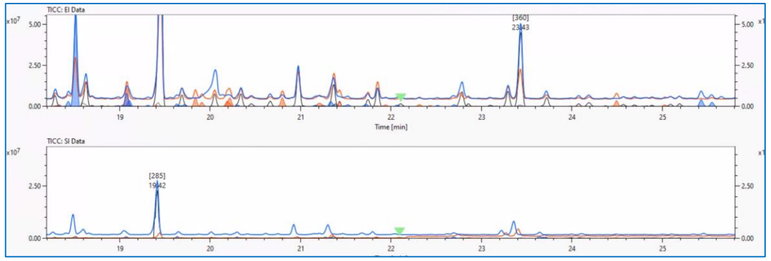
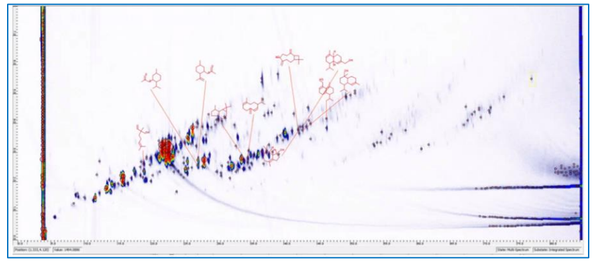




ESSENTIAL SEPARATIONS:

ANALYSIS OF AROMA OILS BY ONE-DIMENSIONAL AND TWO-DIMENSIONAL GAS CHROMATOGRAPHY AND MASS SPECTROMETRY

Multidimensional Chromatography Workshop
Los Angeles, CA January 2024

Robert (Chip) Cody

JEOL mass spectrometers used for this study

Q1600GC UltraQuad™ SQ-Zeta
GC-MS (Single Quadrupole)
Ionization used: EI, CI

TQ2000GC AccuTOF GC-Alpha
GC-HRTOFMS
GCxGC-HRTOFMS
Ionization used: EI, CI, FI

1. JMS-Q1600GC UltraQuad™ SQ-Zeta

Front

High-precision Hyperbolic-shaped Quadrupole

- Superb ion transmission enhances high sensitivity
- Reduces ion-quenching when introducing a large volume of ions, allowing a wide dynamic range

Pre-filter

- Suppresses the degradation of resolution and sensitivity due to contamination of the quadrupole

Dual Filament

- Reduces instrument down-time during filament exchange

Draw-in Lens

- Reduces diffusion of ions from the quadrupole outlet and reduces chemical noises
- Improved sensitivity by re-acceleration of ions

Secondary Electron Multiplier Detector

- Detector is resistant to degradation in an atmospheric environment to maintain high performance over a long period of time

Split Flow Turbo Molecular Pump

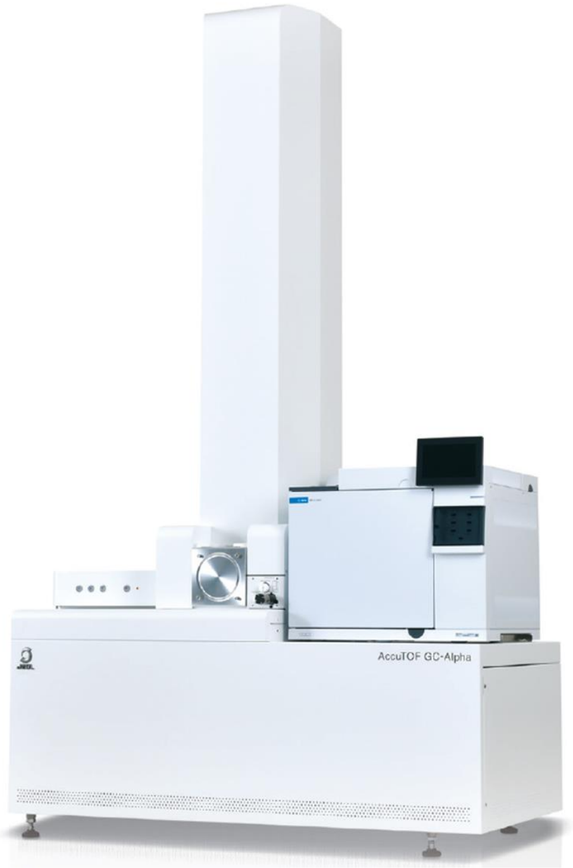
- Quick evacuation after column replacement and maintenance work
- Use of mega bore, packed column is possible
- Compatible with the latest Low-Pressure GC (LPGC) column

No tools required
Easy maintenance!

High pumping capacity!

2: GC-MS High-Resolution Time-of Flight Mass Spectrometer: AccuTOF™ GC-Alpha

- Simple ion optics
- High mass resolving power (>30,000)
- High mass accuracy (sub-mmu)
- High speed (50 Hz) -- compatible with GCxGC
- Wide mass range (*m/z* 4 up to *m/z* 6400)
- All ion sources and direct sample introduction methods
- Field Ionization (FI) and Field Desorption (FD) options
- Combination ion sources (EI/FI and EI/PI)
- Easy ion source exchanges with very fast pump-down
- The most powerful data analysis software available

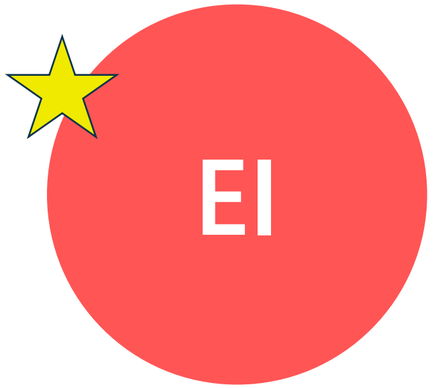


Dual-stage reflectron

Ionization Methods for GC-MS

Used in this talk

AccuTOF GC-Alpha only



Electron Ionization

- Hard ionization
- Good for structural analysis using the generated fragment ions
- Able to change the ionization energy, typically use 70eV

Chemical Ionization

- Soft ionization
- Effective for compounds with higher proton affinities
- Negative-ion mode is available
- Reagent gas required

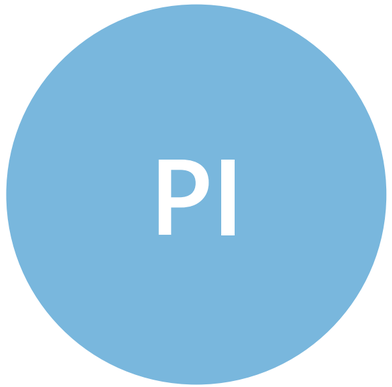


Photo Ionization

- Soft ionization
- Ionization energy is about 10.3eV (D2 lamp)
- Good for aromatic compounds
- No reagent gas required

Field Ionization

- Softest ionization
- Energies imparted to the molecules are less than 1eV
- Effective even for lower polarity molecules
- No reagent gas required

Software used

- Data acquisition:
  - JEOL msPrimo (single quadrupole)
  - JEOL msAxel (AccuTOF GC-Alpha)
- GC-MS data analysis with deconvolution
  - msFineAnalysis iQ (single quadrupole)
  - msFineAnalysis AI (AccuTOF GC-Alpha)

GC x GC data analysis

- GC Image
- AnalyzerPro XD (SpectralWorks Ltd.)
  - *GC-MS, GCxGC-MS & Direct analysis MS*

- Databases
  - NIST23 main and replicate libraries
  - Wiley main and replicate libraries
  - Wiley Food, Flavors, Fragrances, and Related Compounds
  - JEOL AI for compounds not in experimental databases
- NIST Hybrid Search

JEOL 1D GC data analysis with chromatographic deconvolution

msFineAnalysis iQ (Single quadrupole)

- Deconvolution
- Correlate EI with CI or PI
- Database search
- RI match
- Isotope match
- Variance analysis

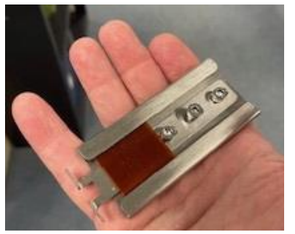
msFineAnalysis AI (HRTOF)

- Deconvolution
- Correlate EI and soft ionization (EI, CI, PI, FI)
- Database search
- Elemental composition
  - Molecular ion and fragment coverage
- RI match
- Isotope match
- Variance analysis
- Structure analysis with AI database

GC conditions

- GC-MS
  - Agilent 8890 GC
  - 30m DB5-MS column (single quadrupole
  - GC x GC column set used in 1D GC mode for the AccuTOF GC-Alpha data
  - Split injection: 200:1
  - Oven:
    1. 50 °C (0 min)
    2. 5°C min^{-1} to 220°C (0 min)
    3. 20°C to 300 °C
    4. Hold 8 minutes

- GCxGC-HRTOFMS
  - ZB-5HT (30m), ZB35-HT (2m)
    - A portion of the ZB35-HT column was used as the modulator loop
  - Zoex ZX-2 thermal modulator, 8 s modulation period
  - InfoMass LLC Column holder
  - Split injection (100:1)
  - Oven:
    1. 60°C (0 min)
    2. 3°C min^{-1} to 315°C (0 min)



