump B Conc.	
5	9.00	Pumps	Pump B Conc.	
6	10.00	Pumps	Pump B Conc.	
7	11.00	Pumps	Pump B Conc.	
8	13.00	Pumps	Pump B Conc.	
9	16.00	Pumps	Pump B Conc.	
10	20.00	Pumps	Pump B Conc.	
11	25.00	Pumps	Pump B Conc.	95
12	25.10	Pumps	Pump B Conc.	55
13	30.00	Controller	Stop	

Gradient Curve

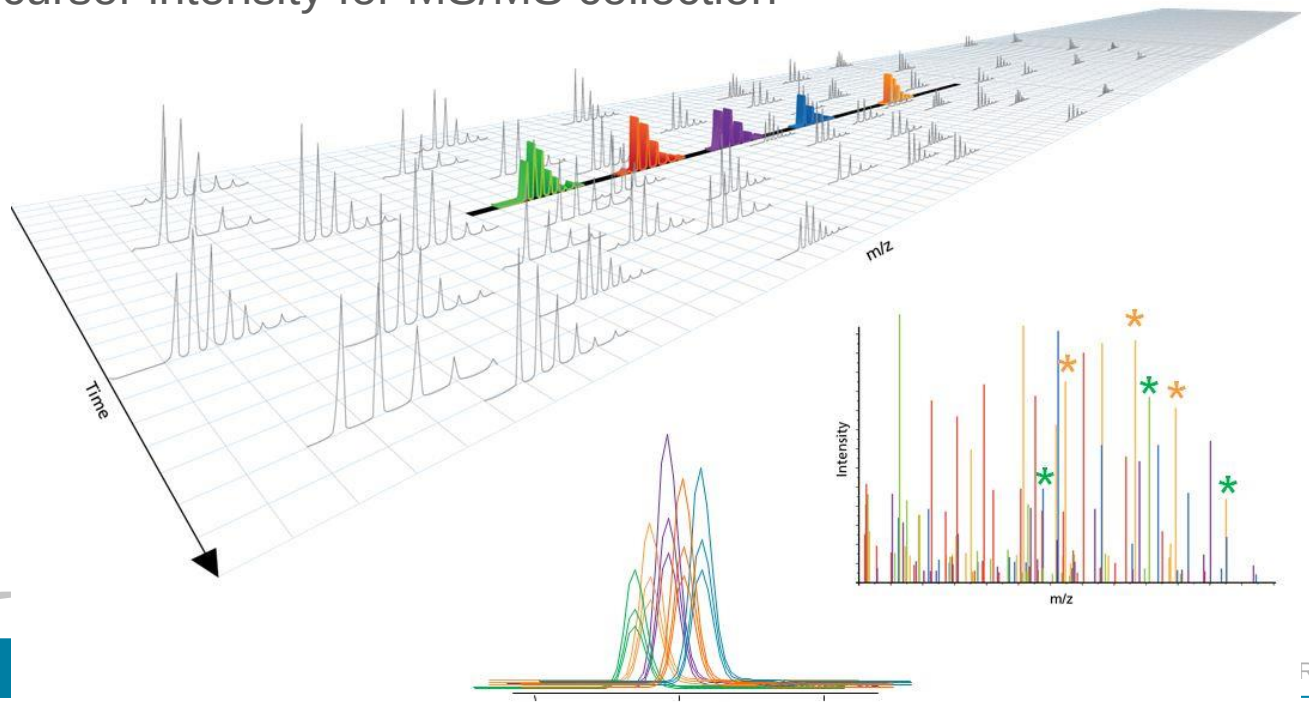


Update Graph

Chromatography was optimized for separation of terpene and cannabinoid isomers

SWATH® Acquisition

- SWATH = Data Independent Acquisition (DIA)
  - For each cycle, the instrument focuses on a mass window of Q1 precursors
  - Acquires MS/MS data from ***all*** precursors detected within that window
  - This mass window is then stepped across the ***entire*** mass range, systematically collecting MS/MS data from every mass and from all detected precursors
  - Unlike IDA (Information Dependent Acquisition), there is no dependence on precursor intensity for MS/MS collection



Variable Window SWATH

Method duration 21 min Total scan time: 1.763727 sec

Estimated cycles: 714

Source and Gas Parameters

Ion source gas 1	60 psi	Curtain gas	35	Temperature	500 °C
Ion source gas 2	60 psi	CAD gas	11		

Experiment SWATH

Polarity	Positive	Spray voltage	5500 V		

TOF MS

TOF start mass	40 Da	Declustering potential	80 V	Collision energy	10 V
TOF stop mass	1000 Da	DP spread	0 V	CE spread	0 V
Accumulation time	0.06 s				

TOF MSMS

TOF start mass	40 Da	TOF stop mass	1000 Da	Dynamic collision energy	☐
Accumulation time	0.03 s	Charge state	1	Enhance dynamic range	☑

Mass Table Autofill SWATH windows...

	Precursor ion start mass (Da)	Precursor ion stop mass (Da)	Declustering potential (V)	DP spread (V)	Collision energy (V)	CE spread (V)
1	39.5000	122.1000	80	0	35	15
2	121.1000	141.9000	80	0	35	15
3	140.9000	156.3000	80	0	35	15
4	155.3000	170.7000	80	0	35	15
5	169.7000	184.2000	80	0	35	15
6	183.2000	200.4000	80	0	35	15
7	199.4000	216.6000	80	0	35	15
8	215.6000	233.2000	80	0	35	15
9	232.2000	249.9000	80	0	35	15
10	248.9000	267.9000	80	0	35	15
11	266.9000	286.3000	80	0	35	15
12	285.3000	303.9000	80	0	35	15
13	302.9000	320.1000	80	0	35	15
14	319.1000	337.6000	80	0	35	15
15	336.6000	353.8000	80	0	35	15
16	352.8000	370.5000	80	0	35	15
17	369.5000	386.2000	80	0	35	15

- Narrower Q1 isolation windows → increased specificity, but more windows needed to cover the *m/z* range
  - Need to balance *m/z* coverage, with impact to cycle time
- Optimizing windows of varying width based on *m/z* for enhancement of specificity by narrowing windows where most ion masses occur
  - Allows for increased mass range to be covered while keeping number of windows (and cycle time) down

SCIEX All-in-One High Resolution MS/MS and NIST'17 MS/MS Mass Spectral Libraries

Enhanced compound coverage across compound classes

The flexibility of the multiple SCIEX software platforms provide scientists a fast way to analyze large batches of MS/MS data for accurate and efficient MS/MS library searching, data mining, and compound database management. In combination with the National Institute of Technology (NIST) MS/MS Spectral Library we have assembled a comprehensive high resolution, accurate mass MS/MS spectral library bundle containing 19,143 compound entries.

Features of the SCIEX All-in-One High Resolution MS/MS Spectral Library

- Includes a SCIEX proprietary 3900 analyte library created using certified reference materials, including compounds commonly tested for in forensics, food, environmental and metabolomics samples.
- The NIST '17 MS/MS Library bundle adds a comprehensive range of different compounds (13,808 small molecules and 1,435 biologically relevant peptides) for enhanced coverage.
- Contains spectra for both positive and negative ionization for compounds that ionize in both polarities.

NIST
National Institute of Standards and Technology

Advantages of the SCIEX All-in-One MS/MS Spectral Library

- Use the integrated MS and MS/MS information to build methods without the need to infuse standards and optimize conditions for a given compound.
- Easily create processing methods for a TOF-MS-IDA-MS/MS workflow or SWATH® Acquisition for use on TripleTOF® and X-Series QTOF Systems.
- Quickly setup XIC tables for quantitation and identification with SCIEX OS Software or MasterView™ software. Build customized libraries by simply selecting only the compounds of interest using the LibraryView™ Software.

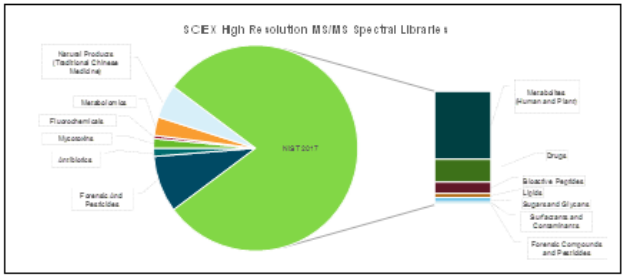


Figure 1. Gain Comprehensive Compound Coverage using the SCIEX All-In-One High Resolution MS/MS Spectral Library bundle containing over 19000 compounds. The inclusion of the NIST '17 MS/MS library increases compound coverage all the way from human and plant Metabolites, to additional Forensic Compounds and Pesticides contained in the proprietary SCIEX MS/MS Spectral Libraries.

