THE EMERALD CONFERENCE
Produced by MJBizScience

Comprehensive comparison of cannabis cultivars using untargeted analytical workflows

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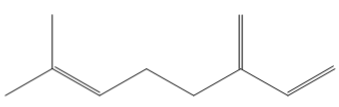
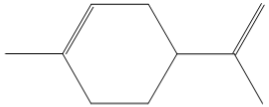
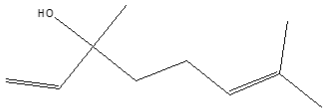
Cannabis aroma volatiles

- Cannabis terpenes and terpenoids
  - ✓ Impart distinctive aromas and flavours







	Myrcene	Limonene	Linalool
Aroma	*Spicy, herbal*	*Citrus, orange*	*Floral, citrus*

"*From the literature, it is therefore difficult to know what to believe when it comes to the odor of limonene.*" (J. Chem. Educ. 2021, 98, 11, 3600–3607)

- Many other aroma volatiles are also present, e.g. esters, aldehydes, etc.

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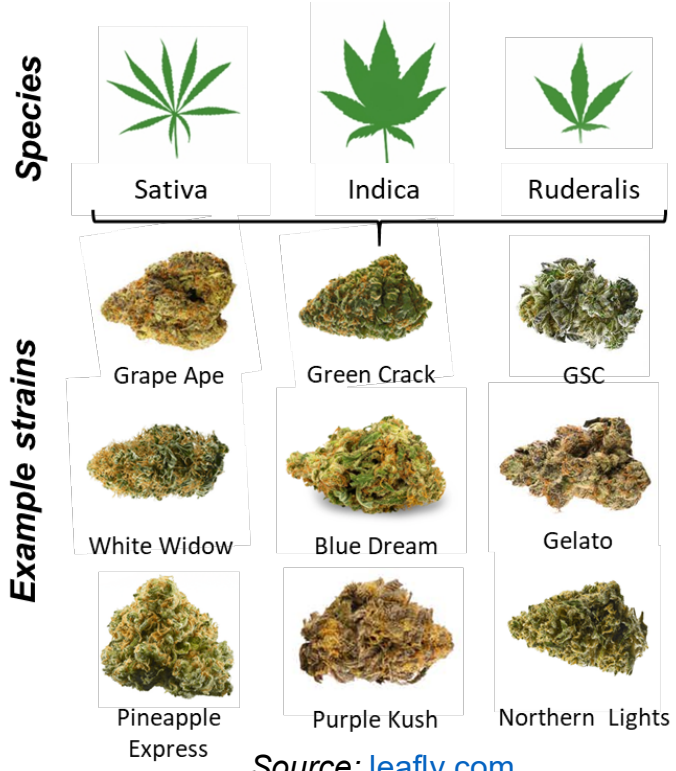
Aroma volatiles vs. cultivars

- Much remains unknown about cultivar distinctions
- Specific aroma profiles can be engineered by plant breeders



Source: leafly.com

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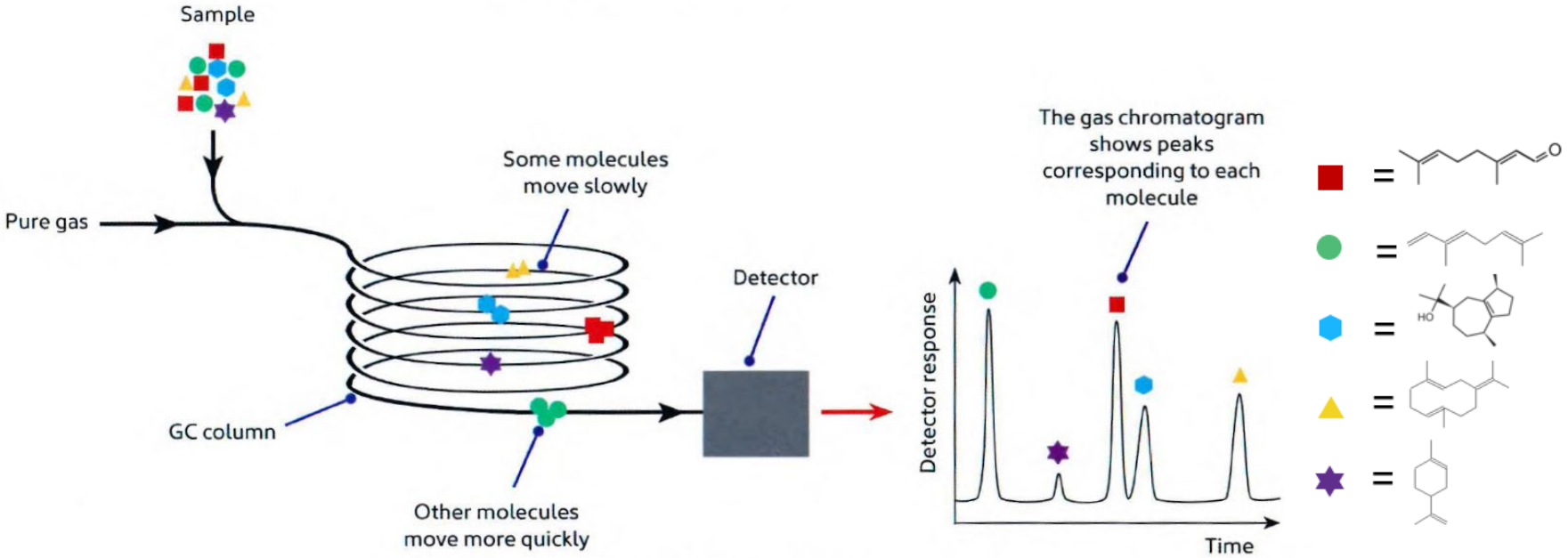
Is GC–MS fit-for-purpose?



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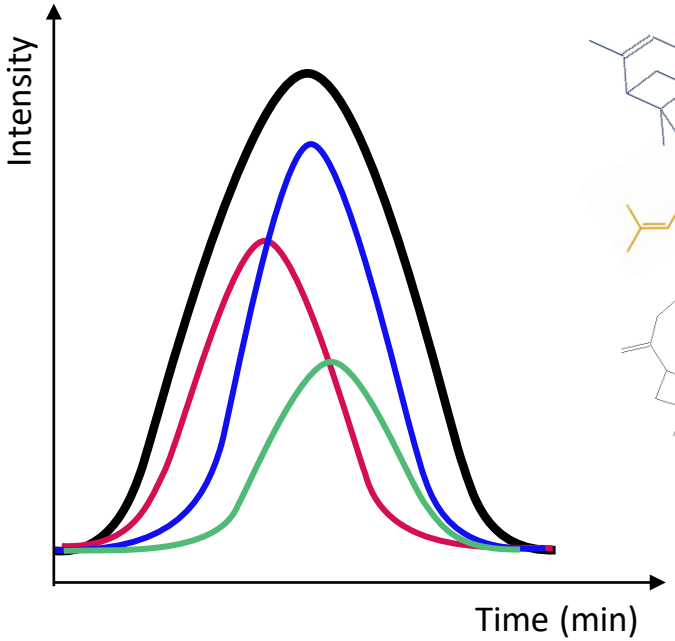
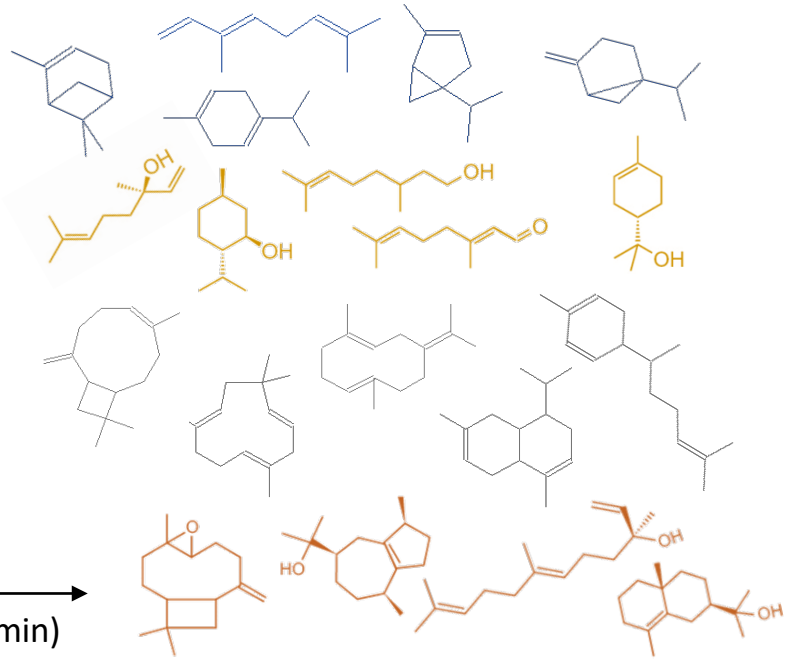
Is GC-MS fit-for-purpose?





