SHIMADZU
Excellence in Science

Cannabis Testing Solutions

Terpene Analysis by HS-GCMS

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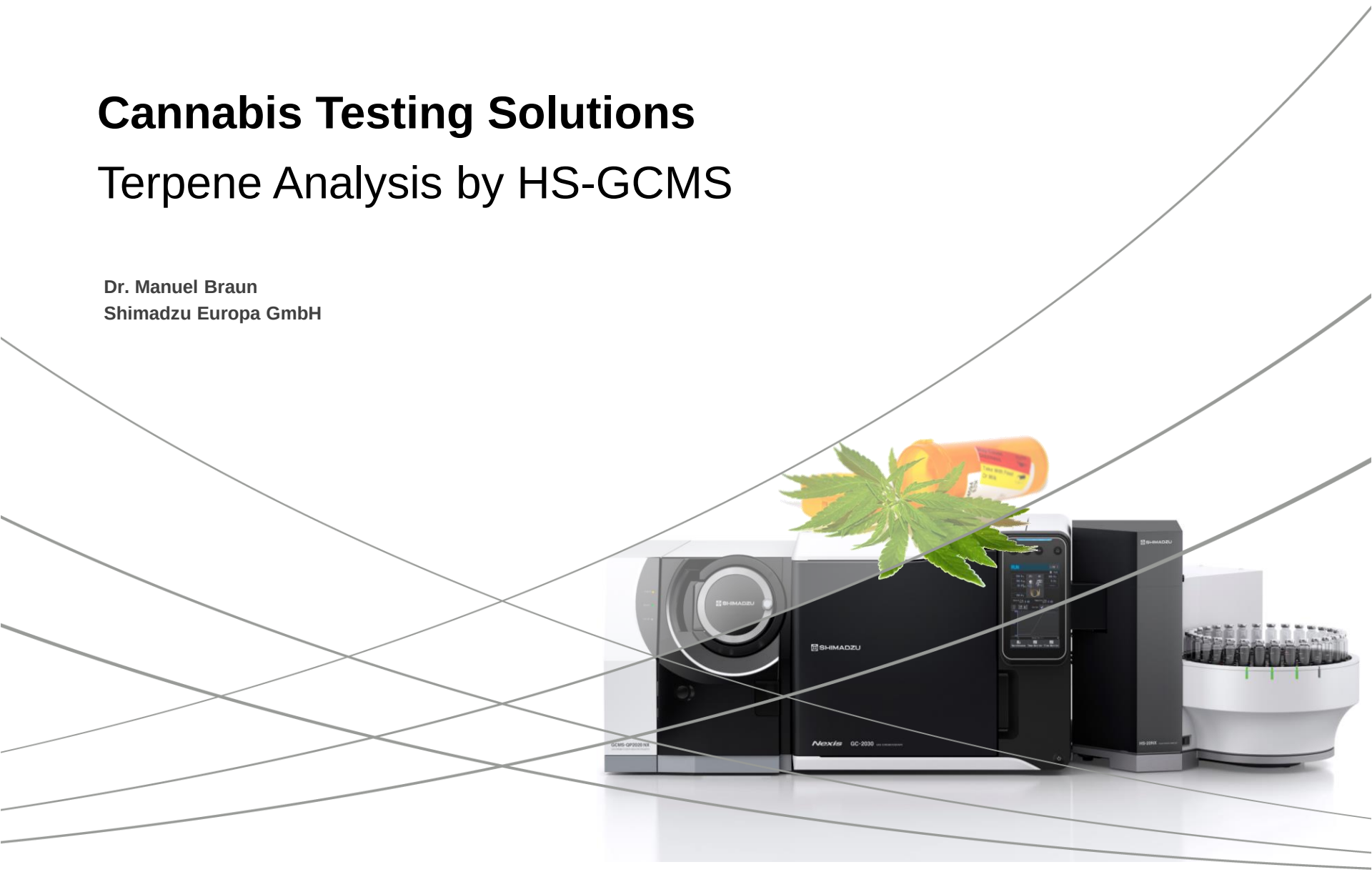
Shimadzu Europa GmbH



Terpenes

- Terpenes – organic, fragrant compounds produced by plants
  - Distinctive flavor and aroma
  - Primary constitute of essential oils of medical plants
  - Medicinal hydrocarbon building blocks
- Terpenes in cannabis
  - Terpenes are produced in trichomes (like THC)

  - Synergistic effect with cannabinoids

GI Grow Biomedical Farm Video, available at Shimadzu's www.GrowYourLab.com

The Synergistic Effect

- Cannabis has over 140 terpene components, many of which are of medicinal interest

“Pine needles”

- Anti-inflammatory
- Enhanced concentration
- Bronchodilator for better cannabinoid absorption

“Lavender”