This comprehensive library, bundled with the NIST '17 MS/MS Library, provides spectra for over **19000 compounds** including pesticides, pharmaceuticals and personal care products (PPCPs), and natural products found in foods and traditional medicines.

Data Processing Workflow: Nontargeted Approach

Statistical Tools to Identify: Which Features Are Distinctive For Strain Classification?

MarkerView™ software to identify important features using PCA

Build Peaks of Interest list from the distinguishing features identified

Import Peaks of Interest list into Analytics

Search MSMS libraries for first-pass identification of the Peaks of Interest

Identify Those Features!

Choose a probable formula from the formula finder based on HR-AM TOF MS data (mass error, elemental composition, hit count)

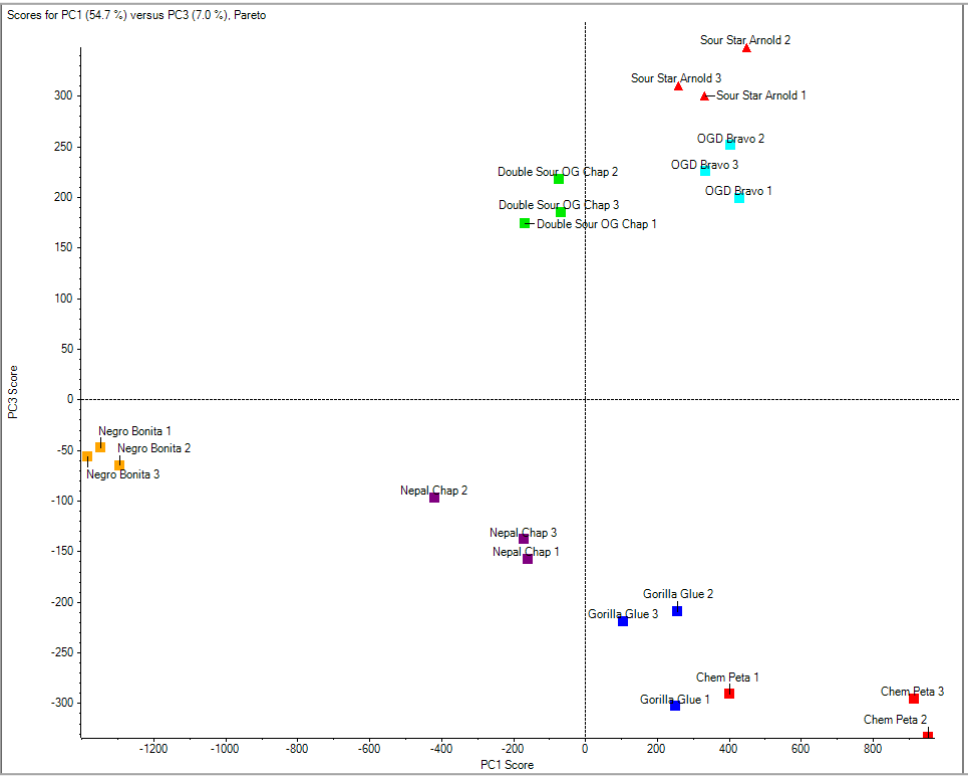
Start ChemSpider session to open the ChemSpider list of hits with that formula

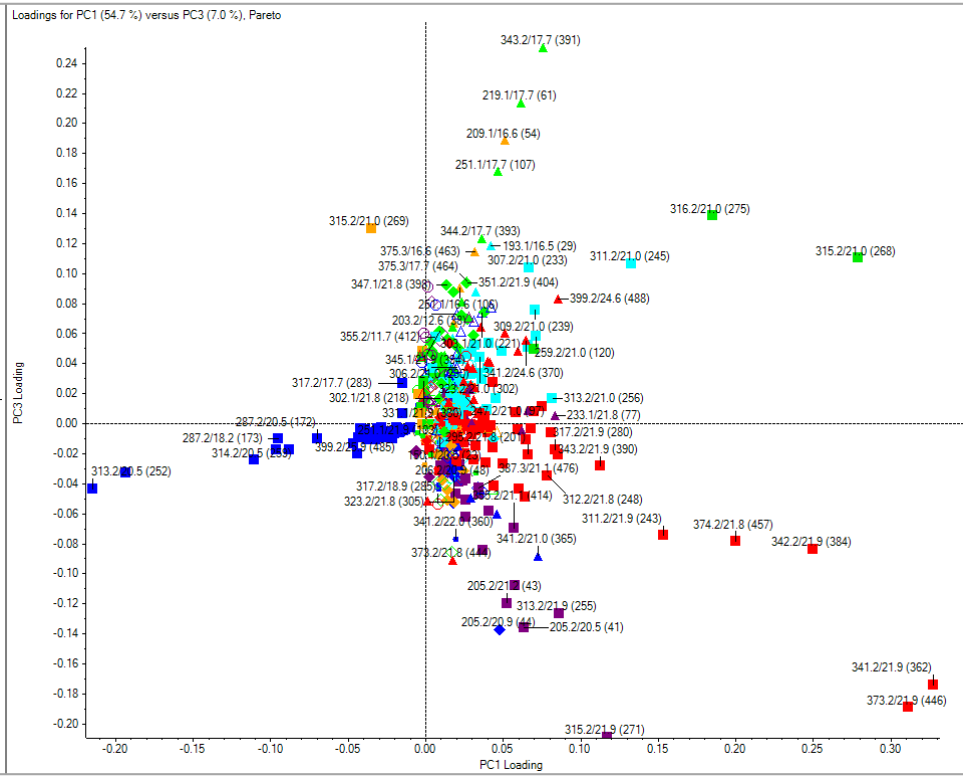
ChemSpider database lists predicted fragments from each structure in the “hit list”

Choose a candidate structure based on the fit of the collected MSMS spectrum to the predicted fragmentation (mass error, number of matched fragment peaks, does the fragment make sense)