Monoterpenes

Monoterpenoids

Sesquiterpenes

Sesquiterpenoids

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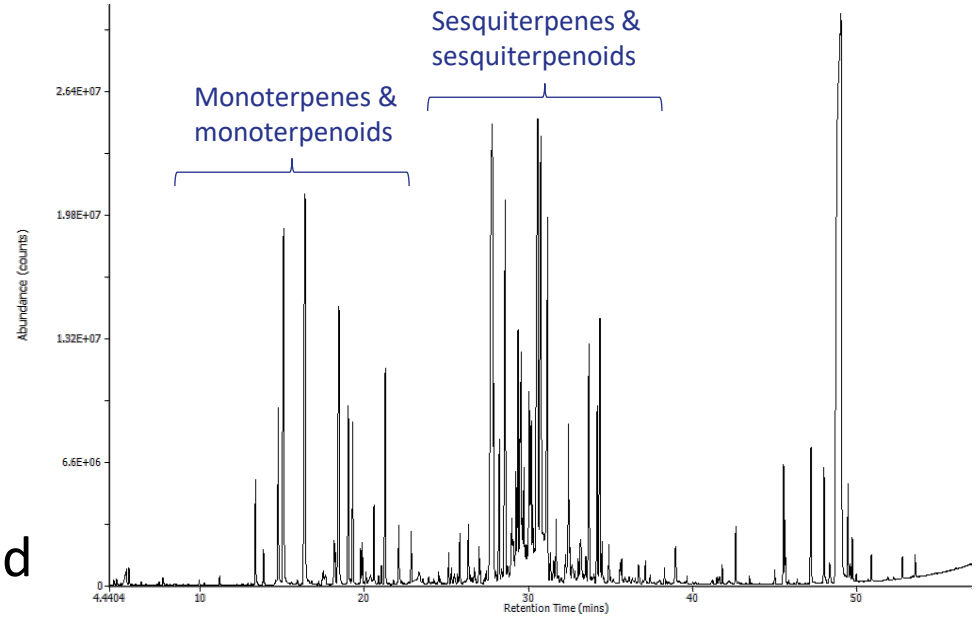
Challenges in terpenes analysis

- Over 200 terpenes have been identified in cannabis
- 1D GC results in coelutions between terpenes and terpenoids
- Abundance of important terpenes can be over-estimated



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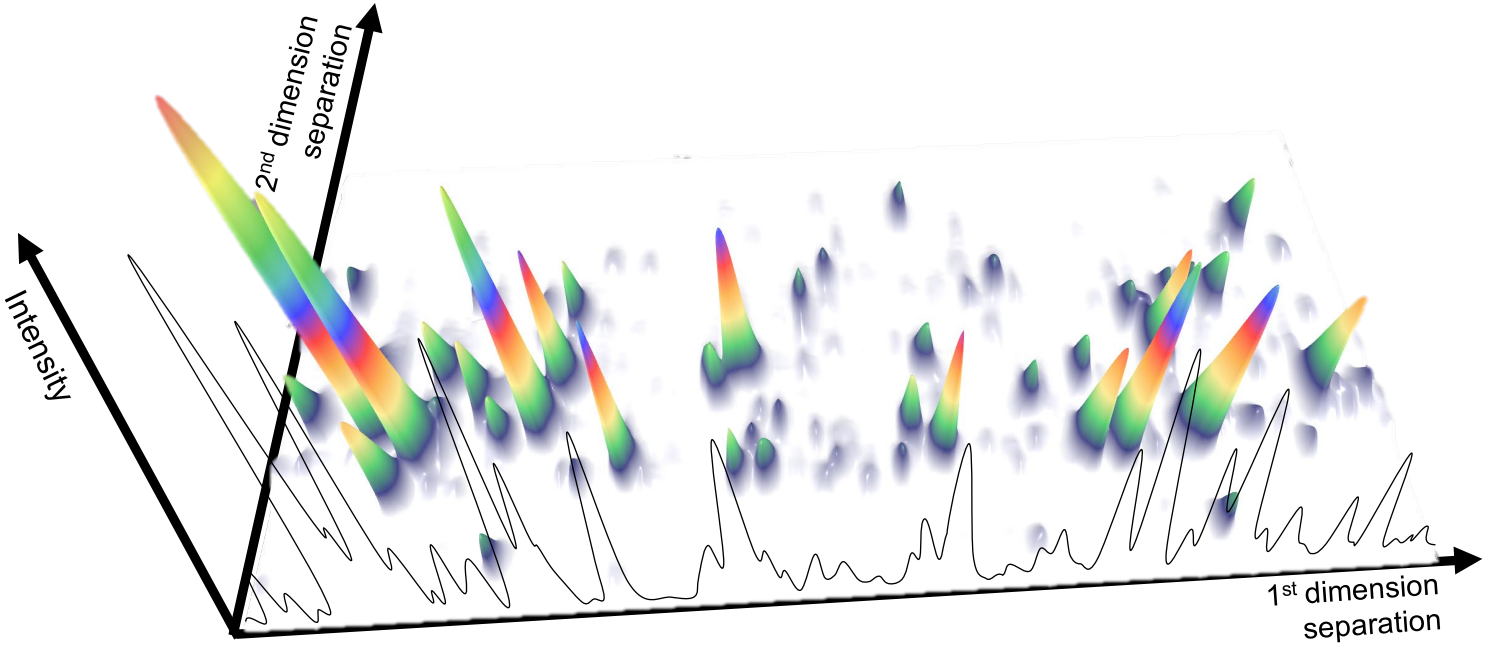
What is GC×GC?



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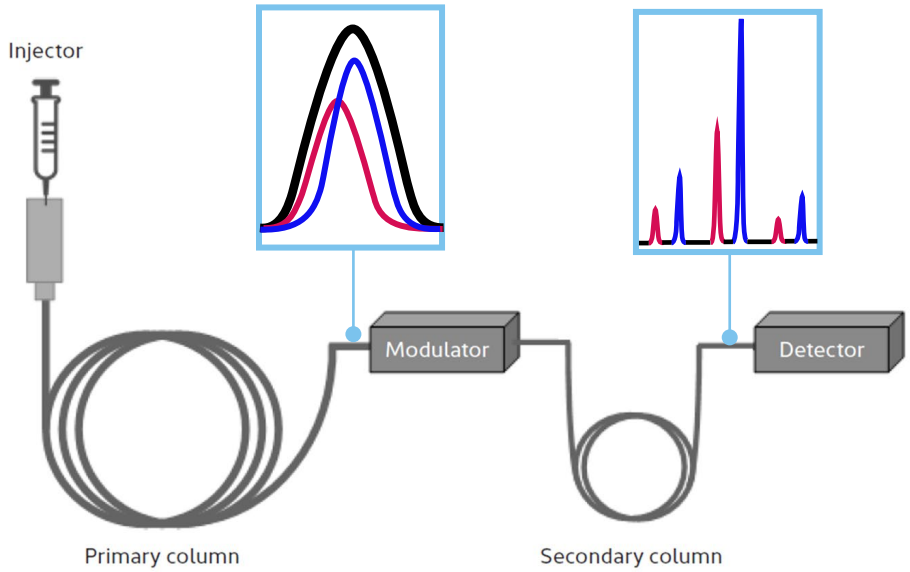