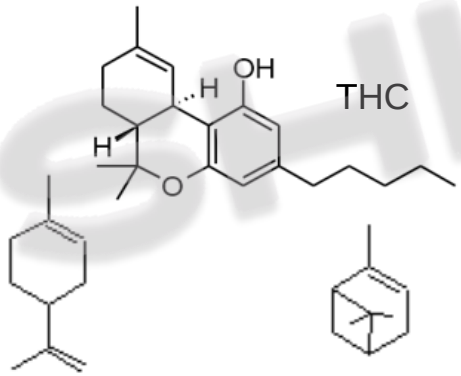
- Anti-anxiety
- Analgesic
- Anticarcinogenic

“Citrus”

- Anti-bacterial
- Anticarcinogenic
- Anti-depression

The Synergistic Effect

- **“Entourage Effect”**
- Terpenes have a synergistic relationship with cannabinoids, which further enhance the therapeutic effect
- The various compounds in cannabis work together to produce a **synergy** of effects



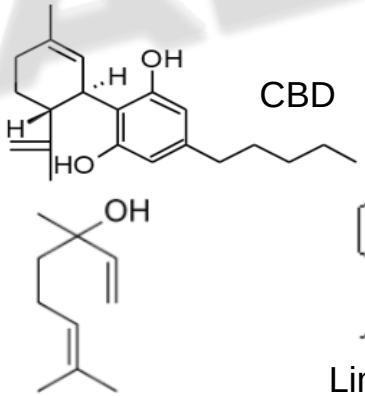
THC

Limonene

Antioxidant

Pinene

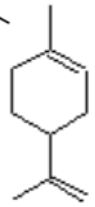
Bronchodilatory



CBD

Linalool

Anticonvulsant



Limonene

Cytotoxic vs breast cancer

Terpene Profiling by GCMS

- Different strains of cannabis plants can be distinguished by different terpene contents

→ Due to the uniqueness of terpene profiles, they can be used by cultivators as a "fingerprint" to partially ID the specific strain in question

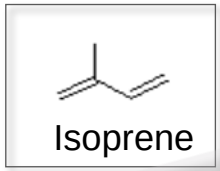
- Sativa vs. Indica

→ Typical Sativa Terpenes: α-Phellandrene and α-Terpinolene

→ Typical Indica Terpenes: myrcene, camphene and limonene

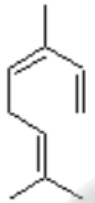
Terpene Profiling by GCMS

- Isoprene rule - General formula of terpenes is $(C_5H_8)_n$

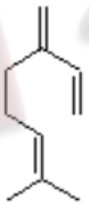


Monoterpenes (n = 2)

acyclic

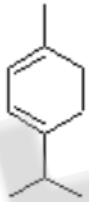


Ocimene

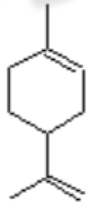


Myrcene

monocyclic



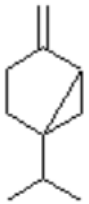
α-Terpinene



Limonene

bicyclic

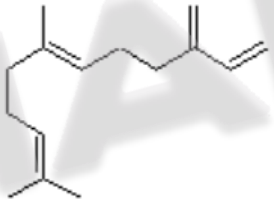
α-Pinene



Sabinene

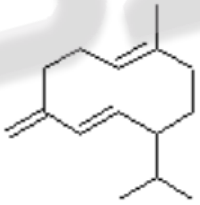
Sesquiterpenes (n = 3)

acyclic



β-Farnesene

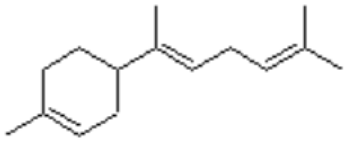
monocyclic



Germacrene D

bicyclic

Carene

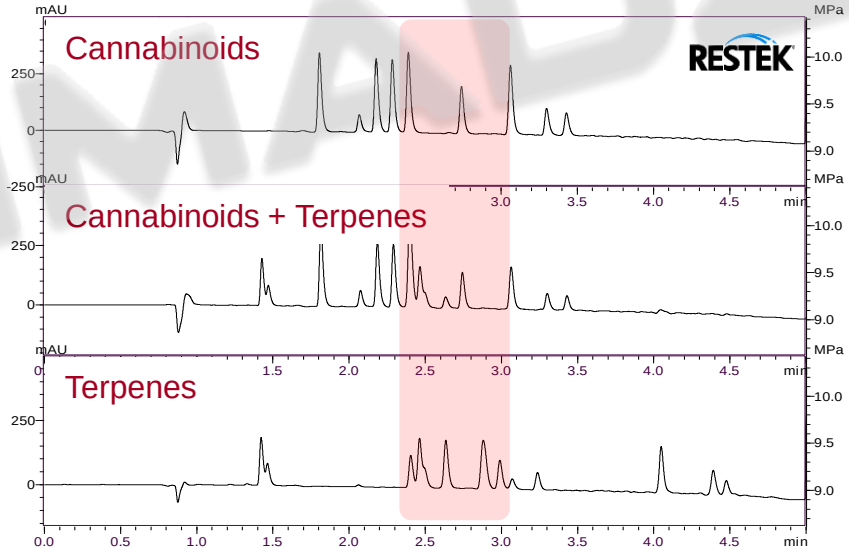


α-Bisabolene

Terpene Profiling by GCMS

- Gas chromatography is the preferred way to test for terpenes
  - Terpenes are volatile which makes them ideal candidates for GC
  - Boiling point between 119 – 200 ºC and therefore easily converted to the gas phase