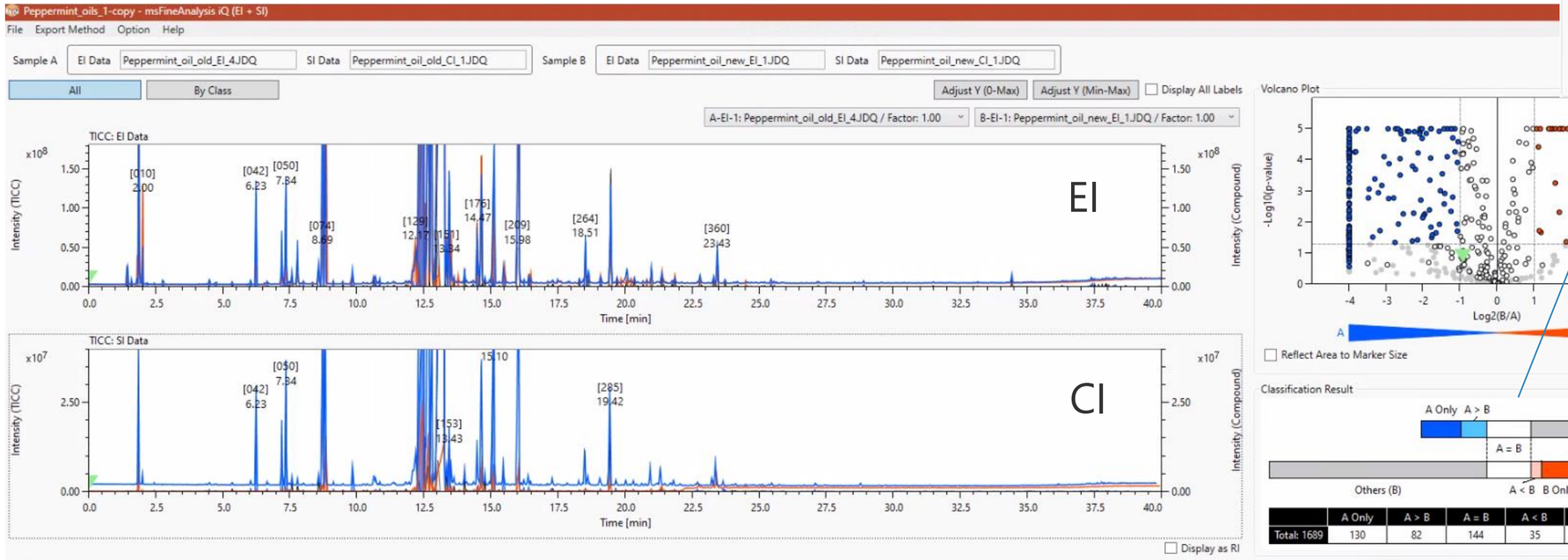
Essential oil samples analyzed

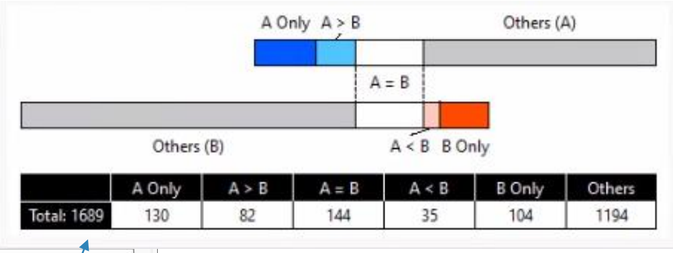
1. Peppermint Oil, Sakae
   - *Mentha piperita*
   - >35 years old
   - Stored at room temperature
2. Peppermint Oil, Fluka
   - *Mentha piperita*
   - < 5 years old
   - Stored in refrigerator
3. Spearmint Oil, Fluka
   - *Mentha spicata*
   - < 5 years old
   - Stored in refrigerator
4. Lavender Oil, Sakae
   - >35 years old
   - Stored at room temperature
5. Lemon Oil
   - *Citrus limon*
   - "JEOL" -- unidentified source
6. Orange Oil
   - *Citrus sinensis*
   - *a.k.a. Citrus × aurantium f. aurantium*
7. "Sweet Orange" Oil
   - *Citrus sinensis*
   - *a.k.a. Citrus × aurantium f. aurantium*

Difference analysis (1D GC and GC x GC)

- **Five EI replicates (Single Quad and HRTOF)**
- **One CI (Single Quad) or FI (HRTOF) measurement**

Single Quadrupole: GC-MS comparison of Peppermint Oils with EI and CI data, msFineAnalysis iQ software





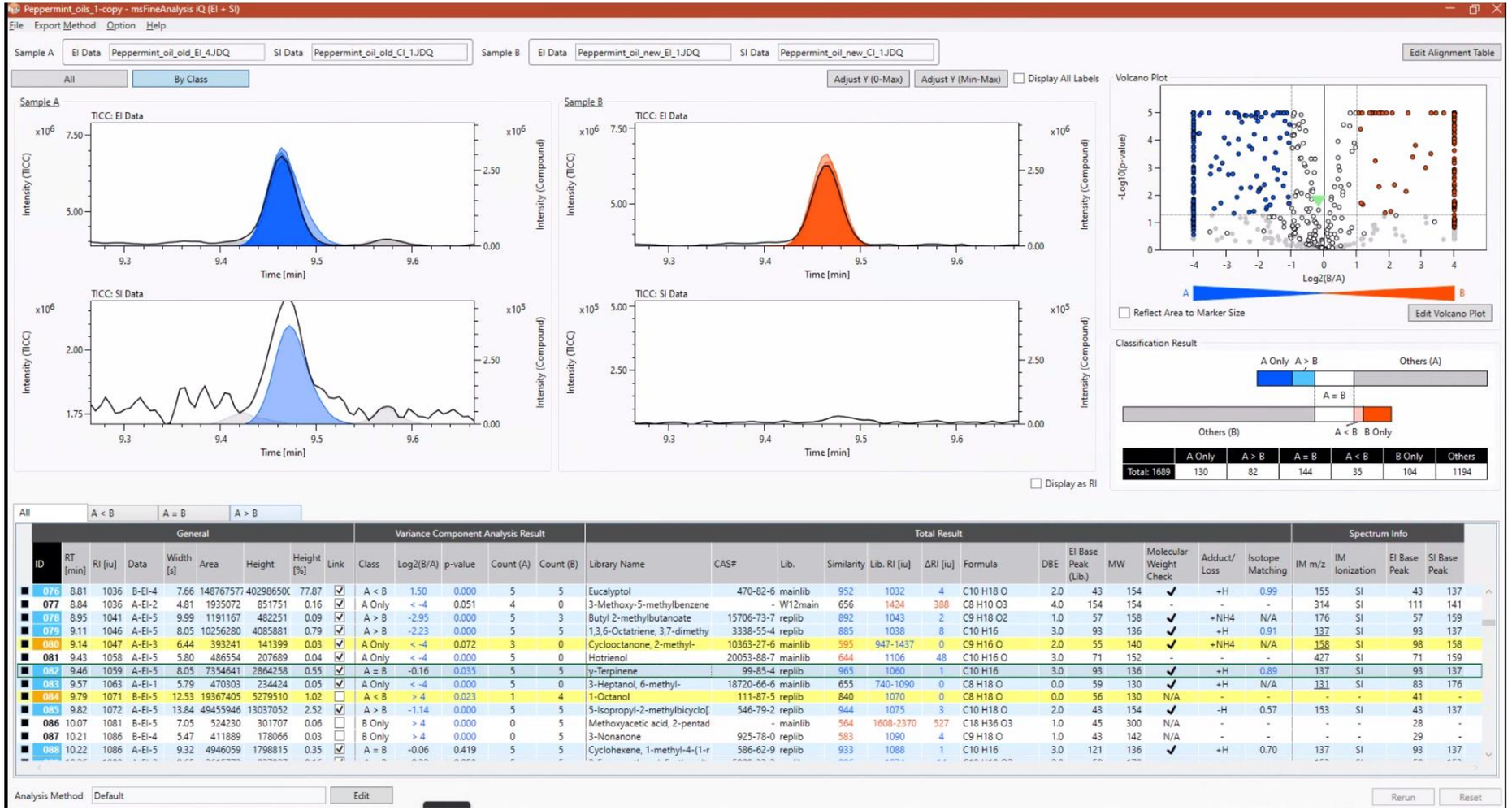
	A Only	A > B	A = B	A < B	B Only	Others
Total: 1689	130	82	144	35	104	1194

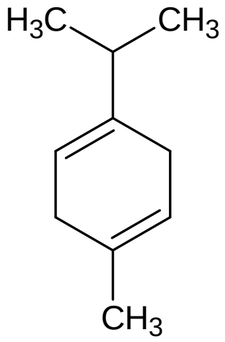
495 reproducible peaks in the 2 samples

- Deconvolution
- Correlate EI and CI
- Database search
- RI match
- Isotope match
- Variance analysis

General									Variance Component Analysis Result					Total Result												Spectrum Info			
ID	RT [min]	RI [iu]	Data	Width [s]	Area	Height	Height [%]	Link	Class	Log2(B/A)	p-value	Count (A)	Count (B)	Library Name	CAS#	Lib.	Similarity	Lib. RI [iu]	ΔRI [iu]	Formula	DBE	EI Base Peak (Lib.)	MW	Molecular Weight Check	Adduct/ Loss	Isotope Matching	IM m/z	IM Ionization	EI Base Peak	SI Base Peak
074	8.69	1031	A-EI-2	7.69	229411871	48934348	9.46	☑	A Only	< -4	0.000	5	0	1,4-Dimethyl-4-vinyl-1-cyclohe	1743-61-9	W12main	703	1015	16	C10 H16	3.0	68	136	-	-	-	425	SI	68	103
075	8.72	1032	A-EI-5	7.37	739333156	232214102	44.87	☑	A = B	-0.94	0.000	5	5	Limonene	138-86-3	W12main	821	1018	14	C10 H16	3.0	68	136	✓	+NH4	0.65	154	SI	39	81
076	8.81	1036	B-EI-4	7.66	148767577	402986500	77.87	☑	A < B	1.50	0.000	5	5	Eucalyptol	470-82-6	mainlib	952	1032	4	C10 H18 O	2.0	43	154	✓	+H	0.99	155	SI	43	137
077	8.84	1036	A-EI-2	4.81	1935072	851751	0.16	☑	A Only	< -4	0.051	4	0	3-Methoxy-5-methylbenzene	-	W12main	656	1424	388	C8 H10 O3	4.0	154	154	-	-	-	314	SI	111	141
078	8.95	1041	A-EI-5	9.99	1191167	482251	0.09	☑	A > B	-2.95	0.000	5	3	Butyl 2-methylbutanoate	15706-73-7	replib	892	1043	2	C9 H18 O2	1.0	57	158	✓	+NH4	N/A	176	SI	57	159
079	9.11	1046	A-EI-5	8.05	10256280	4085881	0.79	☑	A > B	-2.23	0.000	5	5	1,3,6-Octatriene, 3,7-dimethy	3338-55-4	replib	885	1038	8	C10 H16	3.0	93	136	✓	+H	0.91	137	SI	93	137
080	9.14	1047	A-EI-3	6.44	393241	141399	0.03	☑	A Only	< -4	0.072	3	0	Cyclooctanone, 2-methyl-	10363-27-6	mainlib	595	947-1437	0	C9 H16 O	2.0	55	140	✓	+NH4	N/A	158	SI	98	158
081	9.43	1058	A-EI-5	5.80	486554	207689	0.04	☑	A Only	< -4	0.000	5	0	Hotrienol	20053-88-7	mainlib	644	1106	48	C10 H16 O	3.0	71	152	-	-	-	427	SI	71	159
082	9.46	1059	A-EI-5	8.05	7354641	2864258	0.55	☑	A = B	-0.16	0.035	5	5	γ-Terpinene	99-85-4	replib	965	1060	1	C10 H16	3.0	93	136	✓	+H	0.89	137	SI	93	137
083	9.57	1063	A-EI-1	5.79	470303	234424	0.05	☑	A Only	< -4	0.000	5	0	3-Heptanol, 6-methyl-	18720-66-6	mainlib	655	740-1090	0	C8 H18 O	0.0	59	130	✓	+H	N/A	131	SI	83	176
084	9.79	1071	B-EI-5	12.53	19367405	5279510	1.02	☐	A < B	> 4	0.023	1	4	1-Octanol	111-87-5	replib	840	1070	0	C8 H18 O	0.0	56	130	N/A	-	-	-	-	41	-
085	9.82	1072	A-EI-5	13.84	49455946	13037052	2.52	☑	A > B	-1.14	0.000	5	5	5-Isopropyl-2-methylbicyclo[	546-79-2	replib	944	1075	3	C10 H18 O	2.0	43	154	✓	-H	0.57	153	SI	43	137
086	10.07	1081	B-EI-5	7.05	524230	301707	0.06	☐	B Only	> 4	0.000	0	5	Methoxyacetic acid, 2-pentad	-	mainlib	564	1608-2370	527	C18 H36 O3	1.0	45	300	N/A	-	-	-	-	28	-
087	10.21	1086	B-EI-4	5.47	411889	178066	0.03	☐	B Only	> 4	0.000	0	5	3-Nonanone	925-78-0	replib	583	1090	4	C9 H18 O	1.0	43	142	N/A	-	-	-	-	29	-
088	10.22	1086	A-EI-5	9.32	4946059	1798815	0.35	☑	A = B	-0.06	0.419	5	5	Cyclohexene, 1-methyl-4-(1-r	586-62-9	replib	933	1088	1	C10 H16	3.0	121	136	✓	+H	0.70	137	SI	93	137