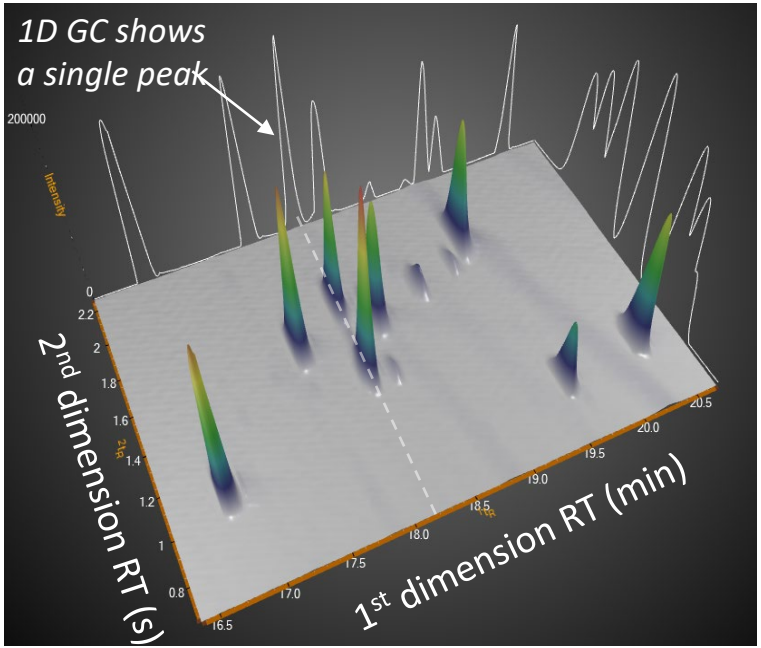

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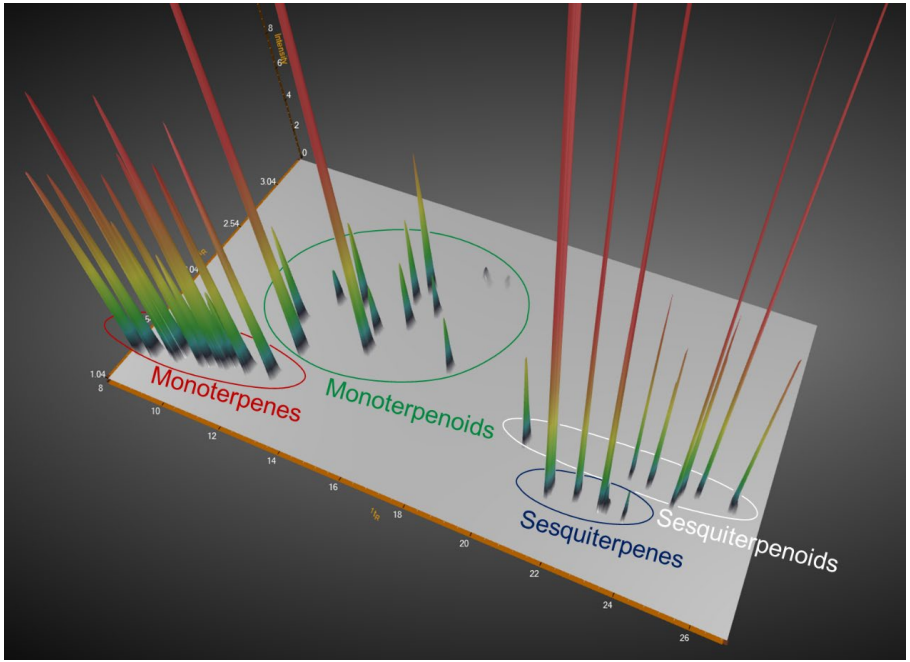
Cannabis terpenes by GC×GC-TOF MS

- Enhanced separation of terpenes and terpenoids
- Comprehensive detail on terpene profile
- Detection of other compounds



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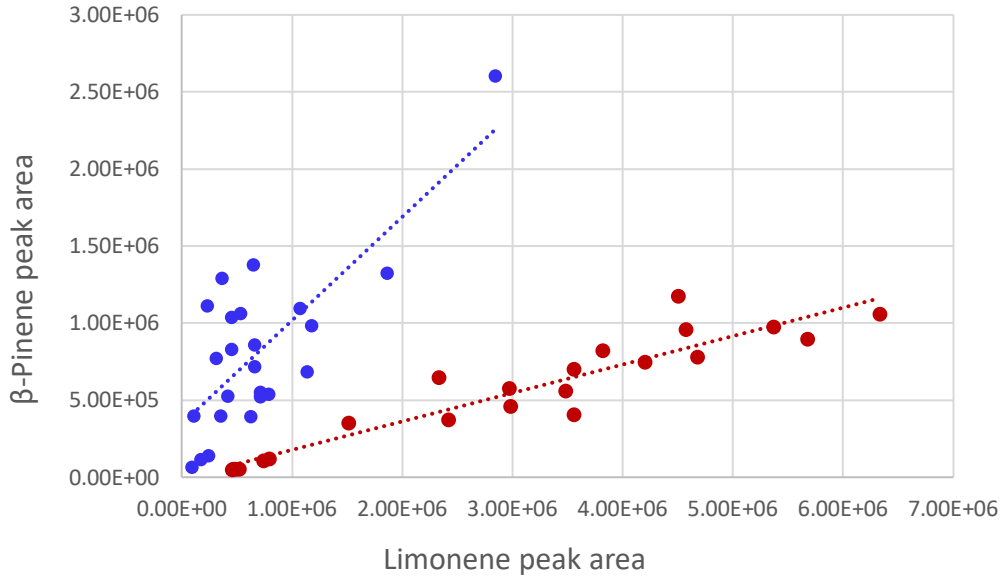
Analysis of cannabis cultivars

- Dried cannabis flowers were obtained from 12 different cultivars
- Extracted with methanol
- Analyzed beta-pine/ limonene ratio
- Looked at other features

Cultivar	BP/LIM Ratio
Pineaple Upside Down Cake	Hi
Blue Dreams	Hi
Northern Wreck	Lo
Acapulco Gold	Hi
P-Funk	Hi
P-Funk x Black Dog	Hi
Lemon OG	Lo
Zkittlez x Black Dog	Hi
Z-Dog	Lo
Orange Valley Delight	Hi
Super Sour Diesel	Lo
GMO x Black Dog	Lo