- Liquid chromatography
  - Limited chromophore
  - Low UV sensitivity
  - Coelution of terpenes and cannabinoids



Headspace-Analysis with GCMS

- Mass Spectrometry (MS) or Flame Ionization Detector (FID)

MS

→ Mass spectrum library for structural identification

→ Can identify unknown peaks or contaminants

FID

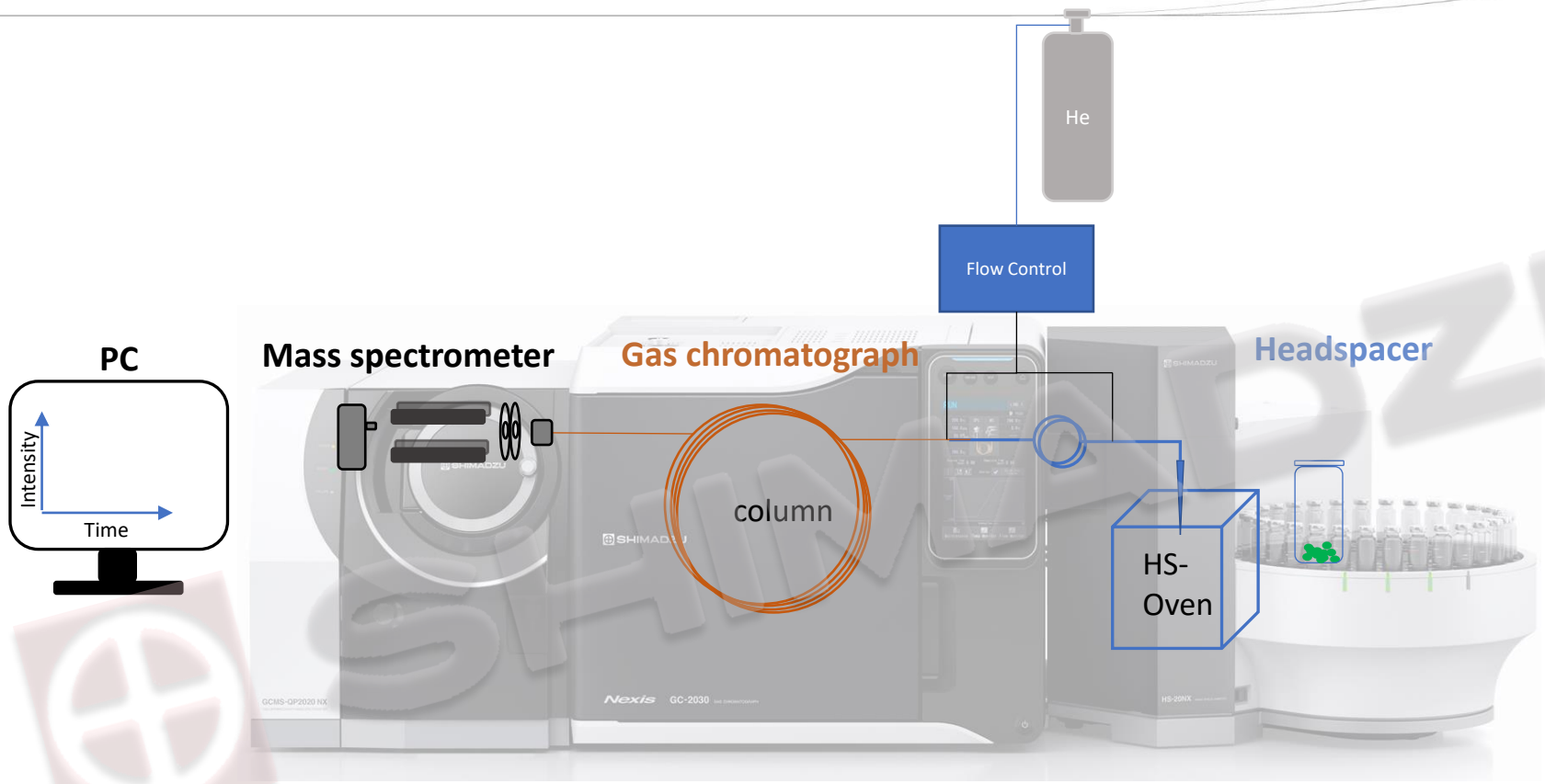
→ No library information

→ Increased in linear range

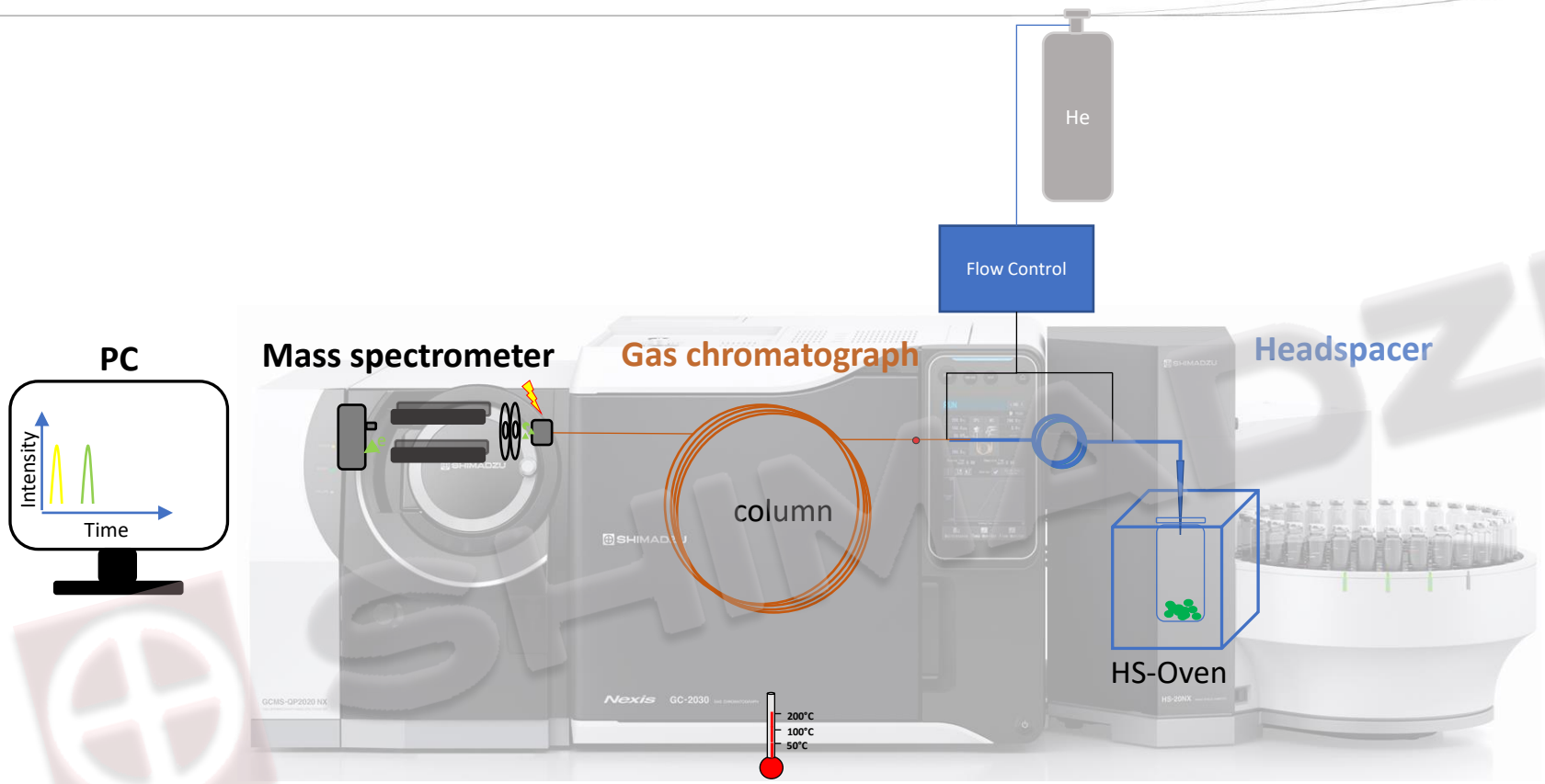
→ Good if you are only quantifying the same terpenes