Step 1: Load up Data into MarkerView

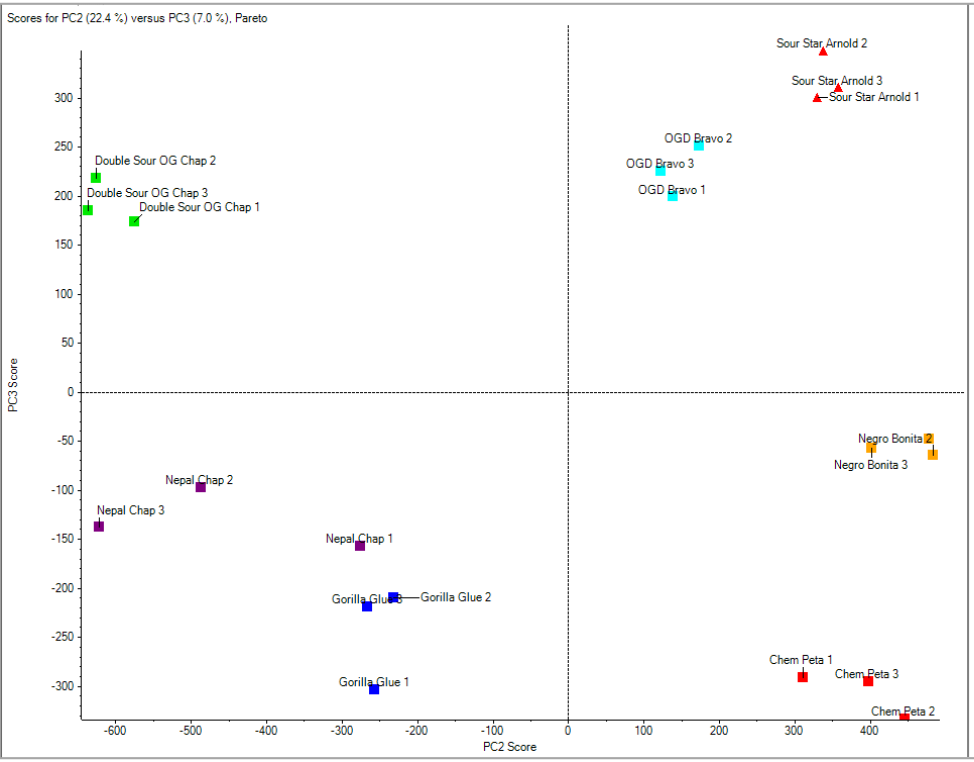
PC1 v PC3, Unsupervised PCA

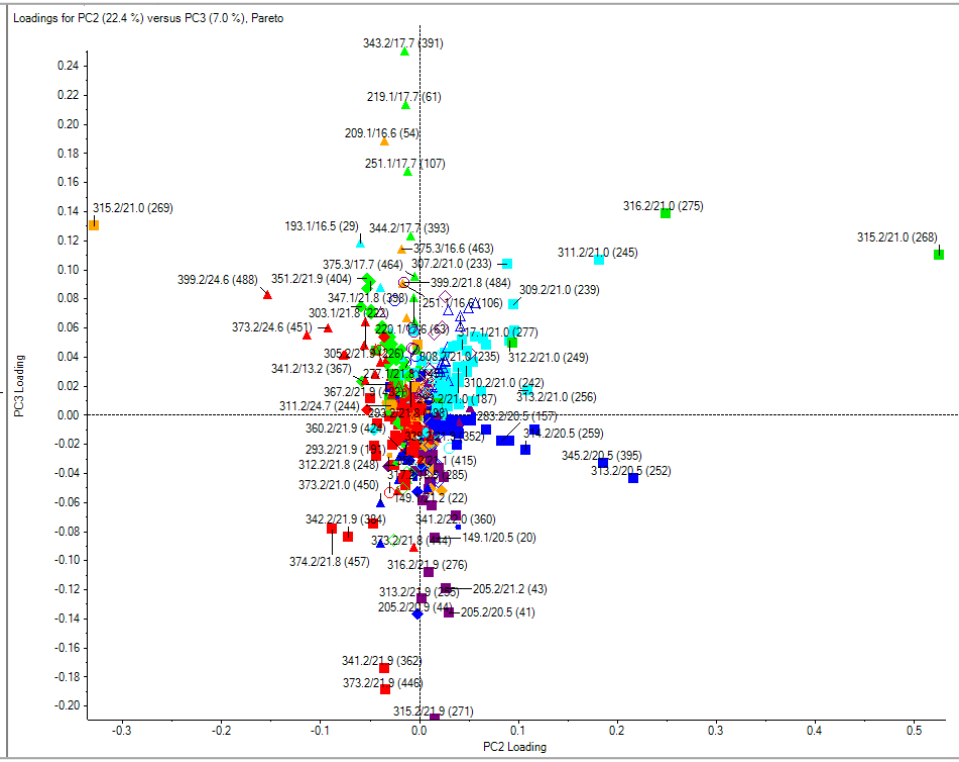




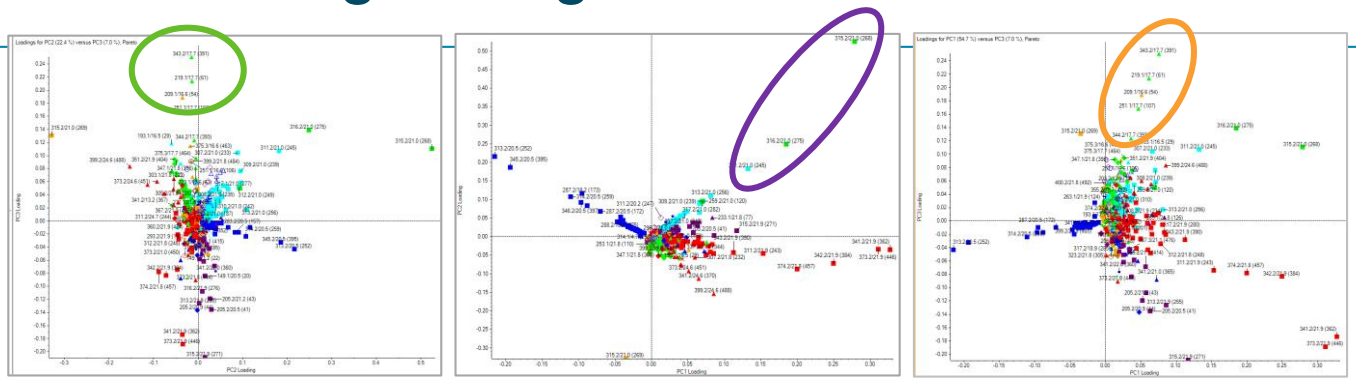
Step 1: Load up Data into MarkerView

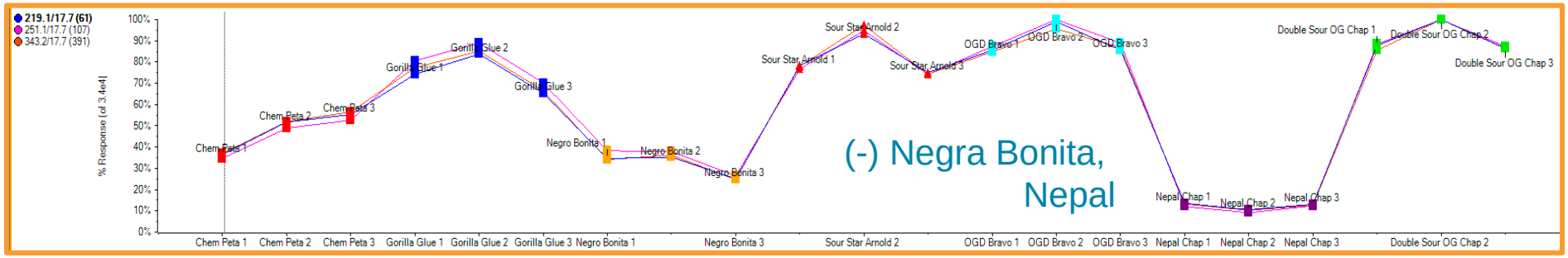
PC2 v PC3, Unsupervised PCA, Data Normalized (MLR method)