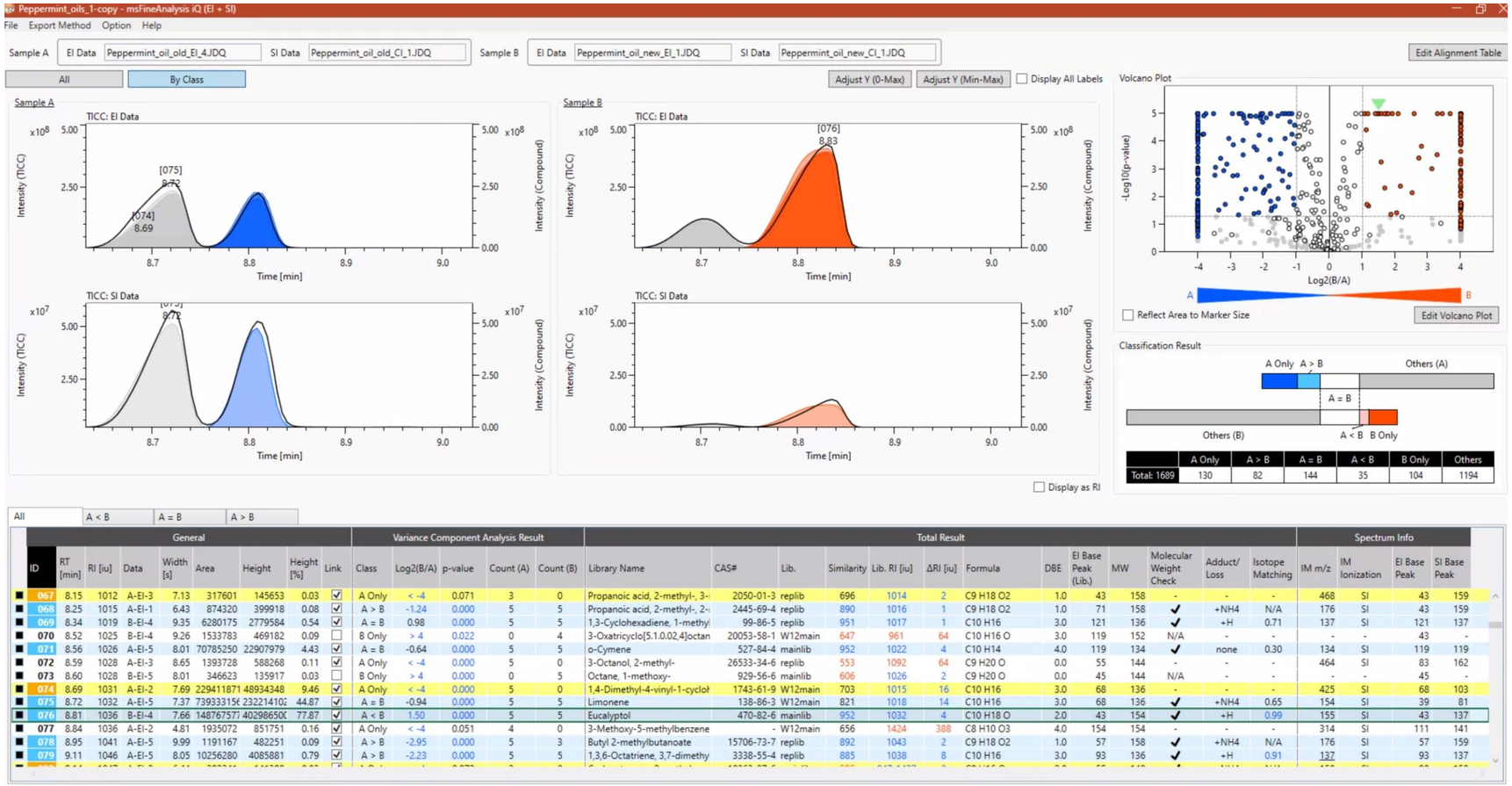
Compare peppermint oil samples with msFineAnalysis iQ
Single quadrupole: EI and CI data

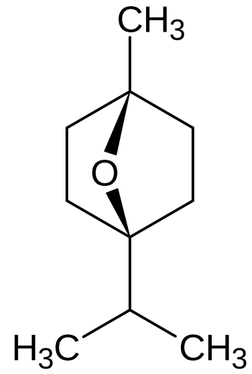




γ-terpinene
Equal in both samples

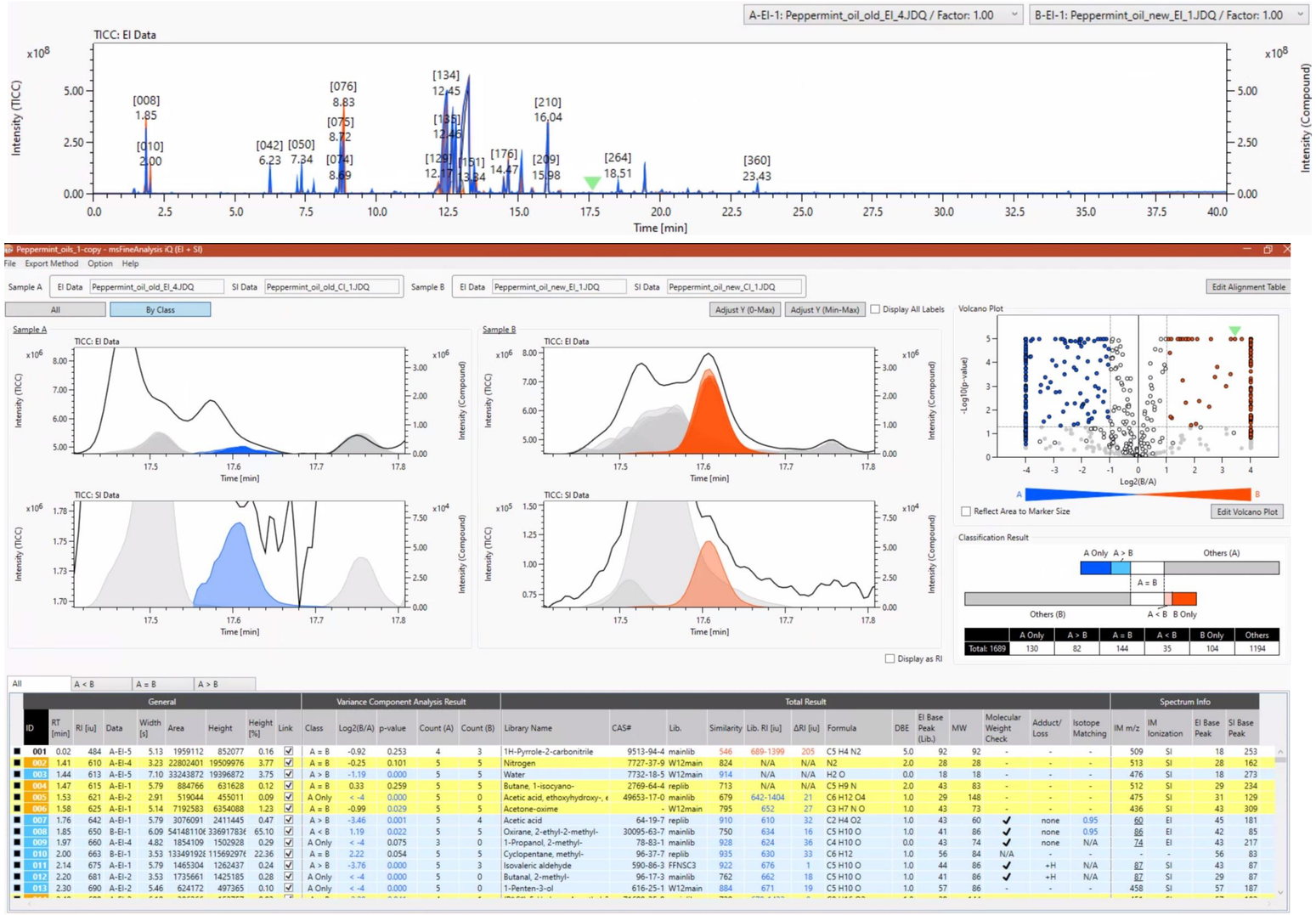
Compare peppermint oil samples with msFineAnalysis iQ
Single quadrupole: EI and CI data