Shimadzu GCMS-QP2020NX
with HS20NX (headspace) autosampler
for the analysis of terpenes

Headspace-Analysis with GCMS



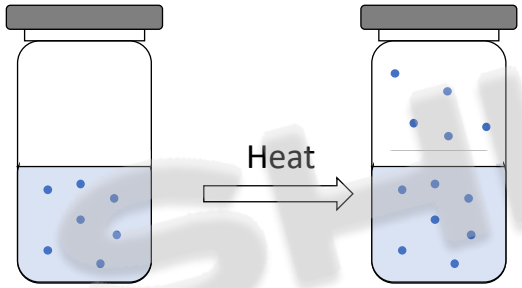
Headspace-Analysis with GCMS



Terpene Profiling by GCMS

- HS is the sampling technique used to extract volatile compounds from a liquid or solid sample

Traditional headspace

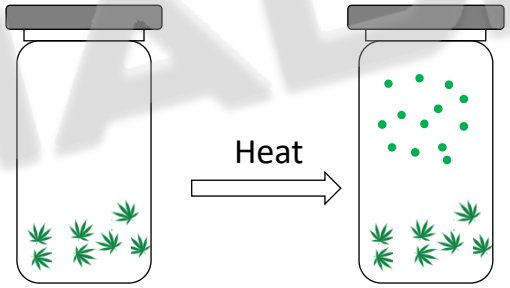


Solution approach

Dissolved in liquid

Equilibrium between gas and liquid

Full Evaporation Technique (FET)