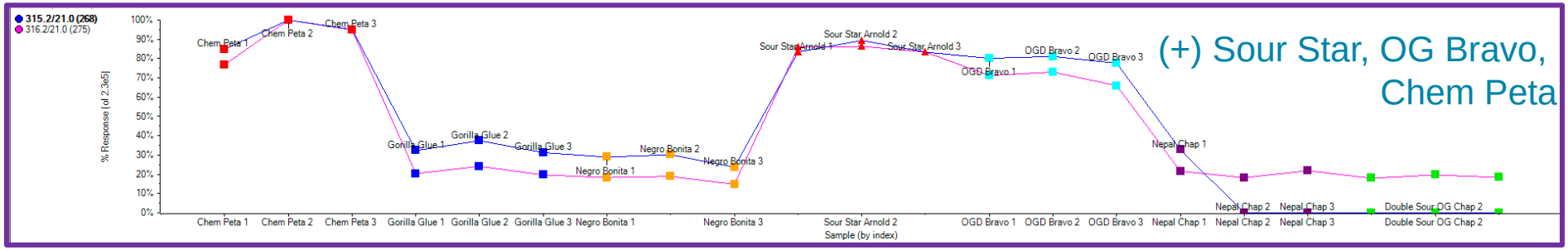


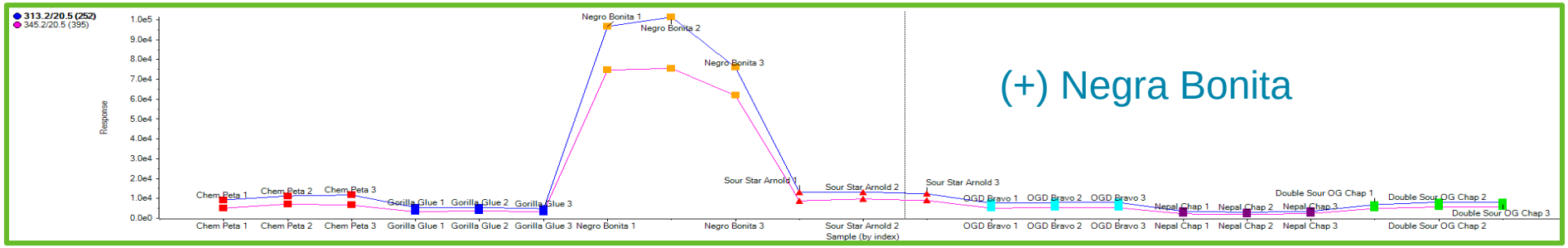


Step 2: Find Distinguishing Features → Peaks of Interest



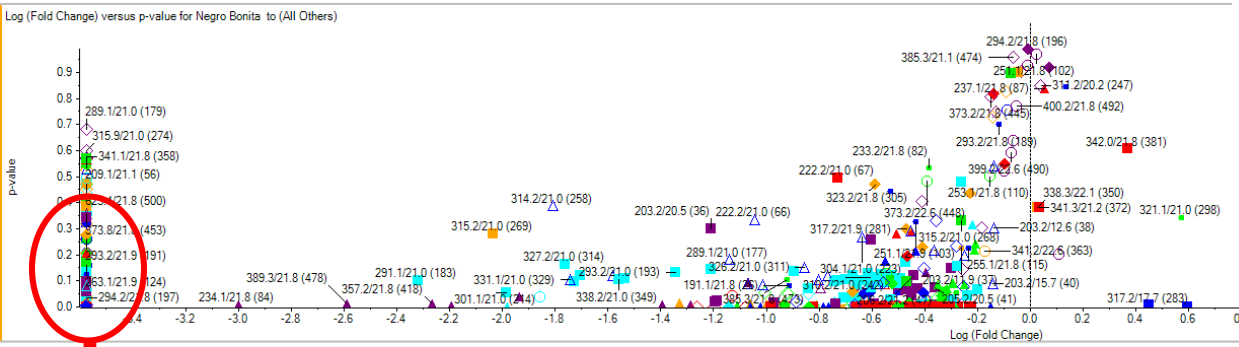






Step 2: Find Distinguishing Features → Peaks of Interest

Perform t-Test

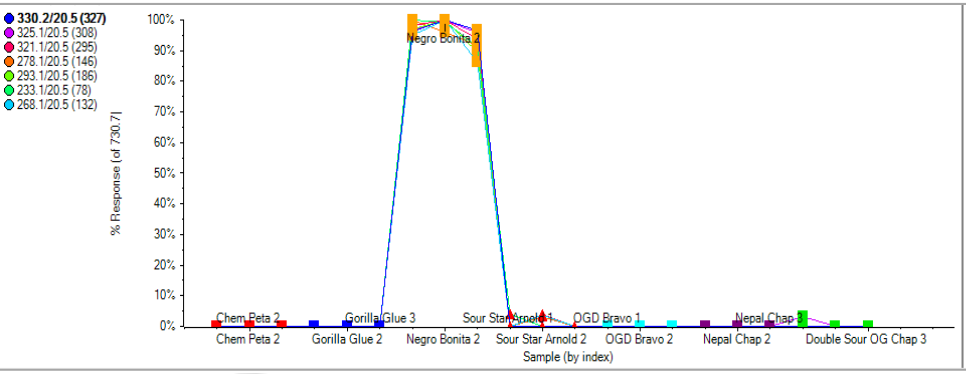


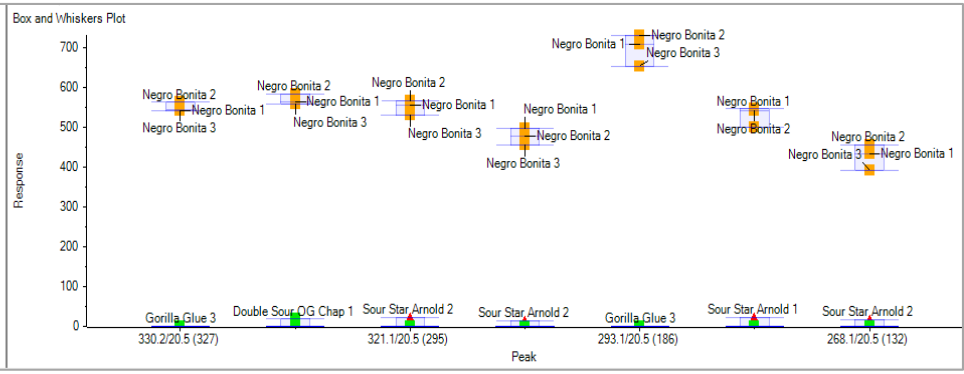
Volcano plot of features

Log (fold change) vs. p-value

Lowest p-value and greatest fold change

Features which are most distinguishing between the two groups compared in the t-Test





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Example: Negra Bonito vs. All Others

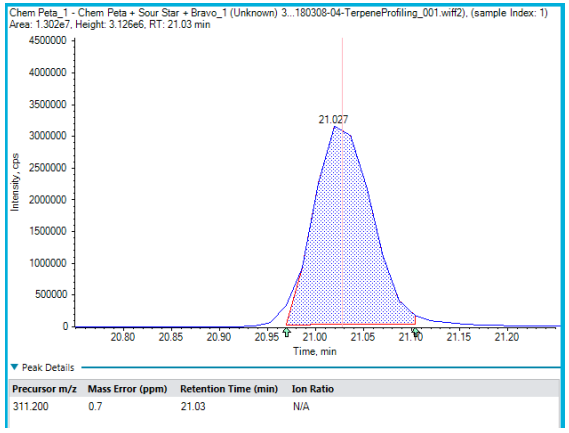
Peaks of Interest

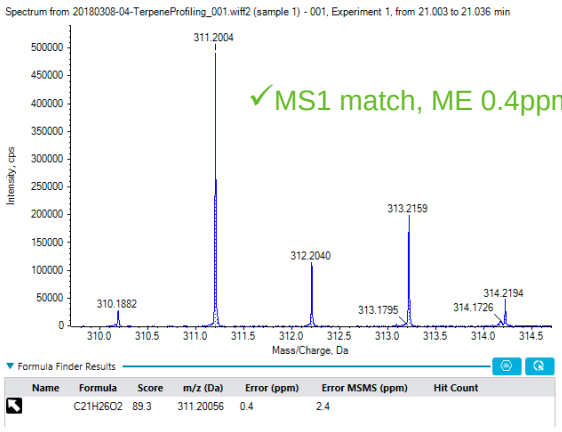
	Peak of Interest	*m/z*	RT
1	**(-) Negro Bonita_1**	341.2107	21.86
2	**(-) Negro Bonita_2**	341.2109	21
3	**(-) Negro Bonita_3**	373.2363	21.86
4	**(-) Nepal_1**	219.1014	17.67
5	**(-) Nepal_2**	251.1277	17.66
6	**(-) Nepal_3**	343.2266	17.67
7	**(+) Chem Peta + Sour Star + Bravo_1**	311.2002	21
8	**(+) Chem Peta + Sour Star + Bravo_2**	315.2309	20.99
9	**(+) Chem Peta + Sour Star + Bravo_3**	316.235	21
10	**(+) Negro Bonita_1**	313.1794	20.52
11	**(+) Negro Bonita_2**	345.2057	20.52
12	**(+) Sour Star + Double Sour_1**	375.2528	16.56

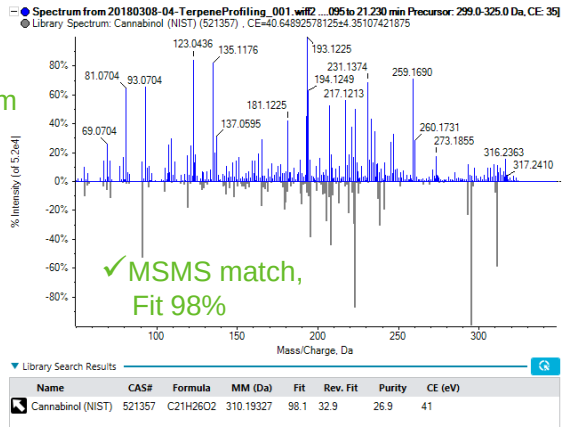
Step 3: Suspect Screening
Some Peaks of Interest Identified by MSMS Library Matches

Peak of Interest #7: (+) in Chem Peta, Sour Star, OG Bravo (*m/z* 311.2003)