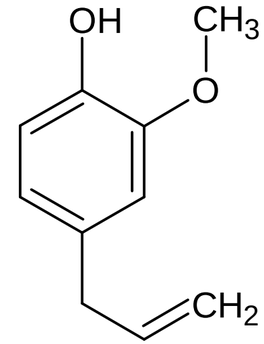


Eucalyptol
More abundant in Fluka

Chromatographic deconvolution for small Eugenol peak



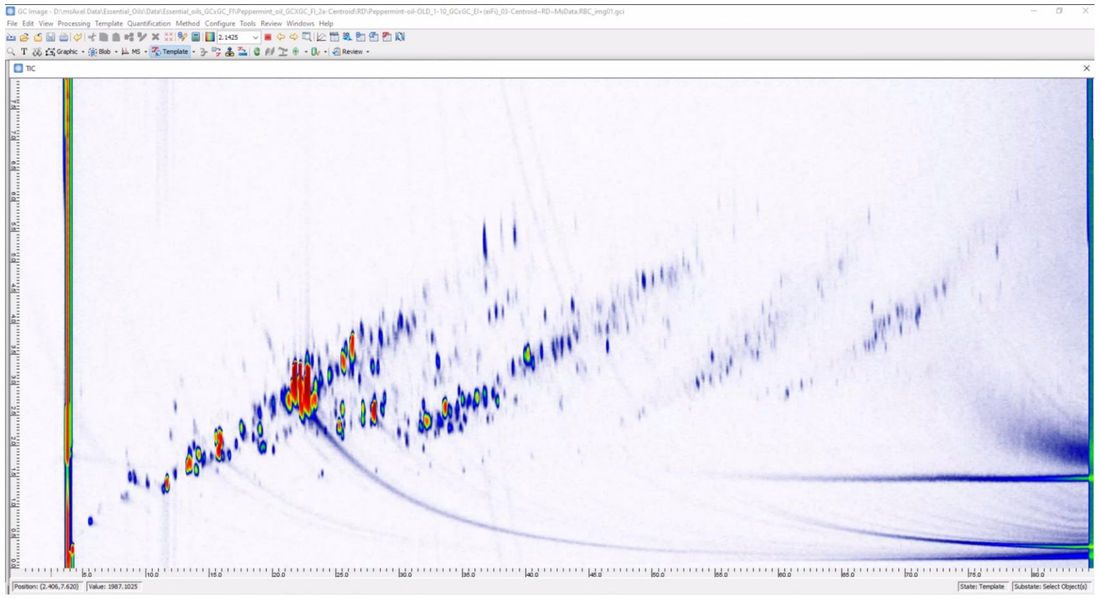
ID	RT [min]	RI [iu]	Data	Width [s]	Area	Height	Height [%]	Link	Class	Log2(B/A)	p-value	Count (A)	Count (B)	Library Name	CAS#	Lib.	Similarity	Lib. RI [iu]	ΔRI [iu]	Formula	DBE	EI Base Peak (Lib.)	MW	Molecular Weight Check	Adduct/ Loss	Isotope Matching	IM m/z	IM Ionization	EI Base Peak	SI Base Peak
001	0.02	484	A-EI-5	5.13	1959112	852077	0.16	☑	A = B	-0.92	0.253	4	3	1H-Pyrrole-2-carbonitrile	9513-94-4	mainlib	546	689-1399	205	C5 H4 N2	5.0	92	92	-	-	-	509	SI	18	253
002	1.41	610	A-EI-4	3.23	22802401	19509976	3.77	☑	A = B	-0.25	0.101	5	5	Nitrogen	7727-37-9	W12main	824	N/A	N/A	N2	2.0	28	28	-	-	-	513	SI	28	162
003	1.44	613	A-EI-5	7.10	33243872	19396872	3.75	☑	A > B	-1.19	0.000	5	5	Water	7732-18-5	W12main	914	N/A	N/A	H2 O	0.0	18	18	-	-	-	476	SI	18	273
004	1.47	615	A-EI-1	5.79	884766	631628	0.12	☑	A = B	0.33	0.259	5	5	Butane, 1-isocyano-	2769-64-4	replib	713	N/A	N/A	C5 H9 N	2.0	43	83	-	-	-	512	SI	29	234
005	1.53	621	A-EI-2	2.91	519044	455011	0.09	☑	A Only	< -4	0.000	5	0	Acetic acid, ethoxyhydroxy-, e	49653-17-0	mainlib	679	642-1404	21	C6 H12 O4	1.0	29	148	-	-	-	475	SI	31	129
006	1.58	625	A-EI-1	5.14	7192583	6354088	1.23	☑	A = B	-0.99	0.029	5	5	Acetone-oxime	-	W12main	795	652	27	C3 H7 N O	1.0	43	73	-	-	-	436	SI	43	309
007	1.76	642	A-EI-1	5.79	3076091	2411445	0.47	☑	A > B	-3.46	0.001	5	4	Acetic acid	64-19-7	replib	910	610	32	C2 H4 O2	1.0	43	60	✓	none	0.95	60	EI	45	181
008	1.85	650	B-EI-1	6.09	54148110	33691783	65.10	☑	A < B	1.19	0.022	5	5	Oxirane, 2-ethyl-2-methyl-	30095-63-7	mainlib	750	634	16	C5 H10 O	1.0	41	86	✓	none	0.95	86	EI	42	85
009	1.97	660	A-EI-4	4.82	1854109	1502928	0.29	☑	A Only	< -4	0.075	3	0	1-Propanol, 2-methyl-	78-83-1	mainlib	928	624	36	C4 H10 O	0.0	43	74	✓	none	N/A	74	EI	43	217
010	2.00	663	B-EI-1	3.53	13349192	11569297	22.36	☑	A = B	2.22	0.054	5	5	Cyclopentane, methyl-	96-37-7	replib	935	630	33	C6 H12	1.0	56	84	N/A	-	-	-	-	56	83
011	2.14	675	A-EI-1	5.79	1465304	1262437	0.24	☑	A > B	-3.76	0.000	5	3	Isovaleric aldehyde	590-86-3	FFNSC3	922	676	1	C5 H10 O	1.0	44	86	✓	+H	N/A	87	SI	43	87
012	2.20	681	A-EI-2	3.53	1735661	1425185	0.28	☑	A Only	< -4	0.000	5	0	Butanal, 2-methyl-	96-17-3	mainlib	762	662	18	C5 H10 O	1.0	41	86	✓	+H	N/A	87	SI	29	87
013	2.30	690	A-EI-2	5.46	624172	497365	0.10	☑	A Only	< -4	0.000	5	0	1-Penten-3-ol	616-25-1	W12main	884	671	19	C5 H10 O	1.0	57	86	-	-	-	458	SI	57	187



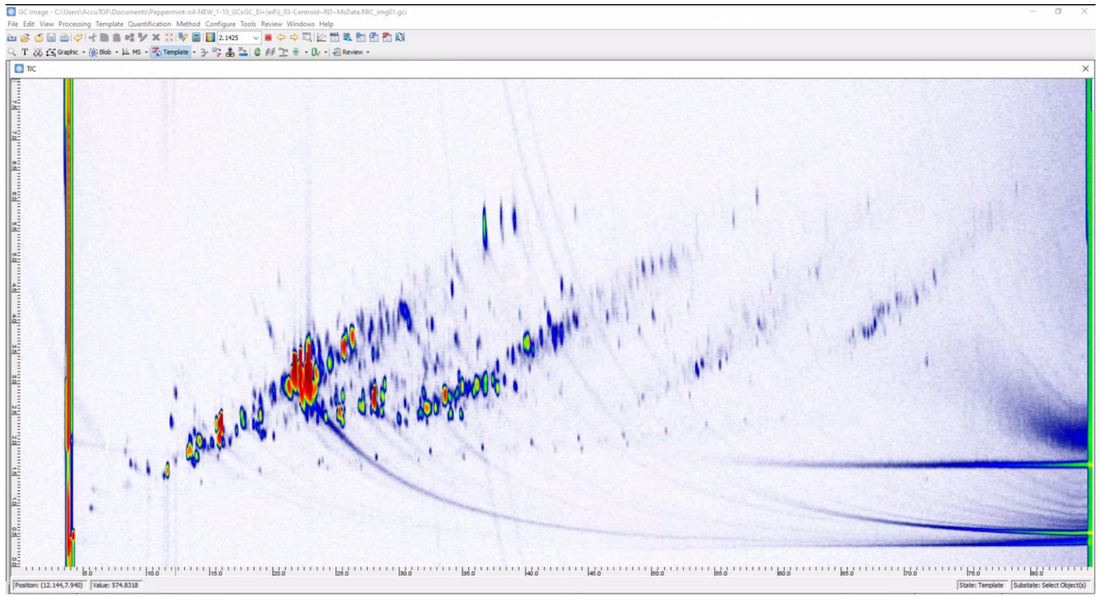
Eugenol
More abundant in Fluka
Height = 0.57%

GC x GC-HRTOFMS chromatograms for the peppermint oils

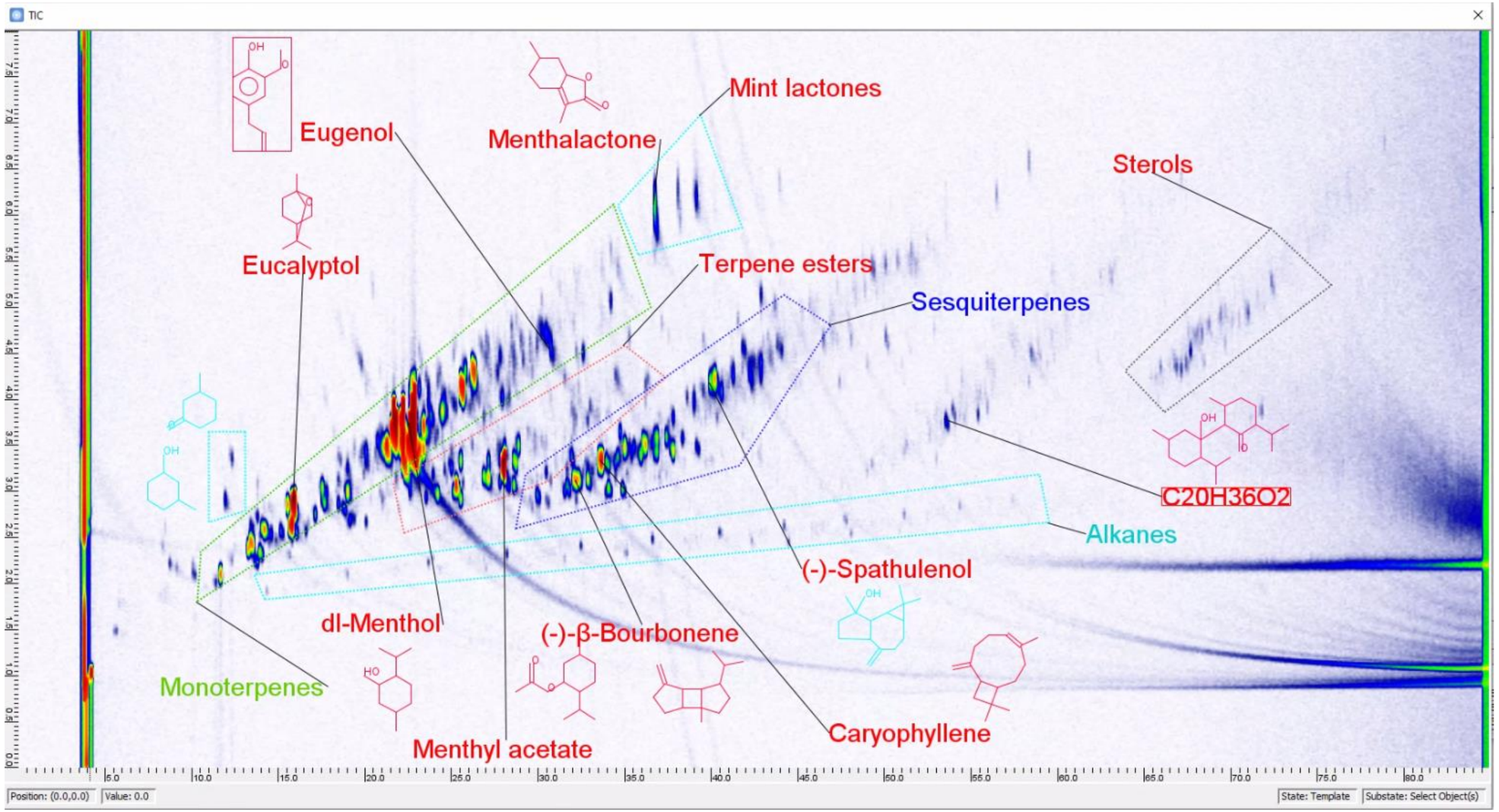
Sakae (>35-year-old sample)