FET

Sample wont dissolve in liquid

Single phase equilibrium

Small sample amount (10-70 mg)

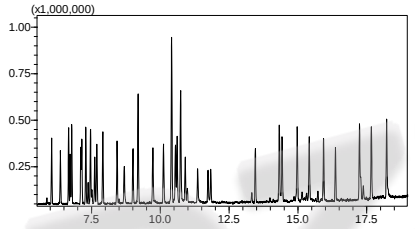
Small standard volume (10 µL)

Terpene Profiling by GCMS

- Standard samples

Dilute standard solution

10 µL volume



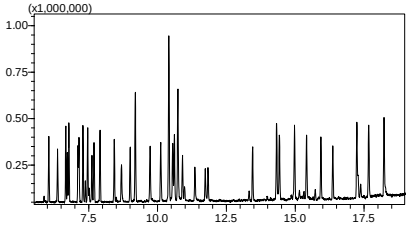
Analyze by GCMS

- Cannabis samples

Weigh out cannabis sample

Crushing

Weigh 10 - 70 mg in HS vial



Analyze by GCMS

Hemp tea measured by HS-GCMS

Crushing

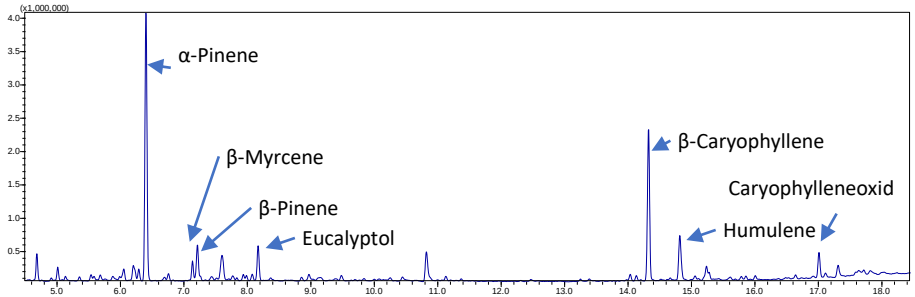
At RT (ZM300)

At -100°C (MM400+MM500)

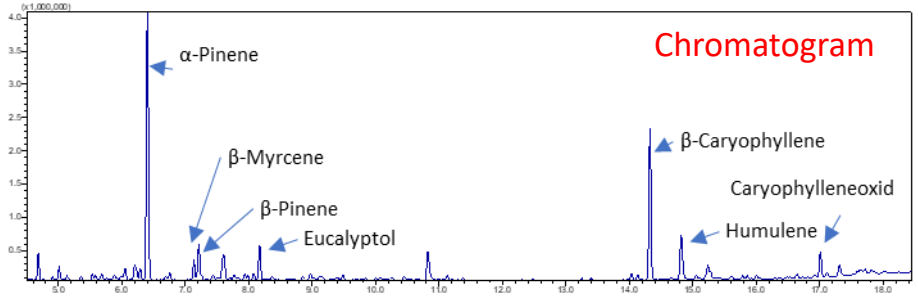
At -196°C (CryoMill)