Diagnostic ratios in cannabis profiling



β-Pinene/Limonene ratio

- Hi
- Lo

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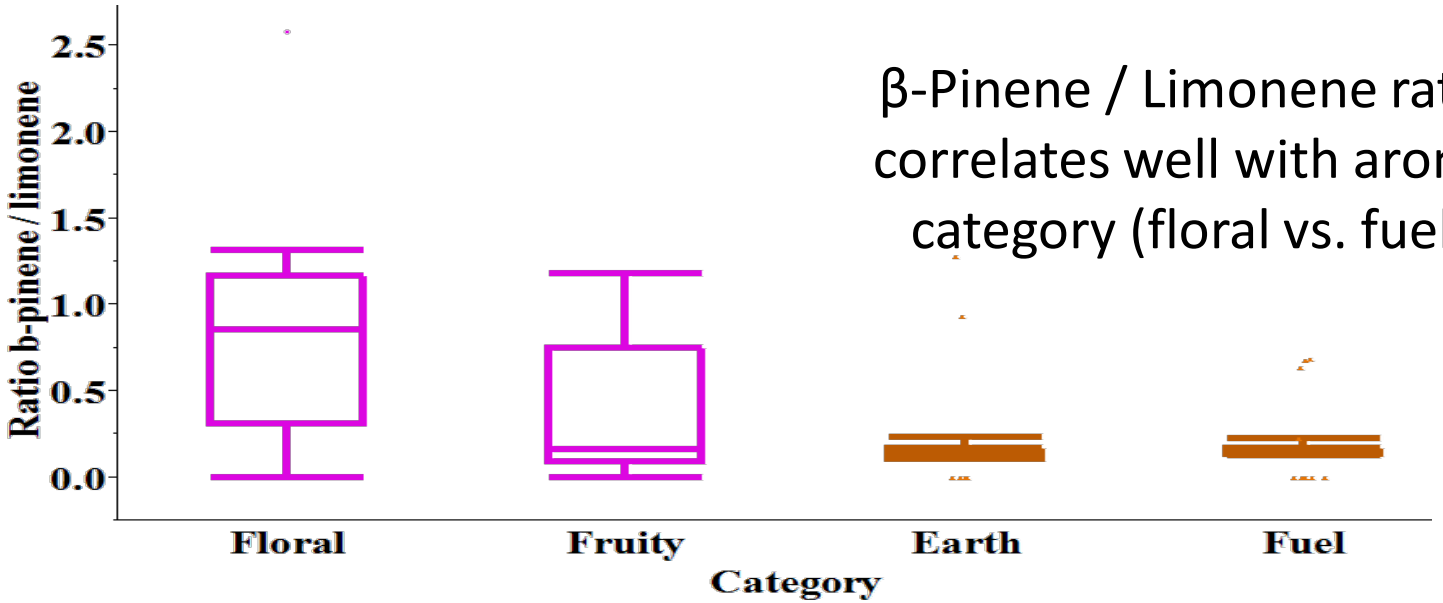
Diagnostic ratios in cannabis profiling



β-Pinene / Limonene ratio correlates well with aroma category (floral vs. fuel)

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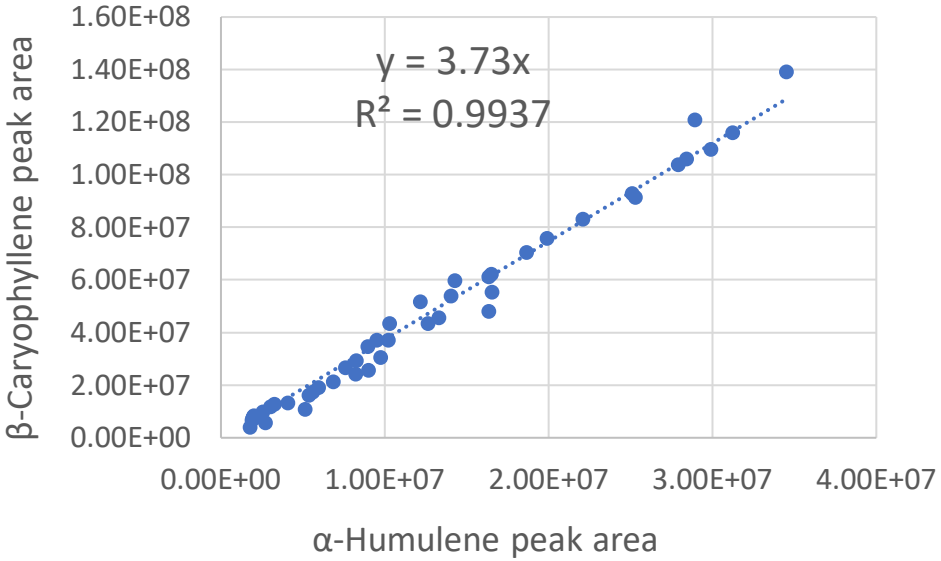
Diagnostic ratios in cannabis profiling



β-Caryophyllene/α-Humulene Ratio

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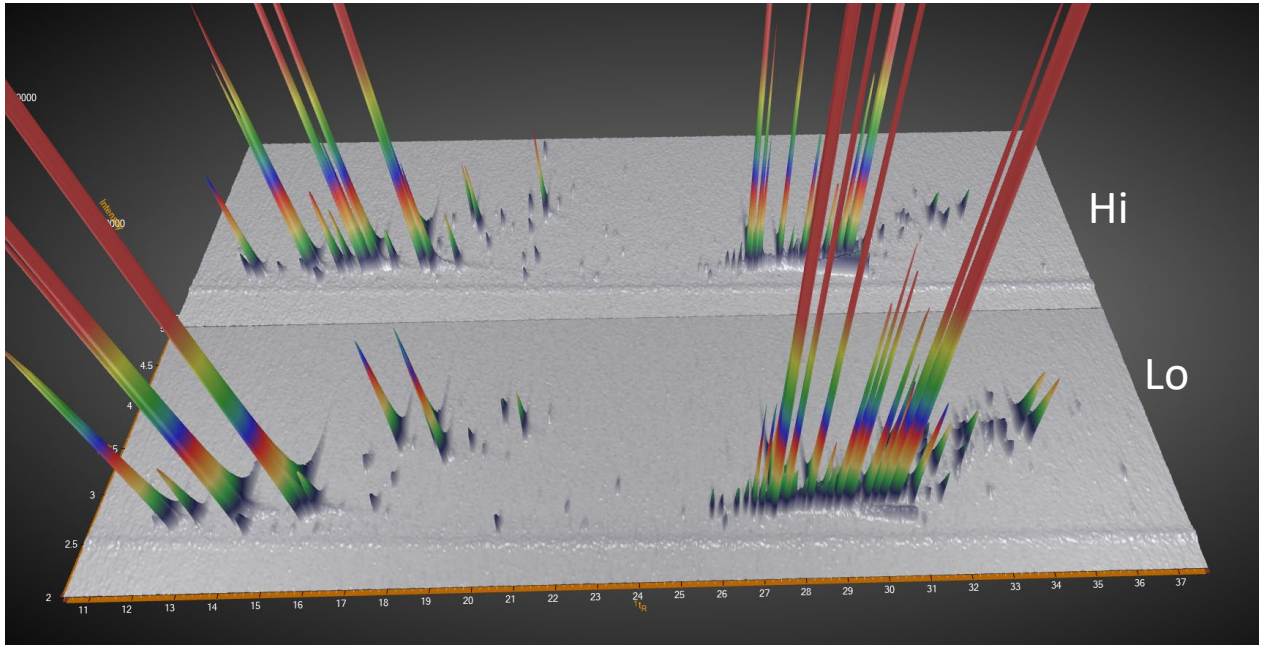
Analysis of cannabis by GC×GC-TOF MS



More detail than a single ratio can provide

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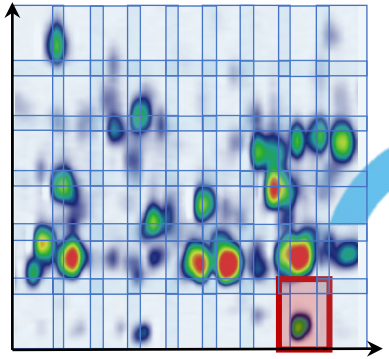
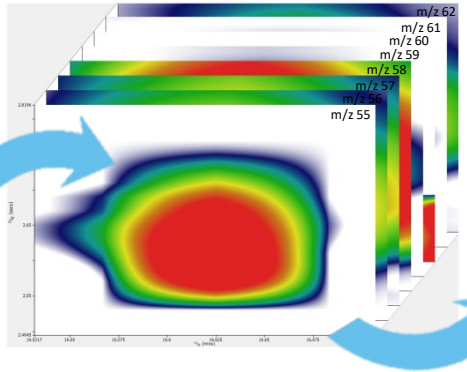
Reducing the dimensionality of data



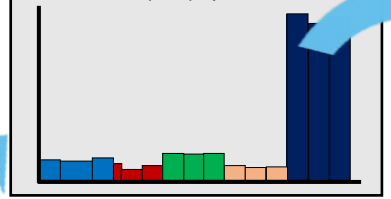
Aligned chromatogram is automatically split in to sections