Fluka (Fresh sample)

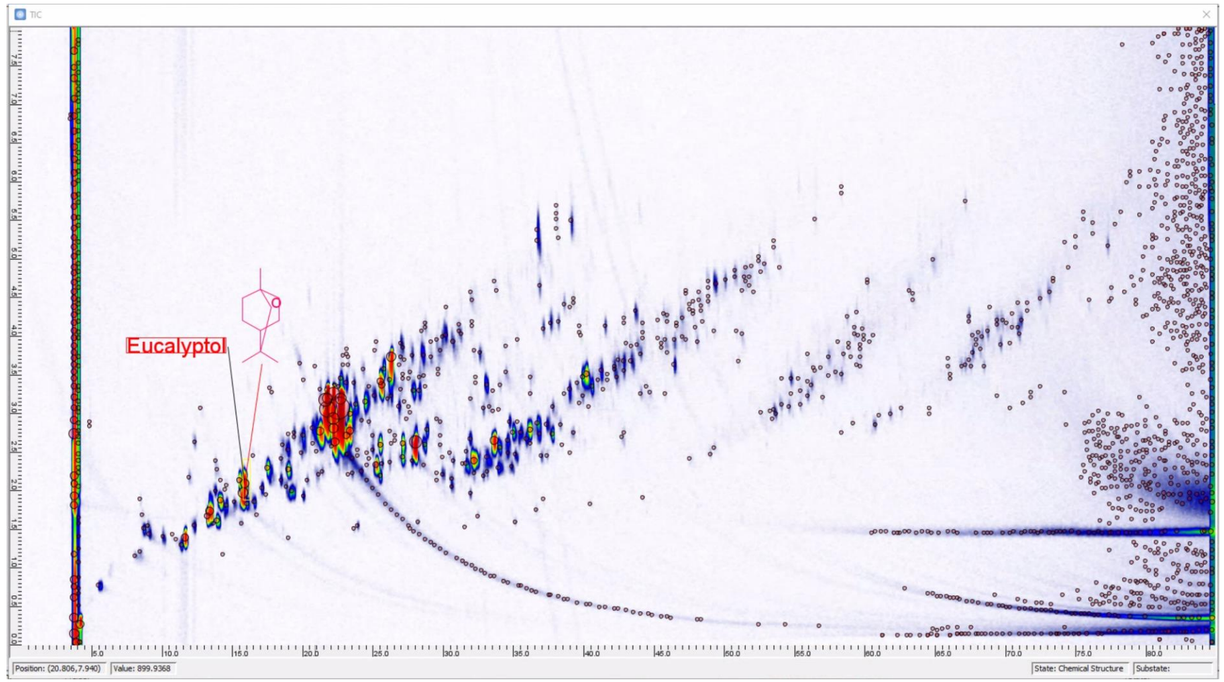


Some details for the Fluka Peppermint Oil

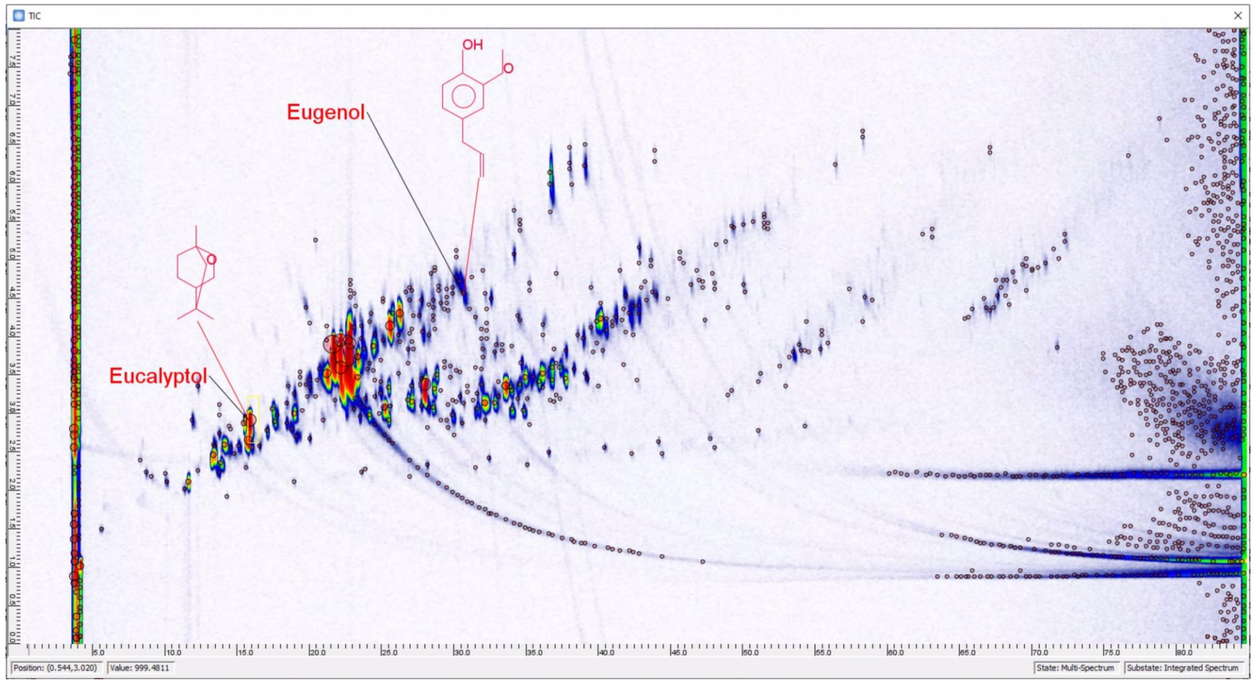


Eucalyptol and Eugenol blobs in Sakae and Fluka samples: Two compounds that were found to differ in the 1D GC-MS data

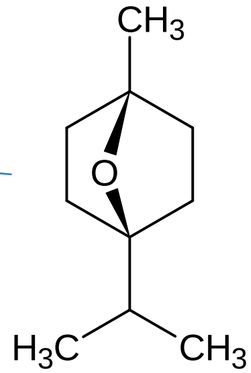
Sakae (>35-year-old sample)



Fluka (Fresh sample)