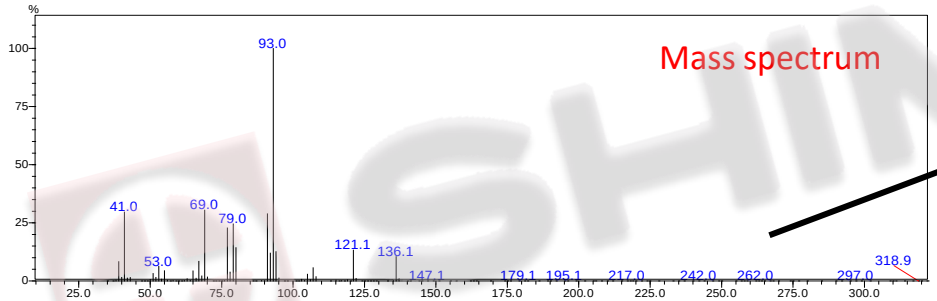
Weighing

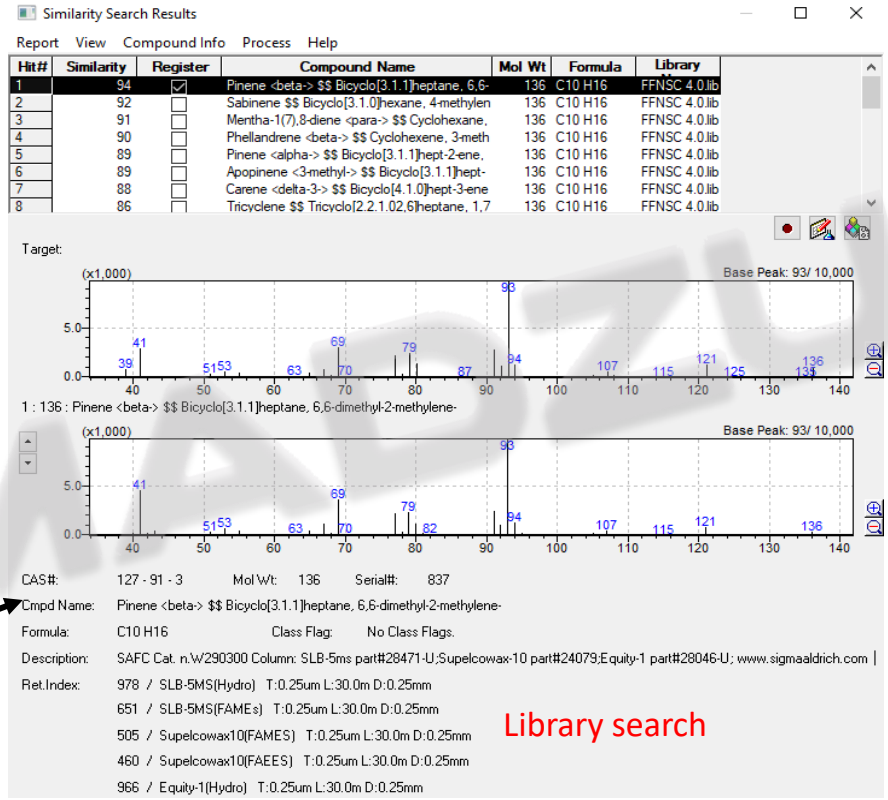
Analyze by GCMS



Hemp tea measured by HS-GCMS





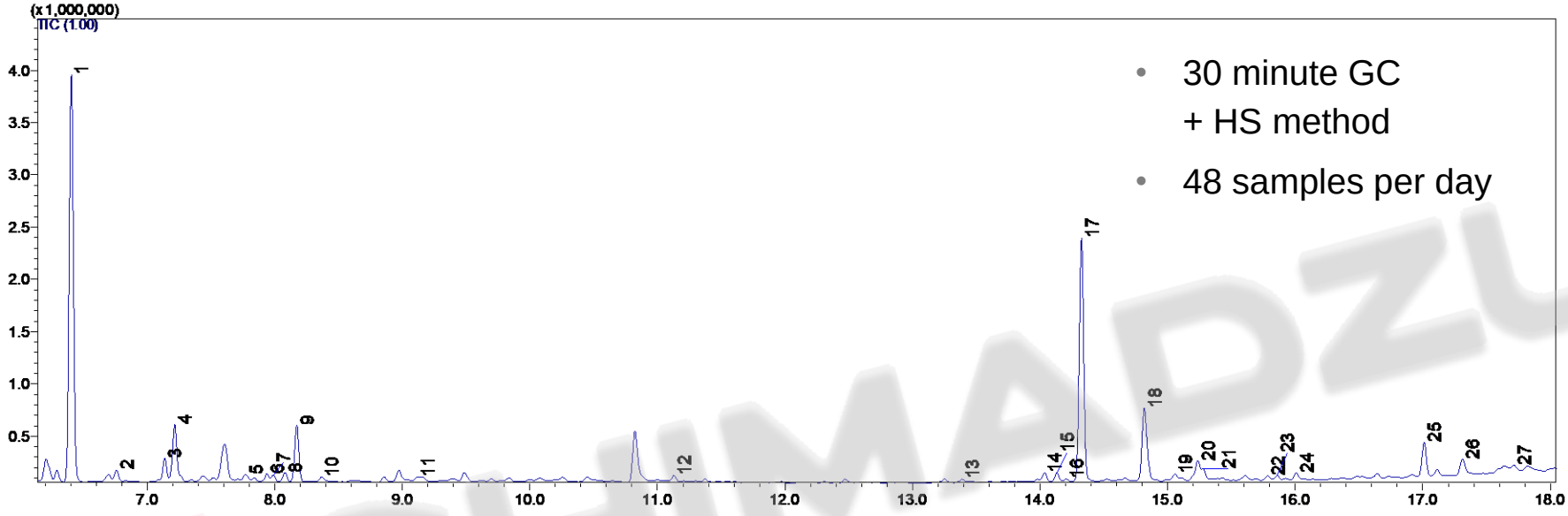


FFNSC4 Library

Flavour & Fragrance Natural & Synthetic Compounds

- **Registered compounds**
  4,030 flavor and fragrances compounds
- **Linear Retention Indices (LRI)**
  Non-polar column (SLB®-5ms)
  Highly-polar column (SUPELCOWAX® 10)
  Non-polar column (Equity®-1)
- **Registered information**
  Mass spectrum, retention index for each column, CAS number, compound name, molecular weight, compositional formula