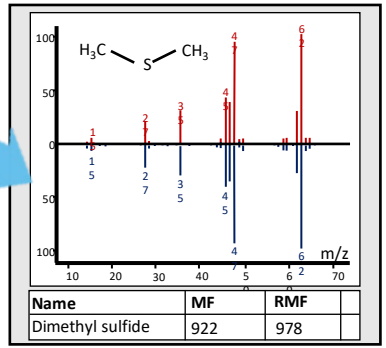
Every m/z channel is automatically compared for every tile of every datafile

ID	Name	Significance
F1135	6.50_3.20_62	1
F1092	6.50_3.20_47	2
F1369	9.33_2.40_94	3

RT 6.50, 3.20, m/z 62



Feature discovery highlights the key differences between sample classes



Name	MF	RMF
Dimethyl sulfide	922	978

Review and identify differences

- Untargeted, tile-based data analysis

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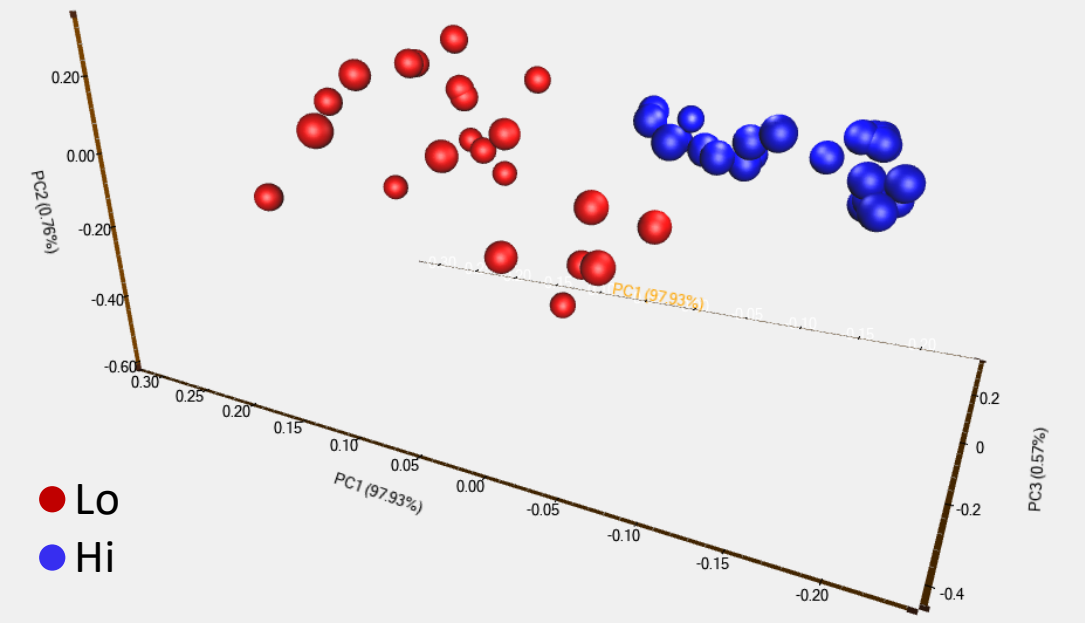
Expanding on the Floral vs. Fuel classification:



Categorization based on beta-pinene / limonene ratio type

- 12 cannabis cultivars analysed in triplicate
- Untargeted, tile-based data analysis

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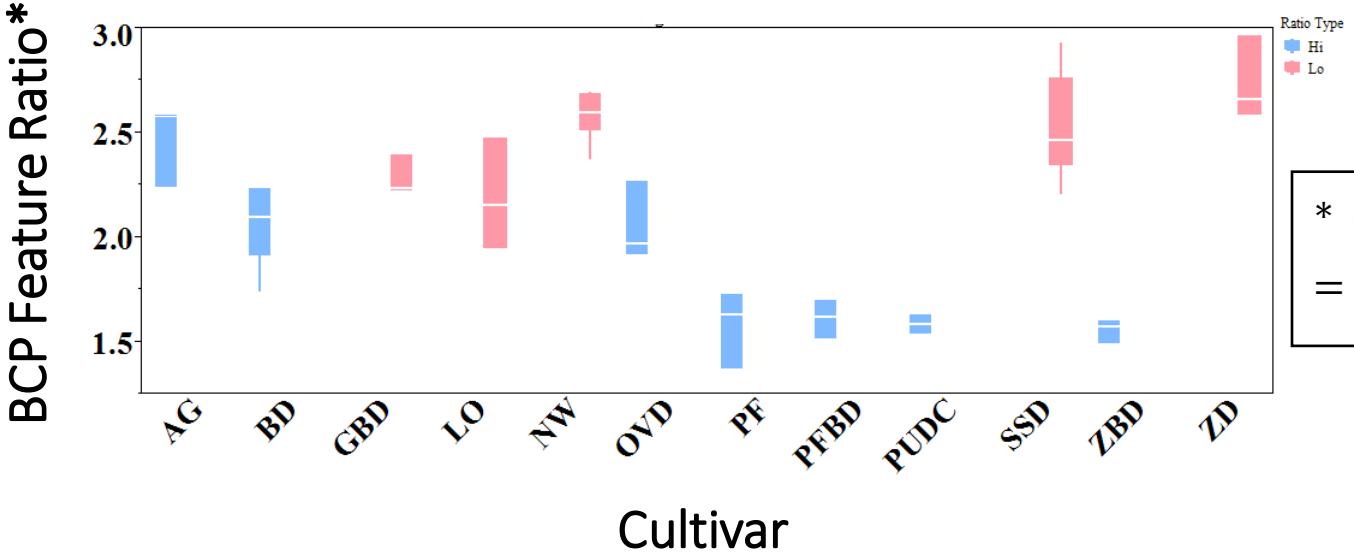
Ratio of 2 BCP Features Correlates Largely with BPIN / LIM Ratio Type



$$* \ \textit{BCP Feature Ratio} = \frac{\text{"27.3, 2.7, 56"}}{\text{"27.3, 0.375, 69"}}$$

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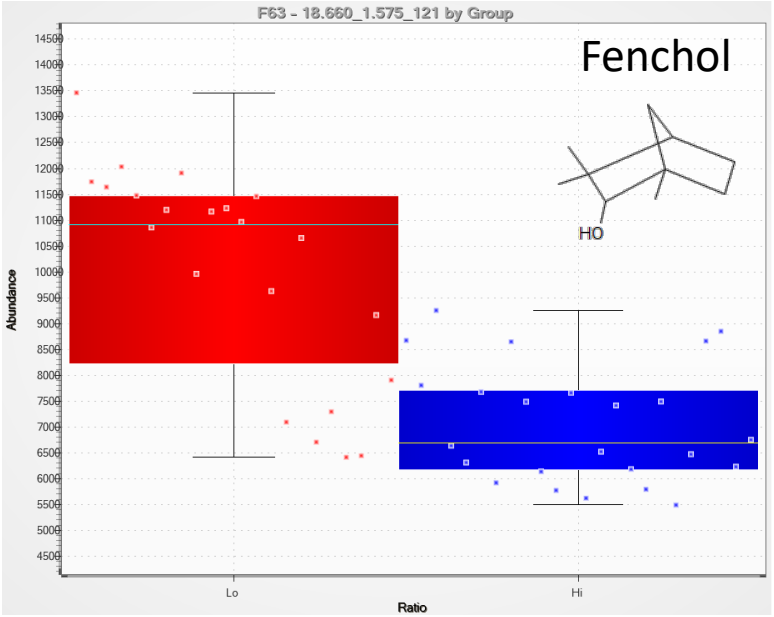
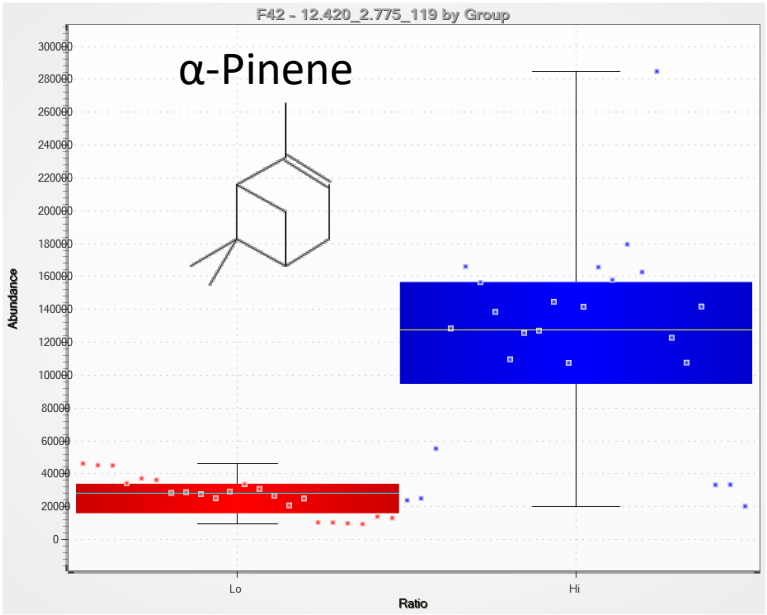
Expanding on the Floral vs. Fuel classification:
Categorization based on beta-pinene / limonene ratio type