- NIST Library Match for Cannabinol (CBN)
- A non-psychoactive cannabinoid usually found only in trace levels

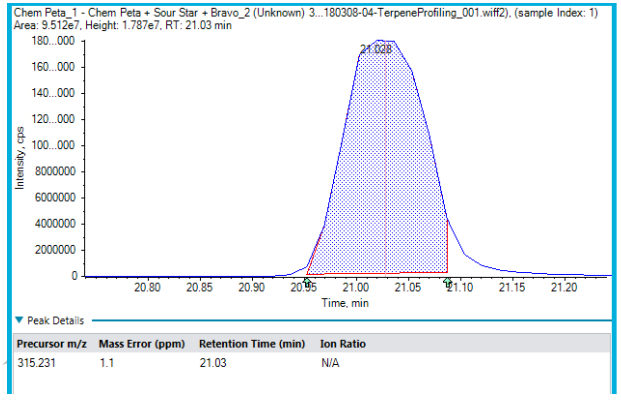


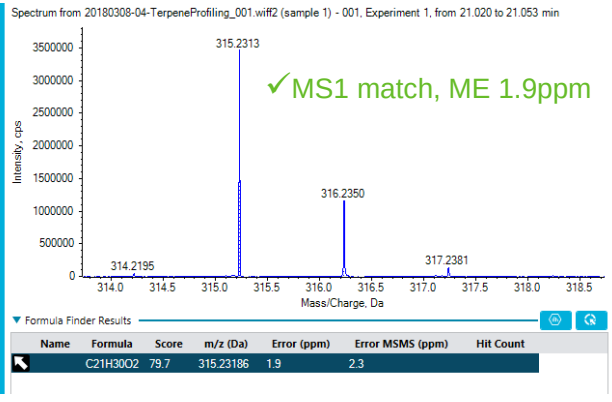


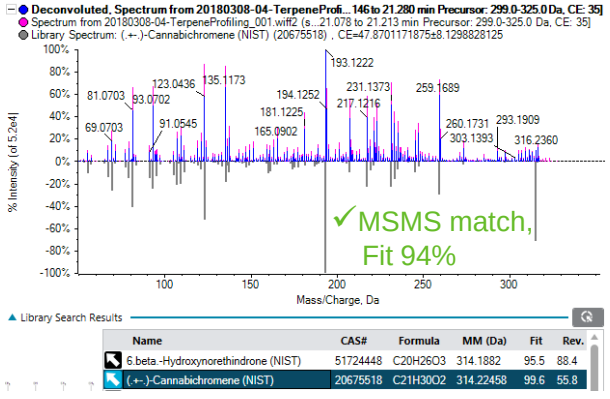


Peak of Interest #8: (+) in Chem Peta, Sour Star, OG Bravo (*m/z* 315.2313)

- NIST Library Match for Cannabichromene (CBC)
- May have anti-inflammatory, anti-viral, antifungal properties
- Contribute to the analgesic effects



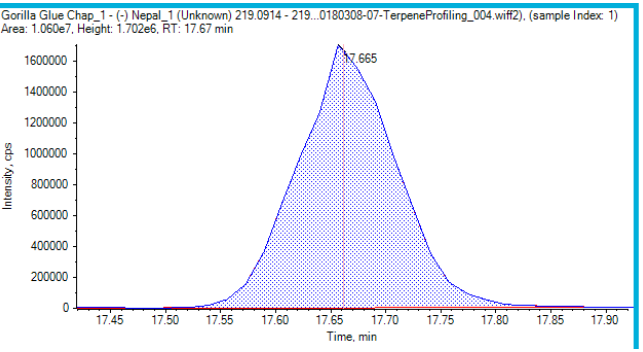


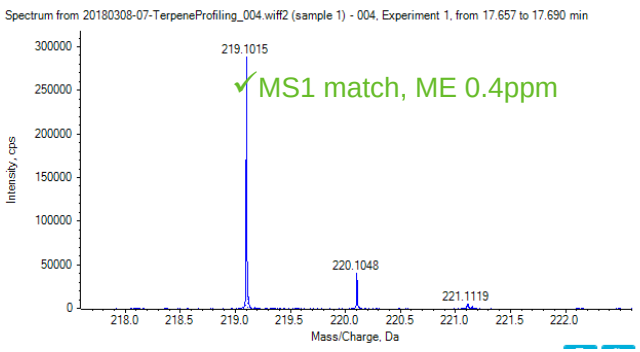


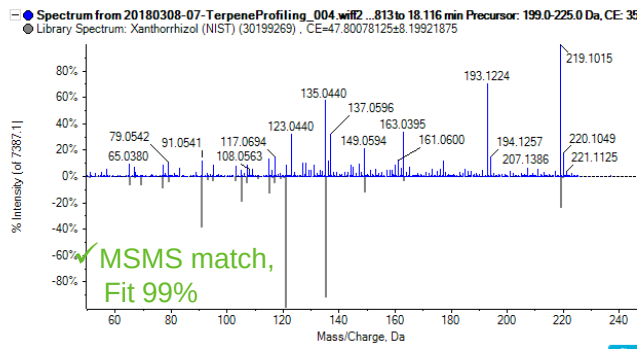
Step 3: Suspect Screening
Some Peaks of Interest Identified by MSMS Library Matches

Peak of Interest #4: (-) in Nepal (*m/z* 219.1015)

- NIST Library Match for Xanthorrizol
- A sesquiterpenoid which exhibits natural antibacterial, antifungal activity



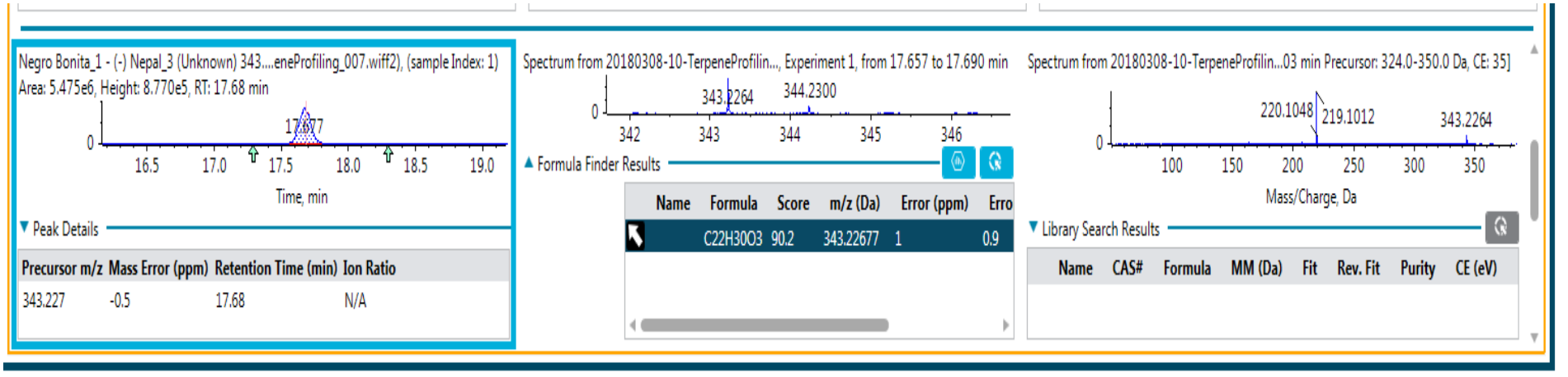




…but some needed some more digging to ID

Peak of Interest #6: (-) in Nepal (*m/z* 343.2266)