Crushing affects Terpene analysis

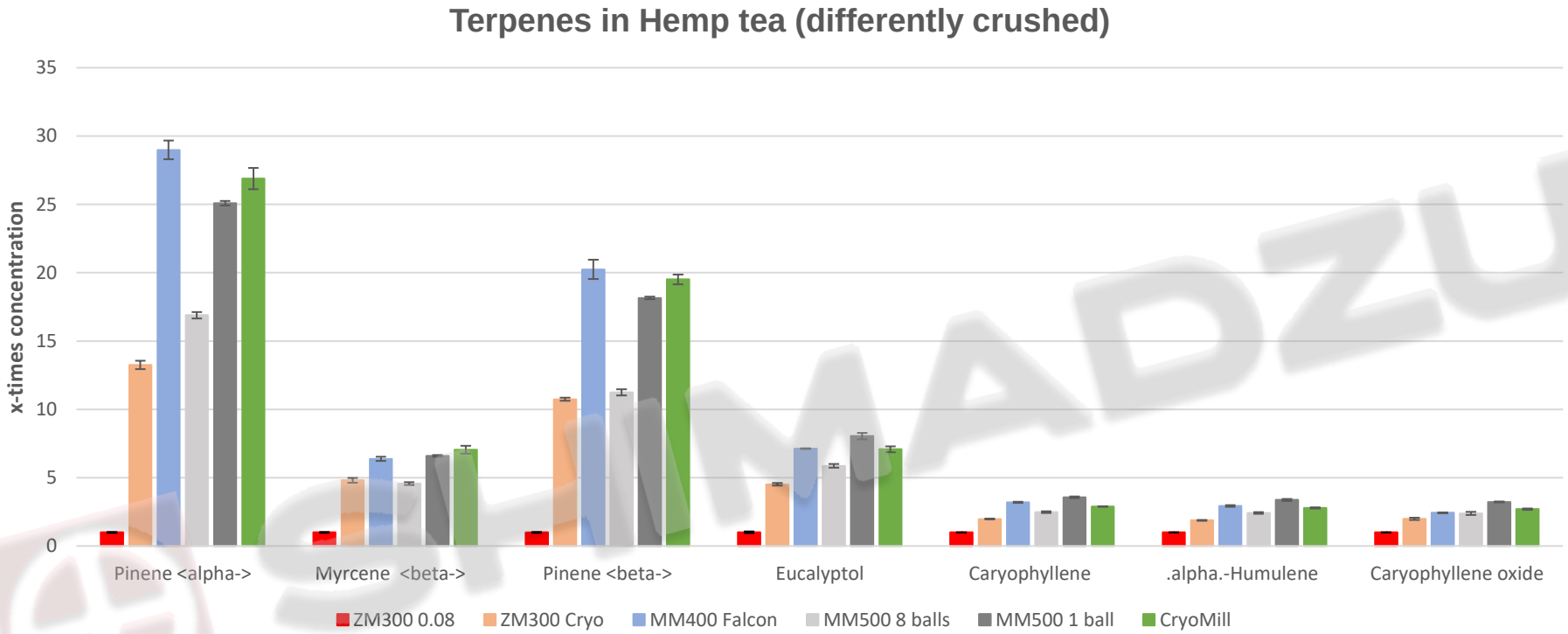


- 30 minute GC + HS method
- 48 samples per day

Peak #	Compound
1	Pinene <alpha->
2	Camphene
3	.beta.-Myrcene
4	Pinene <beta->
5	Carene <delta-2->
6	Limonene
7	Cymene <para->
8	Carene <delta-3->
9	Eucalyptol
10	Terpinene <gamma->
11	Sabinene hydrate <trans->
12	Terpineol <alpha->
13	Ylangene <alpha->

Peak #	Compound
14	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-(1R*,4Z,9S*)]-
15	Bergamotene <alpha-, cis->
16	Farnesene <(E)-, beta->
17	Caryophyllene <(E)->
18	.alpha.-Humulene
19	Selina-4,11-diene
20	Selinene <beta->
21	.alpha.-Selinene
22	Selina-4(15),7(11)-diene
23	Selina-3,7(11)-diene
24	Nerolidol <(E)->
25	Caryophyllene oxide
26	Humulene epoxide II
27	.alpha.-Bisabolol

Milling affects Terpene analysis



-190°C at the start

-20°C

-100°C

-100°C

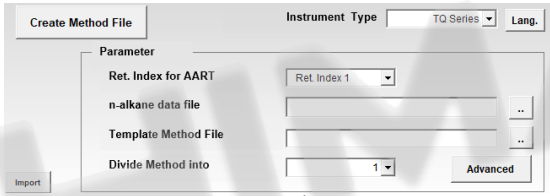
-196°C

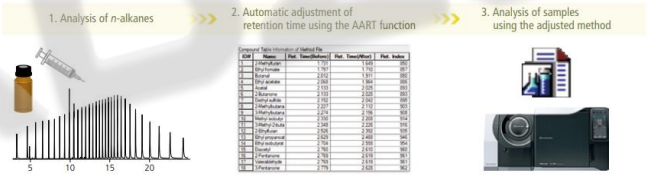
Smart Aroma Database

Database for GC-MS(/MS) Aroma Analysis

Smart Aroma Database Database with 500 Aroma compounds

- A method (SIM or MRM) could be created without measuring standards first
- Only an n-Alkanstandard is necessary to calculate retention times