Floral
Fuel

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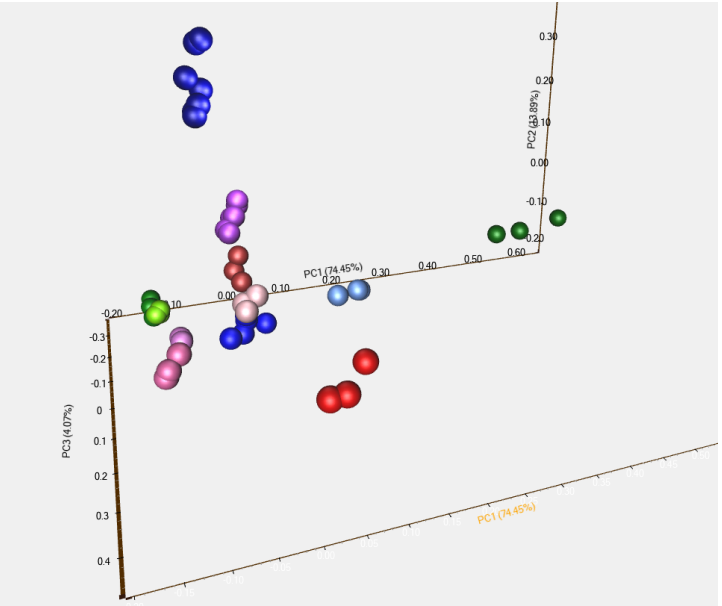
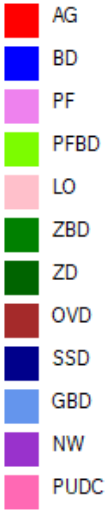
Distinguishing individual cultivars





- All cultivar replicates cluster closely
- Z-Dog (ZD) is clearly differentiated by PC1
- Super Sour Diesel (SSD) is clearly differentiated by PC2

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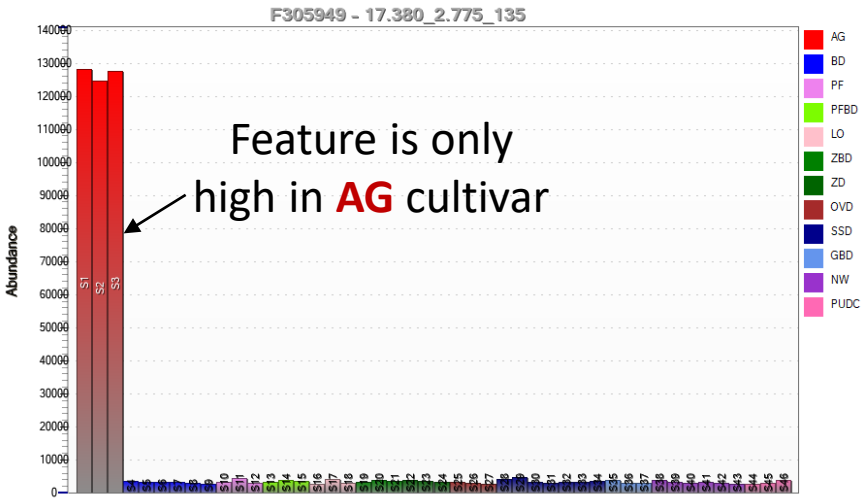
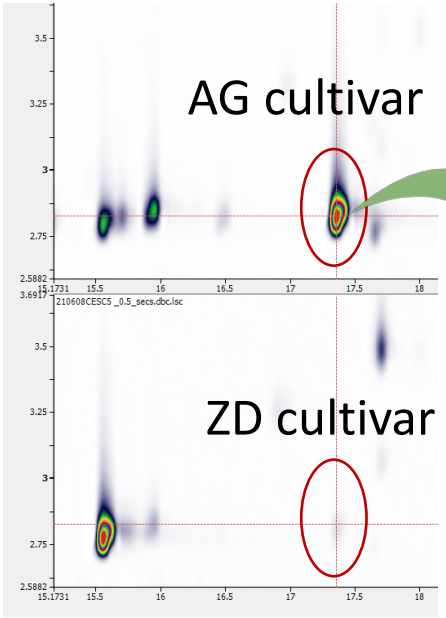
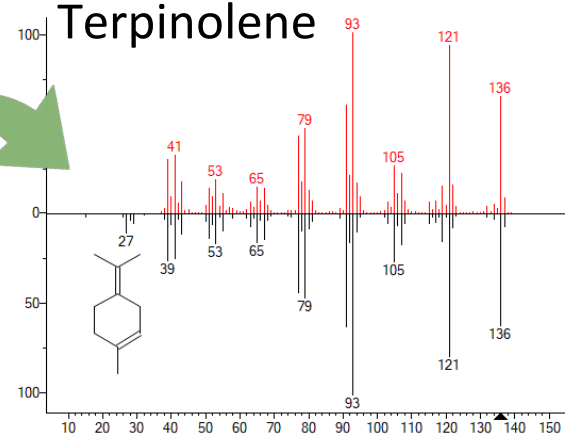
Distinguishing individual cultivars







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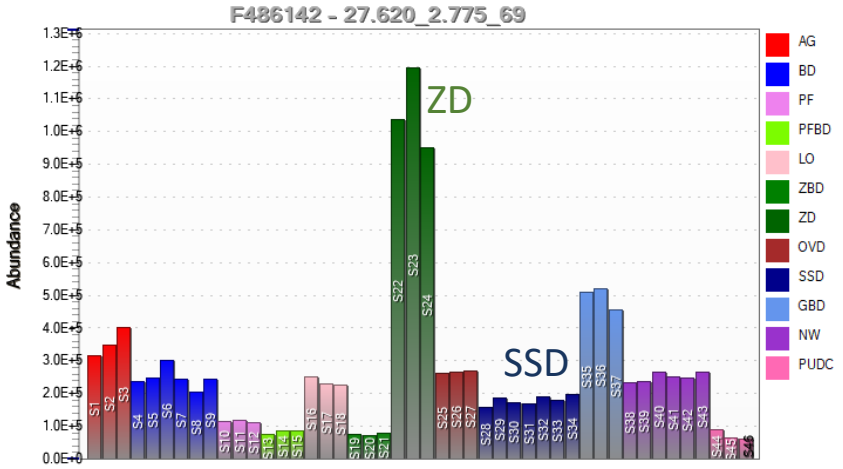
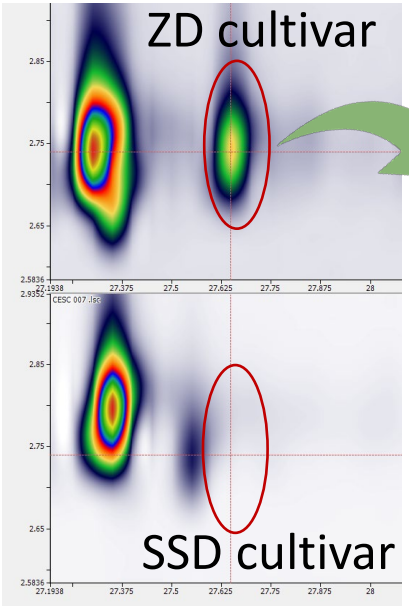
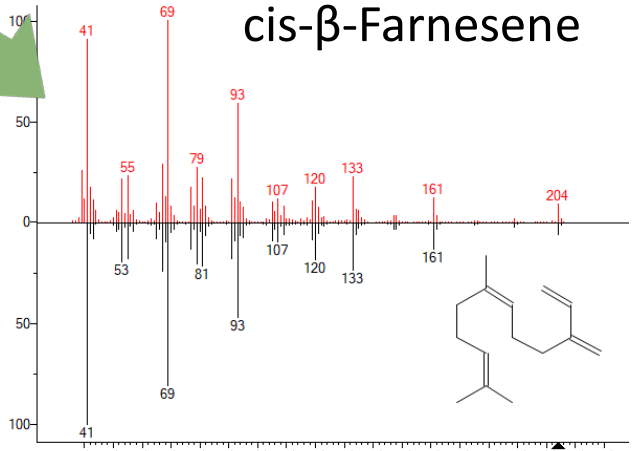
Distinguishing individual cultivars