Index	Sample Name	Component Name	Precursor Mass	Area Ratio of...	Mass Error...	Library Confidence	Found At Mass	Mass Error (pp...	Library Hit	Library Score	Formula Finder Results	Formula Finder Sc...
6	MeOH Blank	(-) Nepal_3	343.227	N/A	■	■	N/A	N/A		N/A		N/A
439	Chem Peta_1	(-) Nepal_3	343.227	N/A	✓	●	343.2268	0.5	No Match	0.0	C22H30O3	99.125
872	Gorilla Glue Chap_1	(-) Nepal_3	343.227	N/A	✓	●	343.2265	-0.3	No Match	0.0	C22H30O3	87.331
1305	Negro Bonita_1	(-) Nepal_3	343.227	N/A	✓	●	343.2264	-0.5	No Match	0.0	C22H30O3	90.203
1738	Sour Star Arnold_1	(-) Nepal_3	343.227	N/A	✓	●	343.2266	0.0	No Match	0.0	C22H30O3	95.001
2171	OGD Bravo_1	(-) Nepal_3	343.227	N/A	✓	●	343.2265	-0.3	No Match	0.0	C22H30O3	88.962
2604	Nepal Chap_1	(-) Nepal_3	343.227	N/A	✓	●	343.2267	0.4	No Match	0.0	C22H30O3	97.504
3037	Double Sour OG Chap_1	(-) Nepal_3	343.227	N/A	✓	●	343.2266	-0.1	No Match	0.0	C22H30O3	93.956



Peak of Interest #6: $C_{22}H_{30}O_3$

No MSMS library match

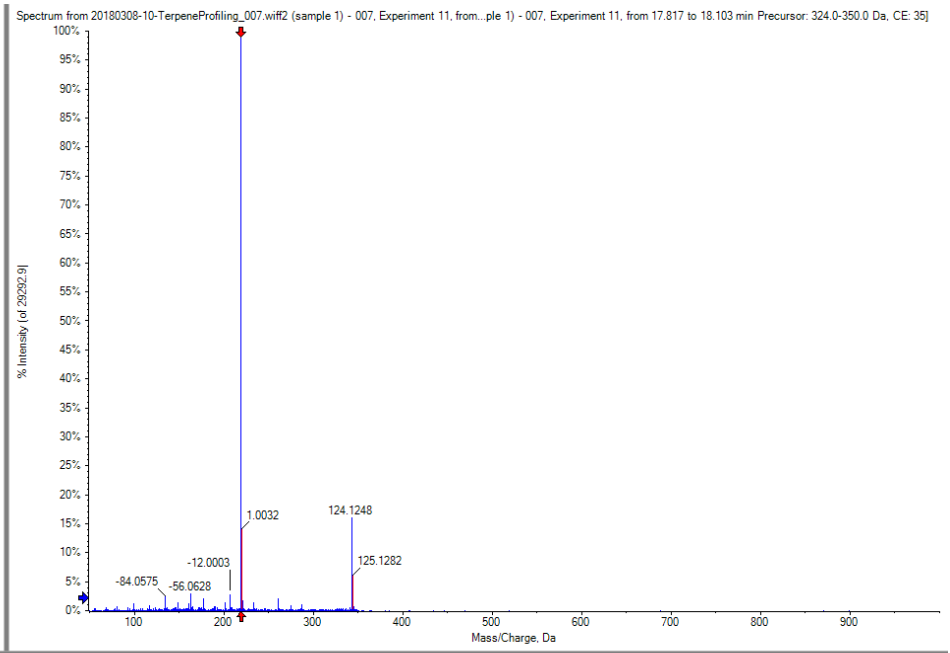
Empirical Formula: $C_{22}H_{30}O_3$
ChemSpider Search with Predicted MSMS Comparison

ChemSpider results for: C22H30O3

1-40 of 526

ChemSpider

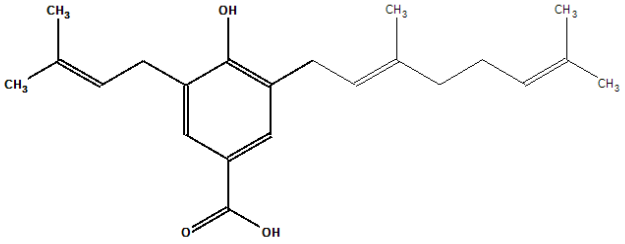
CSID	Common Name	Molecular Weight
166817	Siccanin	342.4718
18023	Megestrol	342.4718
62152	Trimegestone	342.4718
92091	Spironolactone intermediate	342.4718
28184844	5-Isopropenyl-2-methyl-2-cyclohexene-1-carboxylic anhydride	342.4718
64512	endrisone	342.4718
248321	4,4,6a,9-Tetramethyl-1,2,3,4,4a,5,6,6a,11b,13b-decahydrobenzo[a]furo[2,3,4-mn]xanthen-11-ol	342.4718
60209	340308DJ8M	342.4718
4534900	2-Hydroxy-6-[(8E,11E)-8,11,14-pentadecatrien-1-yl]benzoic acid	342.4718
197094	4P9MHB40VF	342.4718
14392179	DWA2JG4TUB	342.4718
8050818	anacardic acid	342.4718
548039	10,13-Dimethyl-1,6,7,8,9,10,11,12,13,14,15,16-dodecahydro-3'H-spiro[cyclopenta[a]phenanthrene-17,2'-furan]-3,5'(2H,4'H)-dione	342.4718
9511768	3OG14YJA8B	342.4718
21675312	3-Hexyl-4,8-dimethyl-7-[(3-methyl-2-buten-1-yl)oxy]-2H-chromen-2-one	342.4718
8535482	myrsinoic acid A	342.4718
3100845	1,1'-[Oxybis(2,1-ethanediyloxy)]bis(2,3,5-trimethylbenzene)	342.4718
198081	1WDZ80D40Y	342.4718
64914	(4aS,6aS,11bR,13aS,13bS)-4,4,6a,9-Tetramethyl-1,2,3,4,4a,5,6,6a,11b,13b-decahydrobenzo[a]furo[2,3,4-mn]xanthen-11-ol	342.4718
135019	(2S)-2-[(8S,9S,10R,13S,14S,17R)-10,13-Dimethyl-3-oxo-6,7,8,9,10,11,12,13,14,15,16,17-dodecahydro-3H-cyclopenta[a]phenanthren-17-yl]pro	342.4718
3360053	1,1'-[Oxybis(2,1-ethanediyloxy)]bis(2,4,6-trimethylbenzene)	342.4718
2667466	3-Oxoandrosta-1,4-dien-16-yl propionate	342.4718
140121	(17beta)-6-Methylene-3-oxoandrost-4-en-17-yl acetate	342.4718
217557	16-Methylene-17-oxoandrost-5-en-3-yl acetate	342.4718
146862	3-oxo-23,24-bisnorchola-1,4-dien-22-oic acid	342.4718
553407	(4aS,6aS,12aR,12bS)-10-Methoxy-4,4,6a,12b-tetramethyl-1,2,3,4,4a,5,6,6a,12a,12b-decahydro-9H-benzo[a]xanthen-9-one	342.4718



Display all Carbon Atoms

Options...

selected composition: $C_{13}H_{15}O_3^+$ (219.1016 Da)



Options...