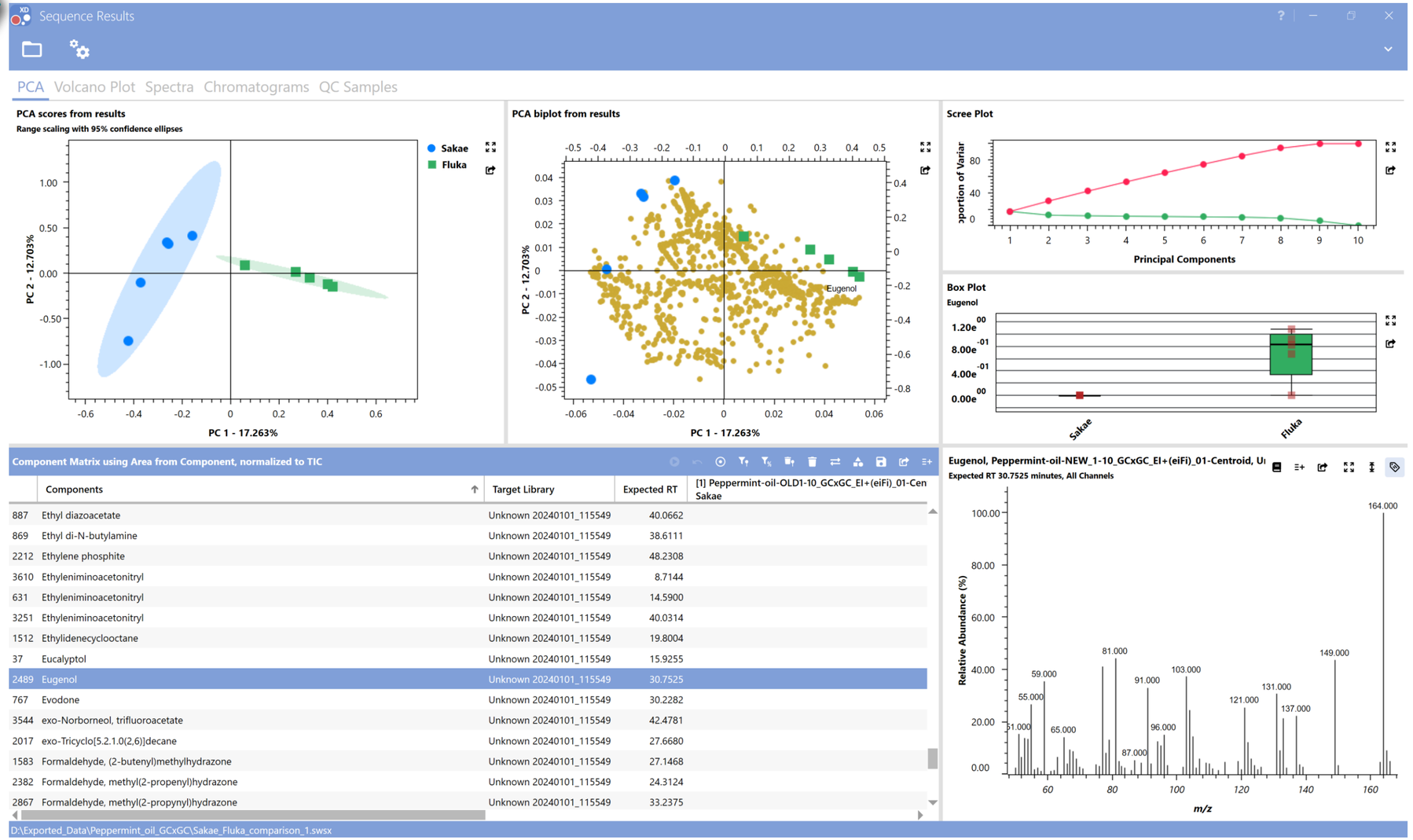


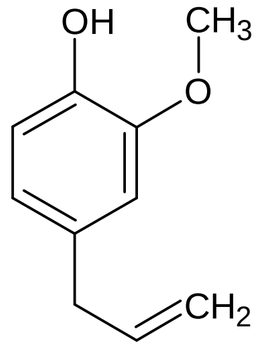
PCA and Eucalyptol entry for GC x GC-MS in AnalyzerPro XD



Eucalyptol
More abundant in Fluka

PCA and Eugenol entry for GC x GC-MS in AnalyzerPro XD



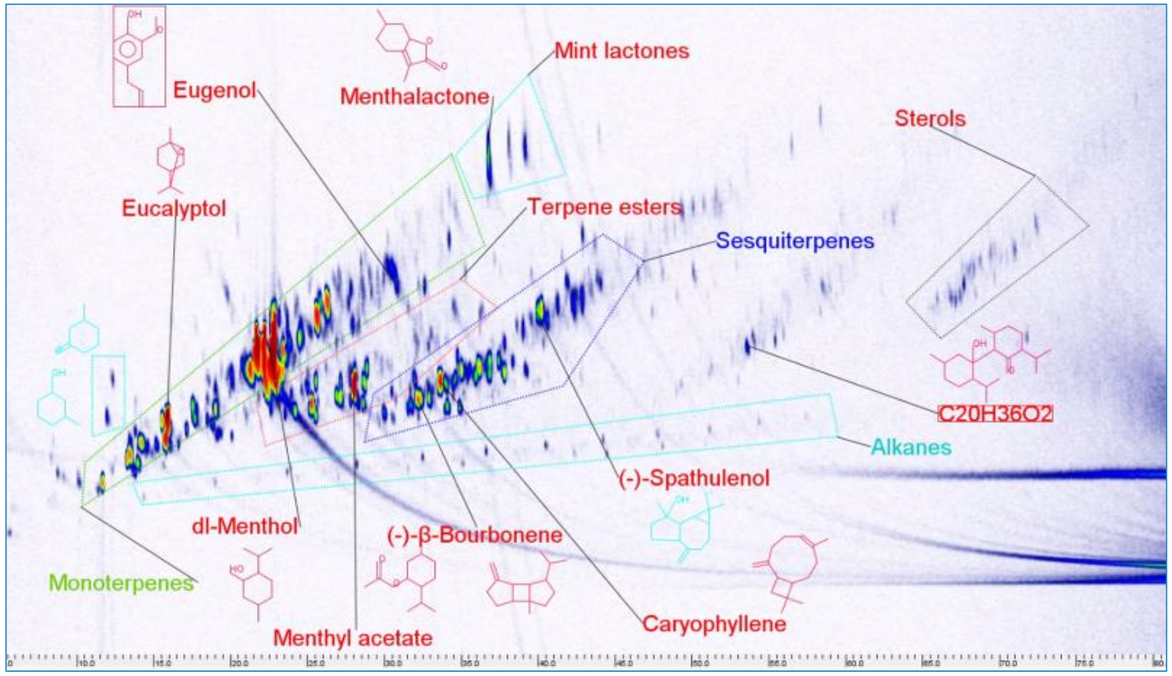


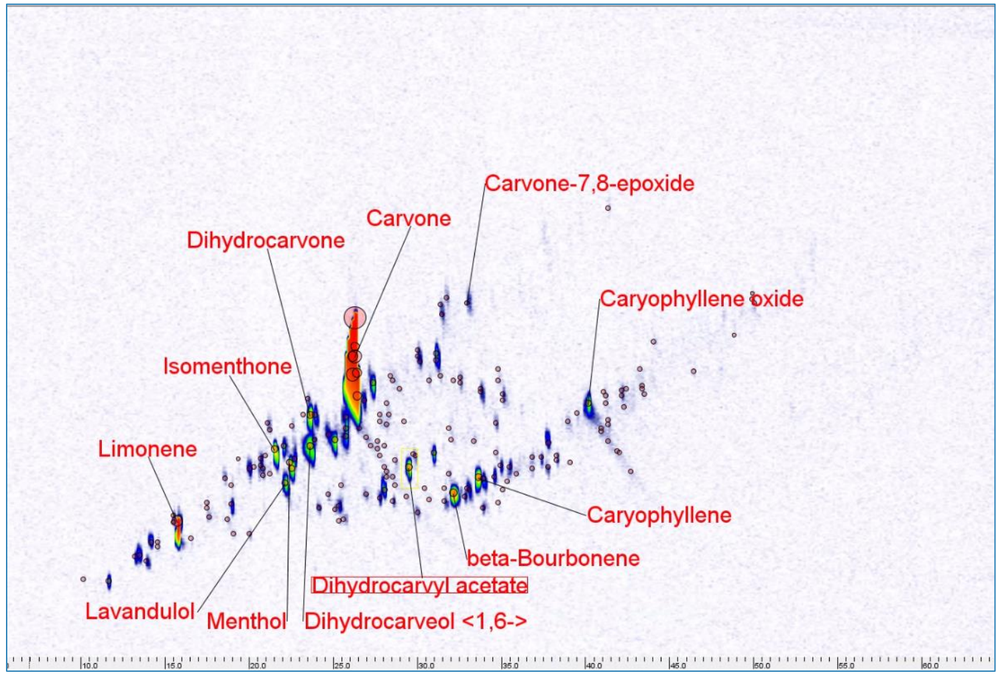
Eugenol
More abundant in Fluka
Height = 0.57%

Peppermint Oil and Spearmint Oil: very different compositions!