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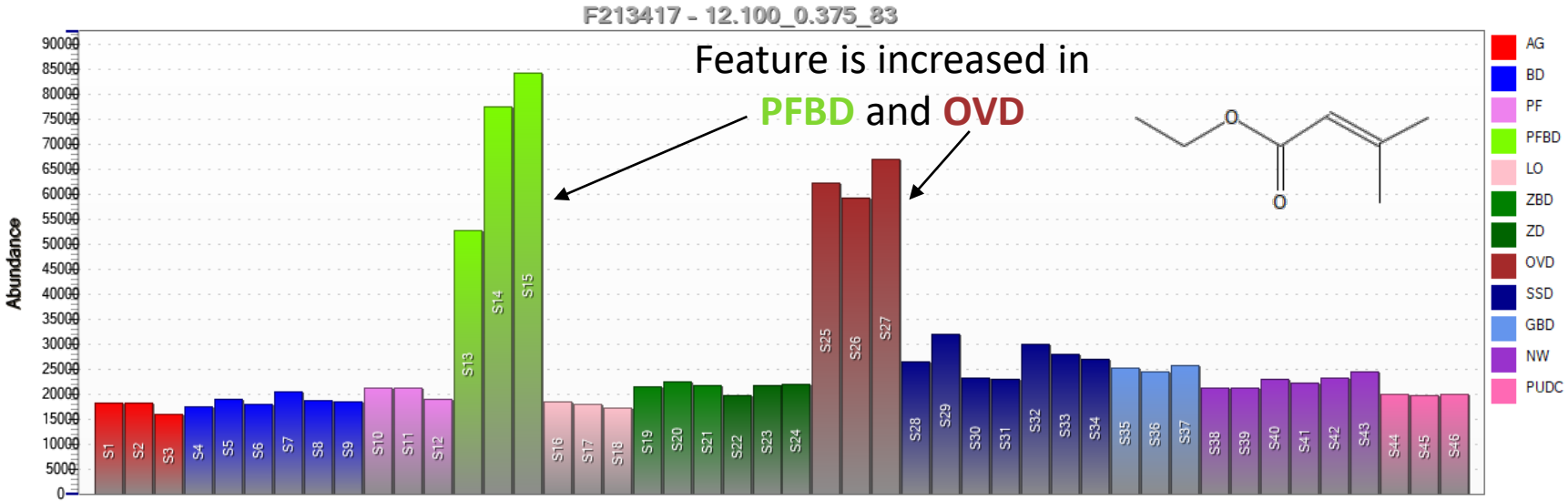
Looking beyond terpenes



- Feature identified as the ester, ethyl 3-methyl-2-butenoate

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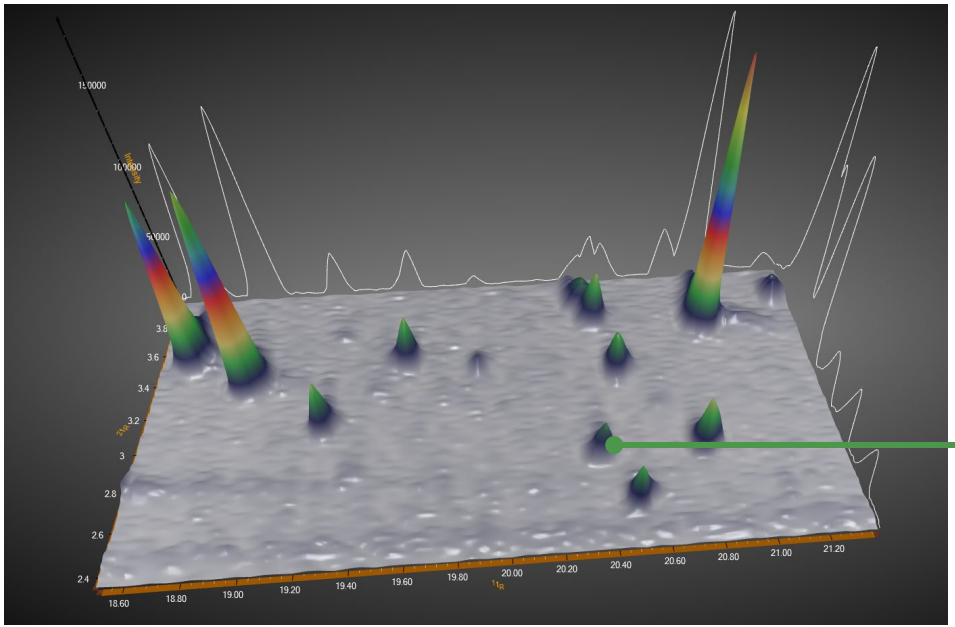
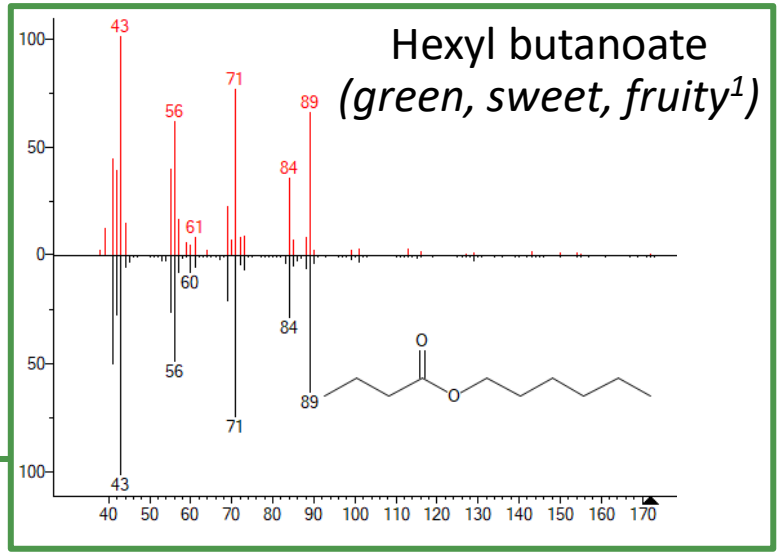
Looking beyond terpenes