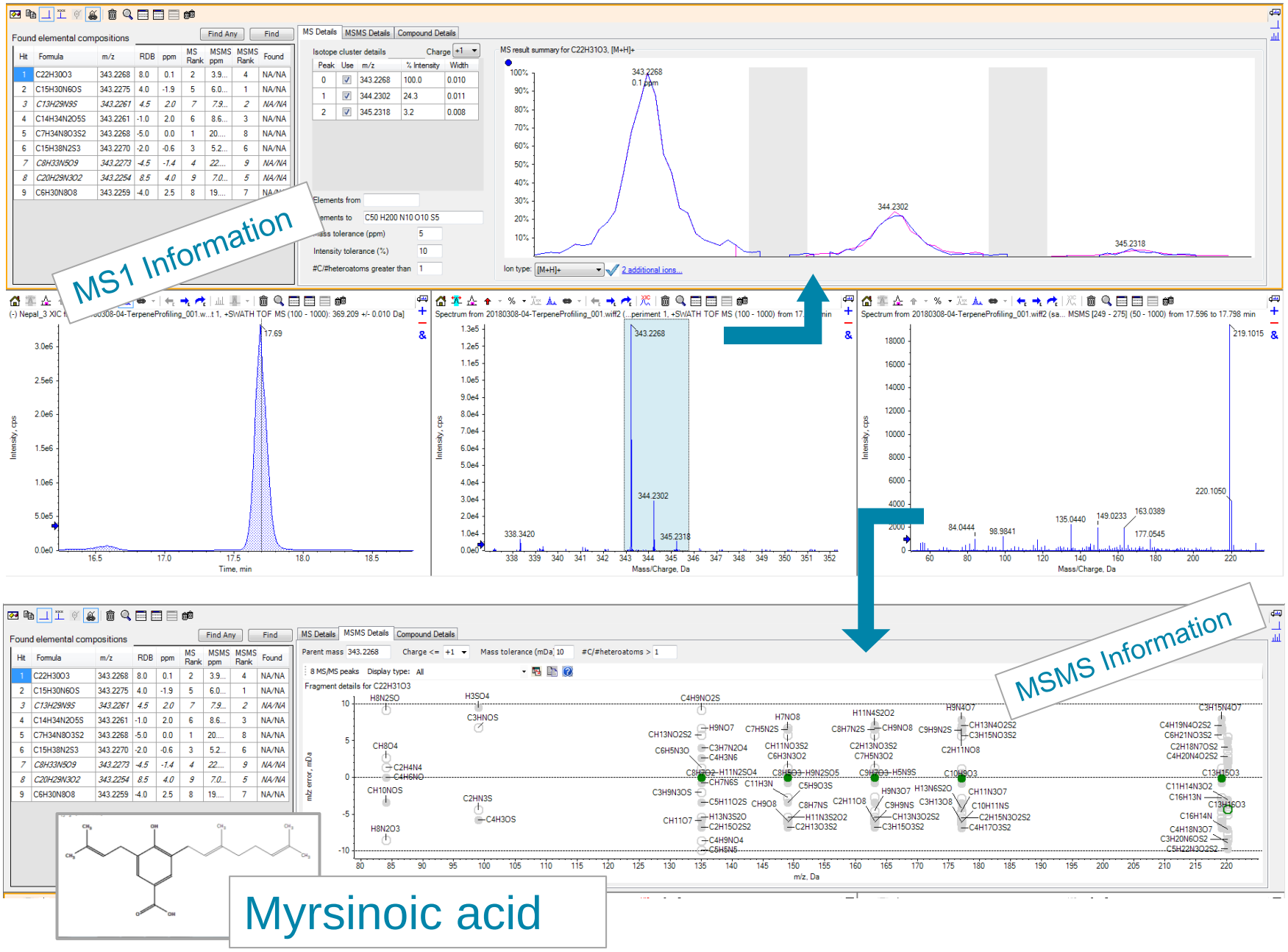
Fragments | Peaks

Mass/Charge	Intensity (%)	Assigned	Error (Da)
135.0441	2.56	☑	0.036
163.0388	2.88	☑	0.000
207.1013	2.80	☑	0.000
219.1012	100.00	☑	0.000
220.1048	14.09	☐	
343.2264	16.08	☑	0.000
344.2298	6.14	☐	

Matches: 5 of 7 peaks, 86.0% of total intensity

Select | Cancel

Myrsinoic acid


Found elemental compositions
Find Any
Find
Hit Formula m/z RDB ppm MS Rank MSMS ppm MSMS Rank Found
1 C22H30O3 343.2268 8.0 0.1 2 3.9... 4 NA/NA
2 C15H30N6OS 343.2275 4.0 -1.9 5 6.0... 1 NA/NA
3 C13H29N9S 343.2261 4.5 2.0 7 7.9... 2 NA/NA
4 C14H34N2O5S 343.2261 -1.0 2.0 6 8.6... 3 NA/NA
5 C7H34N8O3S2 343.2268 -5.0 0.0 1 20.... 8 NA/NA
6 C15H38N2S3 343.2270 -2.0 -0.6 3 5.2... 6 NA/NA
7 C8H33N5O9 343.2273 -4.5 -1.4 4 22.... 9 NA/NA
8 C20H29N3O2 343.2254 8.5 4.0 9 7.0... 5 NA/NA
9 C6H30N8O8 343.2259 -4.0 2.5 8 19.... 7 NA/NA
MS Details
MSMS Details
Compound Details
Isotope cluster details
Charge +1
Peak Use m/z % Intensity Width
0 343.2268 100.0 0.010
1 344.2302 24.3 0.011
2 345.2318 3.2 0.008
Elements from
Elements to C50 H200 N10 O10 S5
Mass tolerance (ppm) 5
Intensity tolerance (%) 10
#C/#heteroatoms greater than 1
MS result summary for C22H31O3, [M+H]+
343.2268
0.1 ppm
344.2302
345.2318
Ion type: [M+H]+
2 additional ions...
MS1 Information
(-) Nepal_3 XIC
20180308-04-TerpeneProfiling_001.w...t 1, +SWATH TOF MS (100 - 1000): 369.209 +/- 0.010 Da]
17.69
Intensity, cps
Time, min
Spectrum from 20180308-04-TerpeneProfiling_001.wiff2 (...periment 1, +SWATH TOF MS (100 - 1000) from 17...
343.2268
344.2302
345.2318
338.3420
Mass/Charge, Da
Spectrum from 20180308-04-TerpeneProfiling_001.wiff2 (sa... MSMS [249 - 275] (50 - 1000) from 17.596 to 17.798 min
219.1015
220.1050
163.0389
149.0233
135.0440
84.0444
98.9841
177.0545
Found elemental compositions
Find Any
Find
MS Details
MSMS Details
Compound Details
Parent mass 343.2268
Charge <= +1
Mass tolerance (mDa) 10
#C/#heteroatoms > 1
8 MS/MS peaks
Display type: All
Fragment details for C22H31O3
m/z error, mDa
m/z, Da
MSMS Information
Myrsinoic acid

Ranking Confidence Level in Unknown Identification

1. Analytical Standard Comparison
2. Accurate Mass/ Isotope/ Fragments consistent with published data
3. Accurate Mass/ Isotope/ Fragments are consistent with proposed structure, but published spectra are not available
4. Accurate Mass and Isotopes are consistent with proposed structure, but unable to attribute the major fragments to the proposed structure

Schymanski, E. L., Jeon, J., Gulde, R., Fenner, K., Ru, M., Singer, H. P., Hollender, J. (2014) Identifying Small Molecules via High Resolution Mass Spectrometry: Communicating Confidence. 2097–2098.

Ranking Confidence Level in Unknown Identification

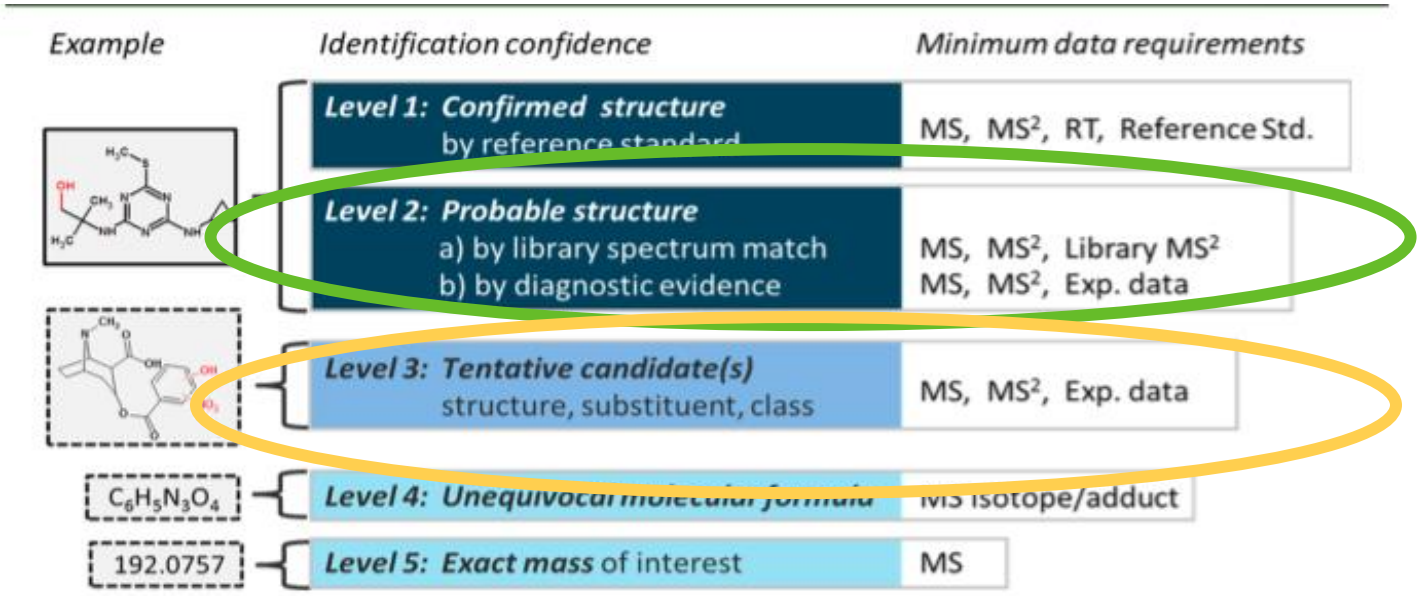


Figure 1. Proposed identification confidence levels in high resolution mass spectrometric analysis. Note: MS^2 is intended to also represent any form of MS fragmentation (e.g., MS^n, MS^n).