Acq. Mode	Compound Name (E)	Ret. Index 1 SH-I-5-Sil MS	Cas#	Comment (E) Odor Quality
Scan	alpha-Pinene	935	80-56-8	pine, turpentine
SIM	Myrcene	990	123-35-3	balsamic, must, spice
MRM	delta-3-Carene	1009	13466-78-9	lemon, resin
Scan	alpha-Terpinene	1017	99-86-5	lemon
Scan	p-Cymene	1025	99-87-6	solvent, gasoline, citrus
Scan	Limonene	1030	138-86-3	lemon, orange

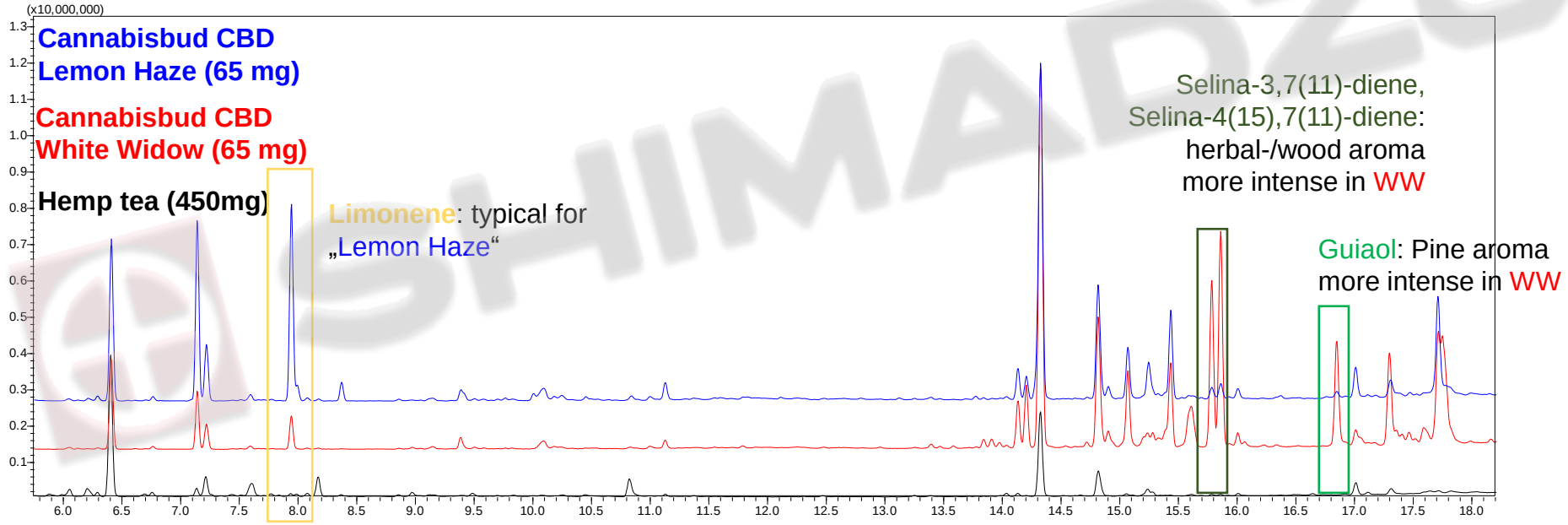
MRM Transition

m/z for SIM or Scan

Ion1				Ion2				Ion1			Ion2		
Type	m/z	CE	Ratio	Type	m/z	CE	Ratio	Type	m/z	Ratio	Type	m/z	Ratio
T	93.00>77.00	12	100.00	Ref.1	93.00>51.00	27	39.91	T	93.0	100.00	Ref.1	121.0	10.43
T	93.00>77.10	12	100.00	Ref.1	69.00>41.10	6	87.54	T	93.1	100.00	Ref.1	91.1	30.08
T	136.10>93.10	9	100.00	Ref.1	136.10>121.10	9	76.90	T	136.1	100.00	Ref.1	121.1	106.83
T	121.10>93.10	6	100.00	Ref.1	136.10>121.10	12	71.07	T	136.1	100.00	Ref.1	121.1	156.15
T	134.10>119.10	9	100.00	Ref.1	119.10>91.10	15	90.53	T	134.1	100.00	Ref.1	119.1	321.93
T	136.00>93.00	15	100.00	Ref.1	107.00>91.00	12	104.64	T	93.0	100.00	Ref.1	121.0	29.51

Hemp tea vs. Cannabis bud

- Measured Hemp tea had a lower concentration of terpenes, and the terpene profile was less complex as in cannabis buds.
- Possible reasons: Different Hemp strain (Finola), Mixture from leaves and buds, different drying procedure, Age of the samples…



Summary

- Terpene analysis is easier over Headspace-GC than via LC
- MS or FID are both suitable detectors
- Cannabis samples
  - The temperature during the milling process of the cannabis samples has a strong effect on the terpene profile and their concentrations
  - Terpene content depends on harvesting, storage and drying conditions.
- Terpenes can provide a reference to the variety (sativa vs. indica) and can provide a "variety-specific" fingerprint

Disclaimer

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