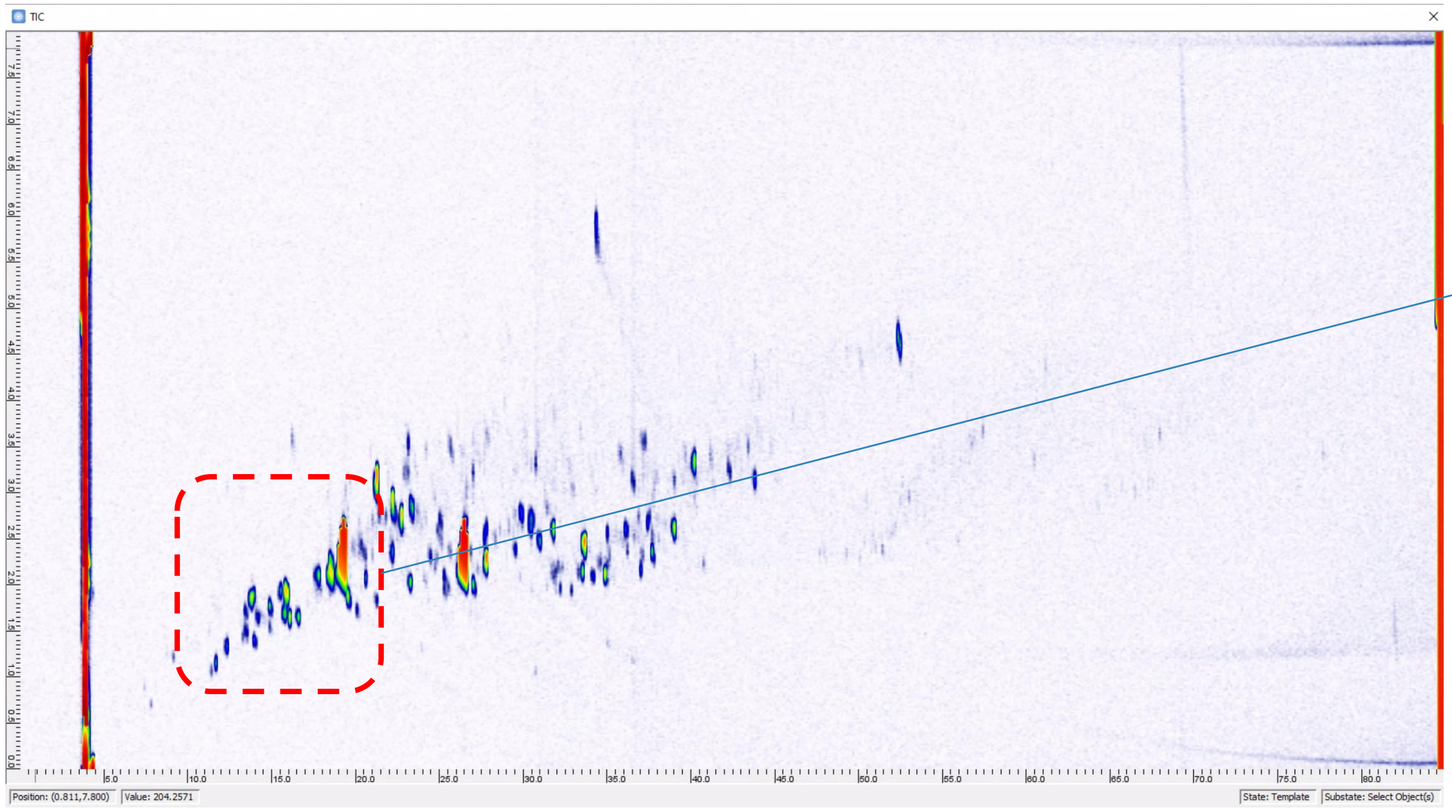
Peppermint Oil

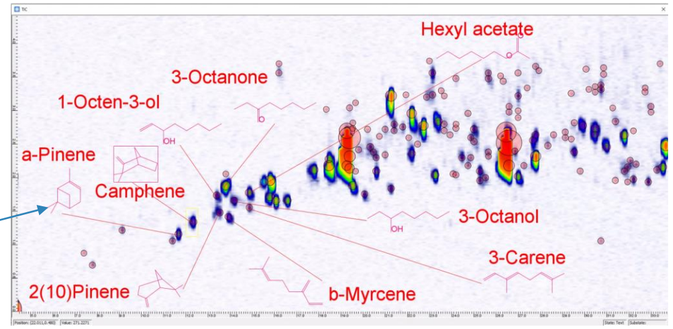
Spearmint Oil





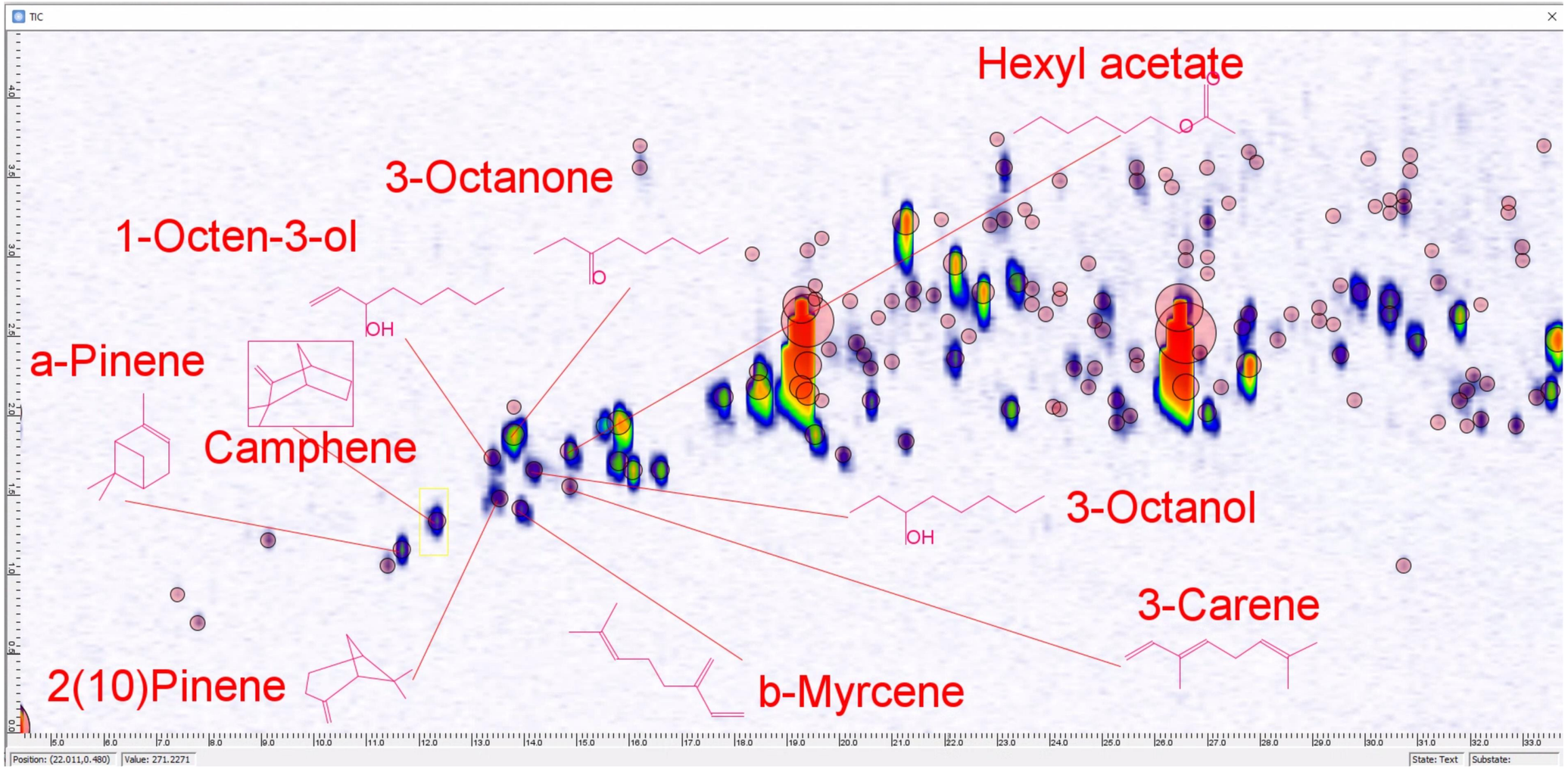
How does GC-MS (LR and HR) with deconvolution compare with GCxGC-HRTOFMS?



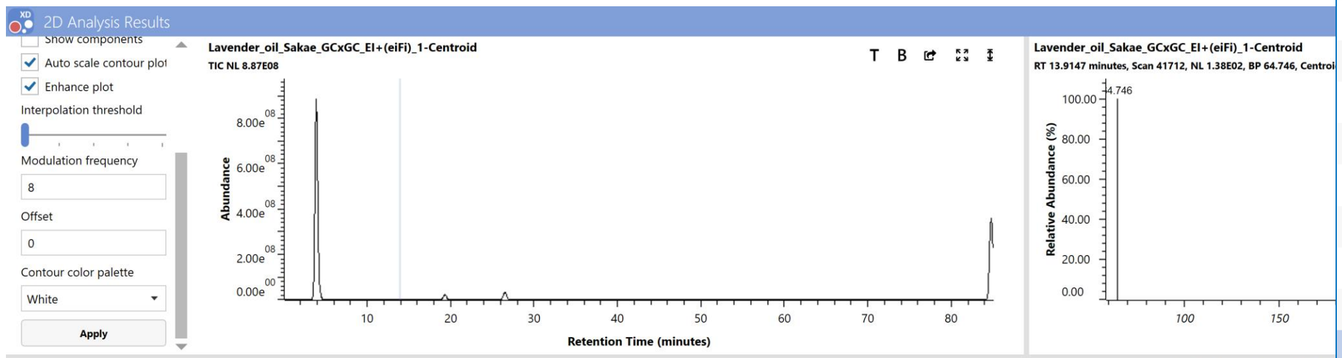


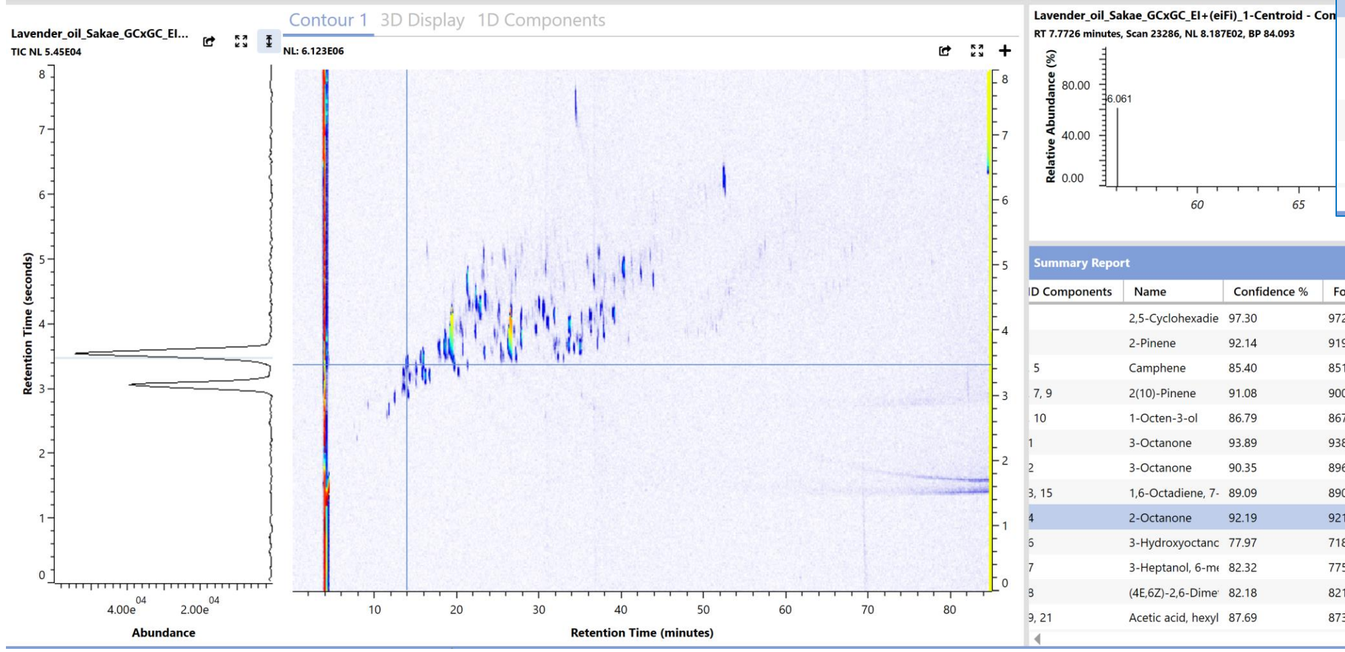
We'll use Lavender Oil as an example. Let's look at a small section of the chromatograms where several components elute closely

GC Image results for the selected region



AnalyzerPro XD results for the selected region



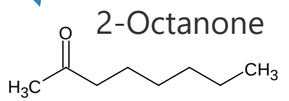


nts	Name	Confidence %	Forward	Reverse	CAS #	Library
	2,5-Cyclohexadie	97.30	972	972	59034-54-7	w12main
	2-Pinene	92.14	919	924	80-56-8	w12rep
	Camphene	85.40	851	858	79-92-5	ffnsc3
	2(10)-Pinene	91.08	900	933	127-91-3	w12rep
	1-Octen-3-ol	86.79	867	867	3391-86-4	w12rep
	3-Octanone	93.89	938	938	106-68-3	w12rep
	3-Octanone	90.35	896	918	106-68-3	replib
	1,6-Octadiene, 7-	89.09	890	890	123-35-3	w12rep
	2-Octanone	92.19	921	921	111-13-7	w12rep
	3-Hydroxyoctanc	77.97	718	921	n/a	w12main
	3-Heptanol, 6-me	82.32	775	933	18720-66-6	w12main
	(4E,6Z)-2,6-Dime	82.18	821	821	7216-56-0	w12main
	Acetic acid, hexyl	87.69	873	883	142-92-7	w12rep