Schymanski *et al*. describes levels of identification criteria for nontargeted screening. With a library hit from a suspect screen, you can get as high as level 2 confidence (no reference standard, but good MS1 and MS2 matches). Without a library match, just your work on in silico fragment prediction of structures etc, you may only get as high as level 3 match.

Level 4 Identifications: Empirical formulae

- Peak of Interest #1: $C_{22}H_{28}O_3$ (492 structures in ChemSpider)
- Peak of Interest #5: $C_{14}H_{18}O_4$ (3,022 structures in ChemSpider)
- Peak of Interest #10: $C_{20}H_{24}O_3$ (16 structures in ChemSpider)
- Peak of Interest #11: $C_{21}H_{28}O_4$ (16 structures in ChemSpider)

Hmmm…. Why bother with the statistical analysis?

Can you just start with the SWATH data and the SUSPECT SCREEN?

765 of 3464 rows | Filters: 3 | Qualify for Rules Filters

Index	Sample Name	Component Name	Precursor Mass	Area Ratio...	Mass Error...	Library Confi...	Found At Mass	Mass Error (...	Library Hit	Library Score	Formula Finder Results	Formula Finder...
755	Chem Peta_1	357.2420 / 21.83 [M+H]+	357.242	N/A		✓	357.2420	N/A	3.beta.-Hydroxy-5-cholenoic a...	100.0	No formula found	0.000
2487	OGD Bravo_1	357.2420 / 21.83 [M+H]+	357.242	N/A		✓	357.2420	N/A	3.beta.-Hydroxy-5-cholenoic a...	100.0	No formula found	0.000
2192	OGD Bravo_1	136.0616 / 1.90	136.062	N/A		✓	136.0616	N/A	Adenine (NIST)	99.5	C5H5N5	85.840
2321	OGD Bravo_1	317.2471 / 16.55	317.247	667.733		✓	317.2474	N/A	Cannabigerol (NIST)	98.6	C21H32O2	95.408
1281	Gorilla Glue Chap_1	397.3829 / 25.79	397.383	77.424		✓	397.3825	N/A	Monobehenin (NIST)	98.5	C29H48	92.728
1759	Sour Star Arnold_1	136.0616 / 1.90	136.062	N/A		✓	136.0615	N/A	Adenine (NIST)	97.9	C5H5N5	82.204
3187	Double Sour OG Chap_1	317.2471 / 16.55	317.247	757.118		✓	317.2472	N/A	Cannabigerol (NIST)	97.7	C21H32O2	88.288
460	Chem Peta_1	136.0616 / 1.90	136.062	N/A		✓	136.0617	N/A	Adenine (NIST)	97.0	No formula found	0.000
2360	OGD Bravo_1	399.2339 / 19.10	399.234	N/A		✓	399.2338	N/A	Methanesulfonamide, N-3-(9H...	96.6	C16H34N2O9	97.624
2366	OGD Bravo_1	399.2340 / 19.61	399.234	N/A		✓	399.2338	N/A	Methanesulfonamide, N-3-(9H...	96.6	C16H34N2O9	97.624
597	Chem Peta_1	317.2474 / 17.62	317.247	N/A		✓	317.2471	N/A	Cannabigerol (NIST)	95.7	C21H32O2	90.542
3013	Nepal Chap_1	397.3829 / 25.79	397.383	75.450		✓	397.3825	N/A	Monobehenin (NIST)	95.5	C29H48	90.778
1888	Sour Star Arnold_1	317.2471 / 16.55	317.247	497.440		✓	317.2473	N/A	Cannabigerol (NIST)	95.0	C21H32O2	94.419
2754	Nepal Chap_1	317.2471 / 16.55	317.247	381.961		✓	317.2469	N/A	Cannabigerol (NIST)	95.0	C21H32O2	85.415
1022	Gorilla Glue Chap_1	317.2471 / 16.55	317.247	357.408		✓	317.2472	N/A	Cannabigerol (NIST)	94.9	C21H32O2	90.432
1455	Negro Bonita_1	317.2471 / 16.55	317.247	159.548		✓	317.2472	N/A	Cannabigerol (NIST)	94.8	C21H32O2	93.263
2762	Nepal Chap_1	317.2474 / 17.62	317.247	N/A		✓	317.2472	N/A	Cannabigerol (NIST)	94.4	C21H32O2	92.589
589	Chem Peta_1	317.2471 / 16.55	317.247	336.278		✓	317.2471	N/A	Cannabigerol (NIST)	93.7	C21H32O2	89.434
3154	Double Sour OG Chap_1	369.1332 / 12.70	369.133	N/A		✓	369.1334	N/A	Methoxyfenozide (NIST)	92.3	C21H20O6	95.444
1544	Negro Bonita_1	337.1794 / 20.57 [M+CH3OH+H]+	337.179	192.132		✓	337.1795	N/A	Acebutolol (NIST)	90.8	C21H20O2	92.439
3351	Double Sour OG Chap_1	233.1169 / 21.83 [M+H]+	233.117	562.790		✓	233.1171	N/A	2,4-Di-tert-amylphenol (NIST)	90.8	C7H16N6OS	66.430
1855	Sour Star Arnold_1	369.1332 / 12.70	369.133	N/A		✓	369.1332	N/A	Methoxyfenozide (NIST)	90.3	C21H20O6	96.888
753	Chem Peta_1	233.1169 / 21.83 [M+H]+	233.117	1411.374		✓	233.1169	N/A	2,4-Di-tert-amylphenol (NIST)	90.2	C14H16O3	87.243
1422	Negro Bonita_1	369.1332 / 12.70	369.133	N/A		✓	369.1333	N/A	Methoxyfenozide (NIST)	90.0	C21H20O6	97.845
609	Chem Peta_1	287.2007 / 18.23	287.201	N/A		✓	287.2004	N/A	Tetrahydrocannabivarin (NIST)	89.3	C19H26O2	91.959
1908	Sour Star Arnold_1	287.2007 / 18.23	287.201	N/A		✓	287.2006	N/A	Tetrahydrocannabivarin (NIST)	88.5	C19H26O2	96.788
2054	Sour Star Arnold_1	357.2420 / 21.83 [M+H]+	357.242	N/A		✓	357.2418	N/A	Ethanethioic acid, S-2-(hexade...	88.5	No formula found	0.000
3233	Double Sour OG Chap_1	341.2110 / 19.69 [M+H]+	341.211	N/A		✓	341.2111	N/A	(+)-Pinoresinol (NIST)	88.3	C22H28O3	98.922

- THOUSANDS of hits, often across multiple sample groups
- Do we need all of them to get to the answer we seek???
- We want to CLASSIFY, DISTINGUISH and find DIFFERENCES that matter

Summary, Potential Applications, Potential Implications

- Unique Cannabis chemovars can be distinguished by their molecular signature
  - Strains cluster together and separate easily by PCA, even with no target masses defined
- Trace cannabinoids CBC and CBN identified as upregulated in Sour Star, Chem Peta, OG Bravo
- Myrsinoic Acid identified as a natural product downregulated in Nepal versus other strains
- Xanthorrhizol identified as a sesquiterpenoid present in multiple strains
  - Not one of the terpenes included in most targeted analyses

Summary, Potential Applications, Potential Implications

- Unique Cannabis chemovars can be distinguished by their molecular signature
  - Intellectual Property?
  - New strain behavior prediction?
  - Authenticity testing? Identifying fraudulent strain labelling?
  - Extraction and manufacturing process refinement?
  - Pharmaceutical/therapy design?
  - Identifying novel natural products?

- Food authenticity
- Metabolomic research
- Workflow: Classification of other types of complex samples

Questions?

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