[1]Good Scents database, http://www.thegoodscentscompany.com/

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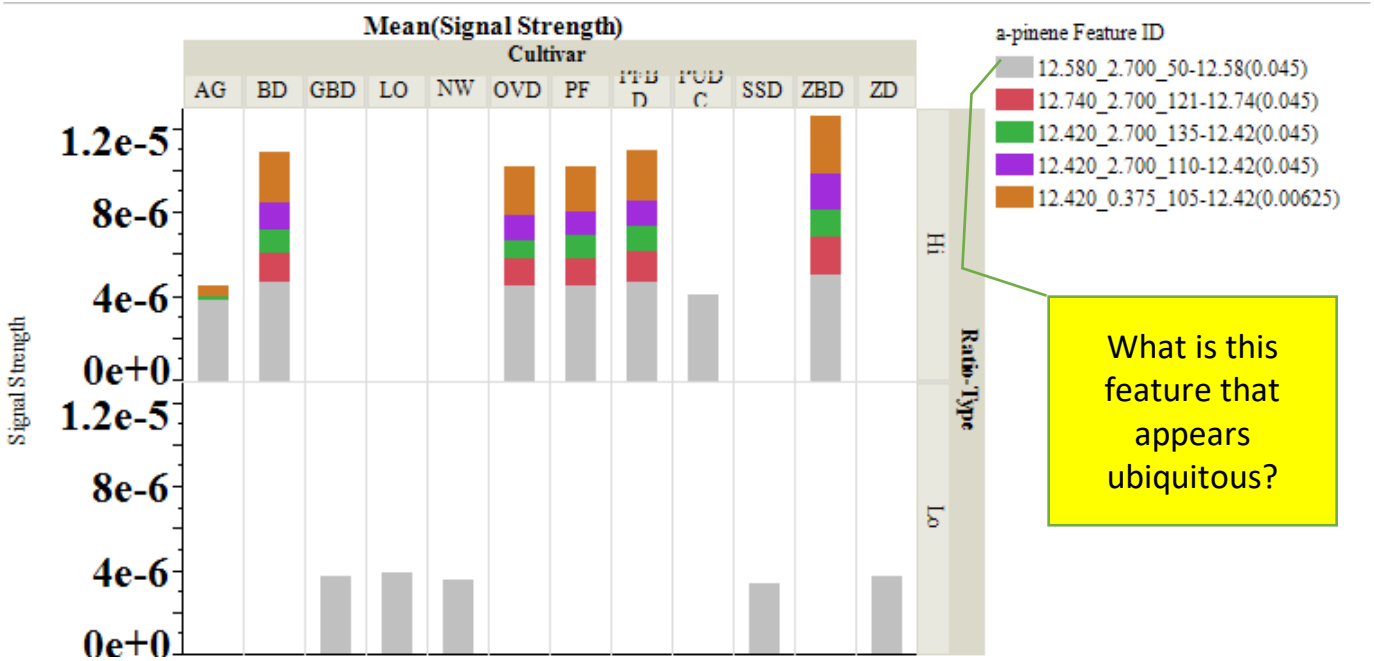
α-Pinene primarily appears as a cluster of features



What is this feature that appears ubiquitous?

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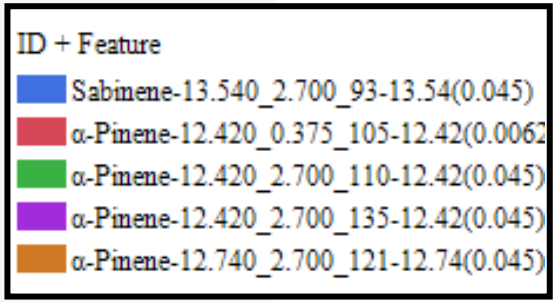
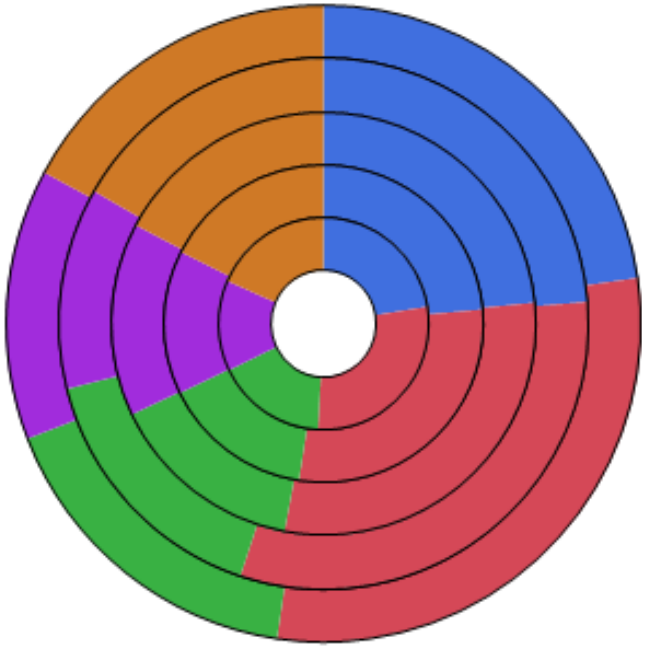
α-Pinene feature cluster includes sabinene . . .

AG (33)
BD (66)
GBD (33)
LO (33)
NW (66)
OVD (33)
PF (33)
PFBD (33)
PUDC (33)
SSD (77)
ZBD (33)
ZD (33)

ID + Feature
Sabinene-13.540_2.700_93-13.54(0.045) (18)
α-Pinene-12.420_0.375_105-12.42(0.00625) (18)
α-Pinene-12.420_2.700_110-12.42(0.045) (18)
α-Pinene-12.420_2.700_135-12.42(0.045) (18)
α-Pinene-12.580_2.700_50-12.58(0.045) (18)
α-Pinene-12.740_2.700_121-12.74(0.045) of Top 50 Untargeted Ratio-type Features
α-Pinene-12.740_2.700_121-12.74(0.045) of Top 50 Untargeted Strain Features mult
β-Myrcene-14.180_0.975_107-14.18(0.01625) (18)
β-Myrcene-14.180_1.575_94-14.18(0.02625) (18)
β-Ocimene-15.940_0.375_94-15.94(0.00625) (18)
β-Ocimene-15.940_1.575_93-15.94(0.02625) (18)





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Summary

- GC×GC–TOF MS provides the enhanced separation necessary for robust profiling of terpenes
- Additional chemical differences were uncovered between the “Floral” and “Fuel” classes
- Unique chemical markers can further distinguish individual cultivars
- Provides improved product definition, through superior cultivar selection and improved labelling and categorization strategies

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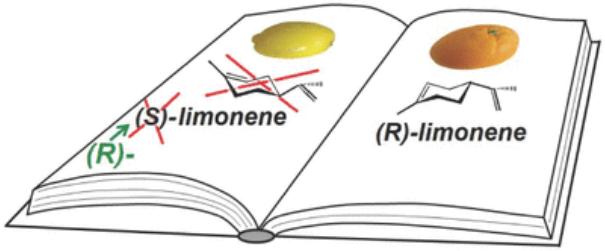
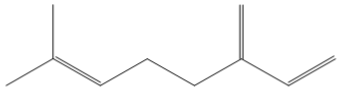
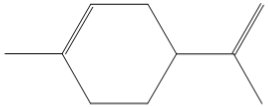
Cannabis aroma volatiles

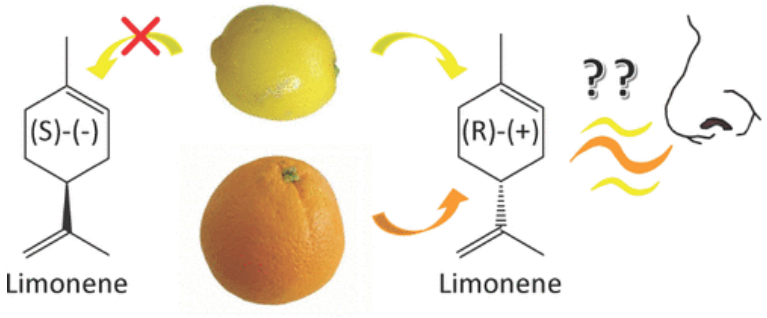


- Cannabis terpenes and terpenoids
  - ✓ Impart distinctive aromas and flavours





	Myrcene	Limonene
Aroma	*Spicy, herbal*	*Citrus, orange*



"*From the literature, it is therefore difficult to know what to believe when it comes to the odor of limonene.*" (J. Chem. Educ. 2021, 98, 11, 3600–3607)

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Thanks for listening! Any questions?

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