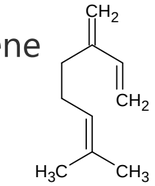
Summary Report

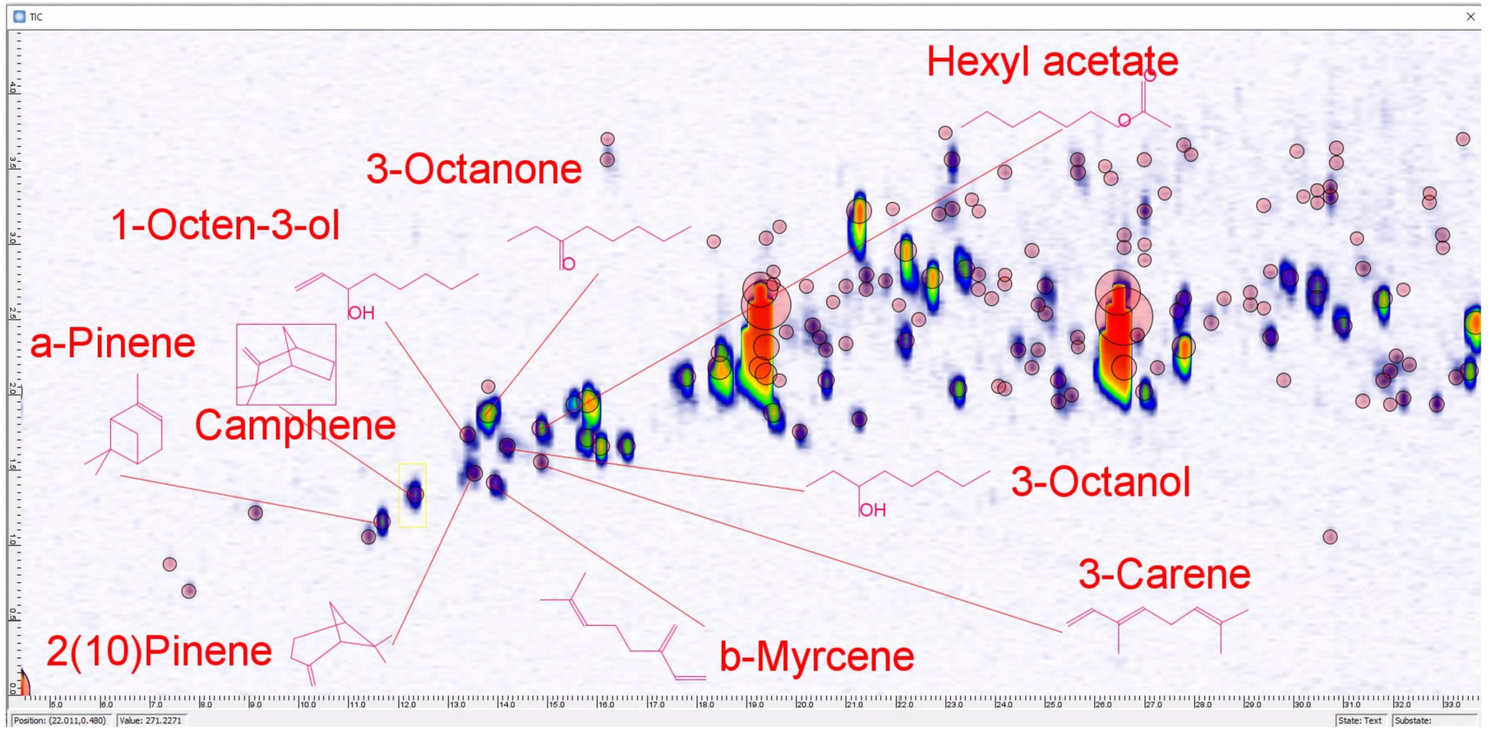
ID Components	Name	Confidence %	Forward	Reverse	CAS #	Library
	2,5-Cyclohexadie	97.30	972	972	59034-54-7	w12main
	2-Pinene	92.14	919	924	80-56-8	w12rep
5	Camphene	85.40	851	858	79-92-5	ffnsc3
7, 9	2(10)-Pinene	91.08	900	933	127-91-3	w12rep
10	1-Octen-3-ol	86.79	867	867	3391-86-4	w12rep
1	3-Octanone	93.89	938	938	106-68-3	w12rep
2	3-Octanone	90.35	896	918	106-68-3	replib
3, 15	1,6-Octadiene, 7-	89.09	890	890	123-35-3	w12rep
4	2-Octanone	92.19	921	921	111-13-7	w12rep
6	3-Hydroxyoctanc	77.97	718	921	n/a	w12main
7	3-Heptanol, 6-me	82.32	775	933	18720-66-6	w12main
8	(4E,6Z)-2,6-Dime	82.18	821	821	7216-56-0	w12main
9, 21	Acetic acid, hexyl	87.69	873	883	142-92-7	w12rep

2-Octanone



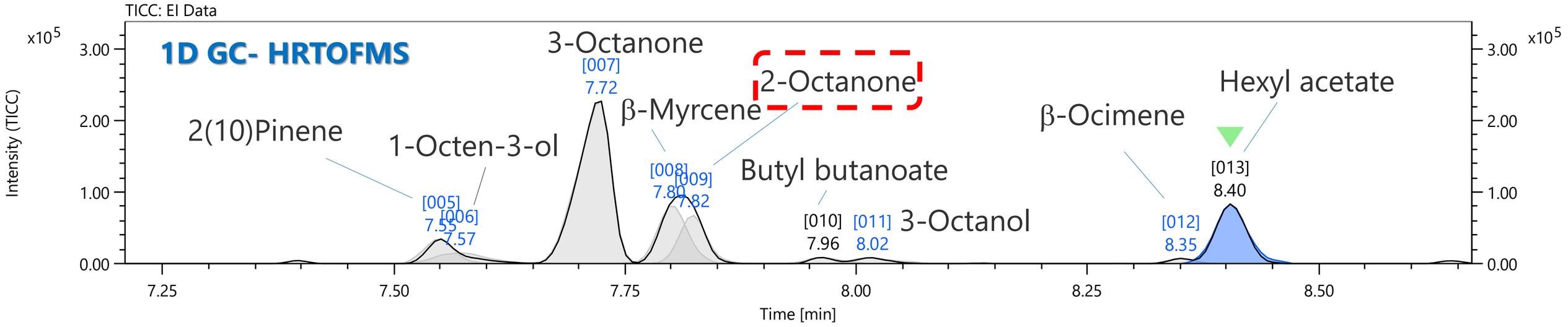
β-Myrcene



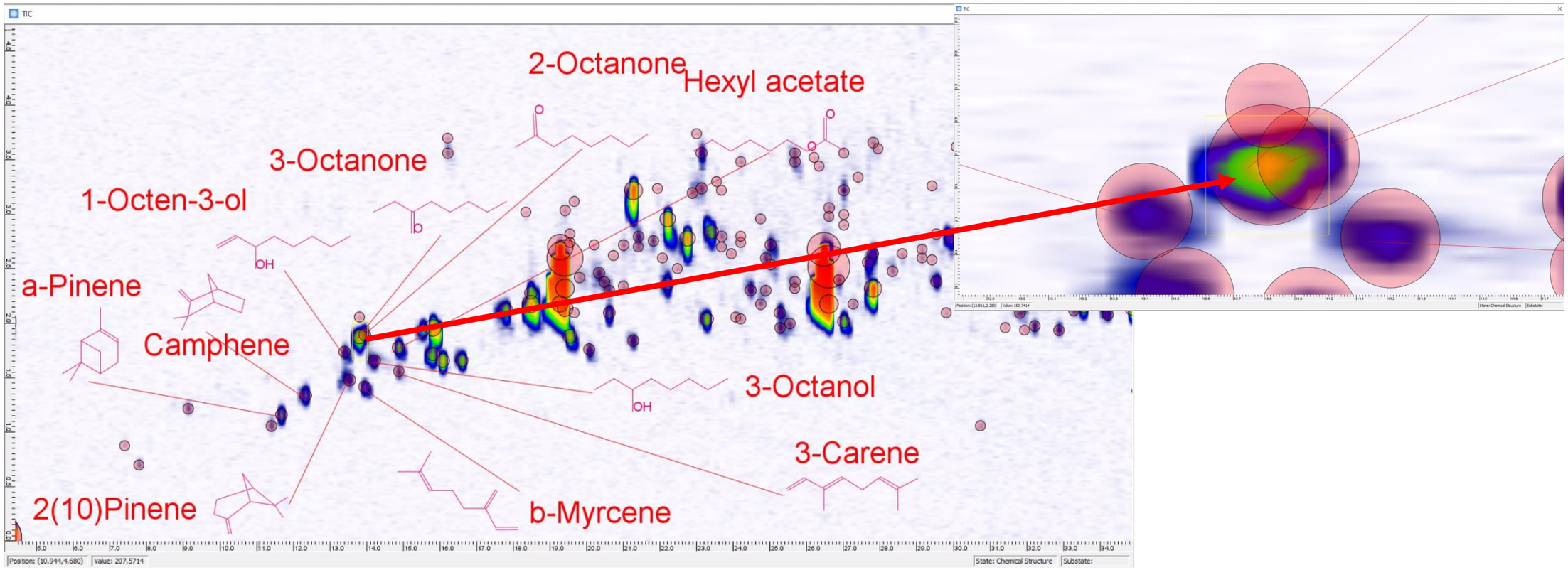


GC x GC – HRTOFMS + GC Image

2-Octanone not detected?



2-Octanone is detected in GC Image after blob deconvolution/unmixing



msFineAnalysis iQ results for GC-single quadrupole MS

Summary for a selected retention time region (lavender oil)

GCxGC/AnalyzerPro XD	GCxGC/GC Image	GC/Quadrupole	GC/HRTOF
2-Pinene (a-Pinene)	YES	YES	YES
Camphene	YES	YES	YES
2(10)-Pinene	YES	YES	YES
1-Octen-3-ol	YES	NO	YES
3-Octanone	YES	YES	YES
3-Octanone (not a separate blob)	YES	YES	YES
b-Myrcene	YES	NO	YES
2-Octanone	NO [1]	YES	NO
3-Hydroxyoctanoic Acid (Butyl Butanoate is Hit #6)	NO [2]	NO	Butyl butanoate
3-Octanol	3-Octanol	NO	3-Octanol
b-Ocimene	YES	YES	YES
Acetic acid, hexyl ester	YES	YES	YES

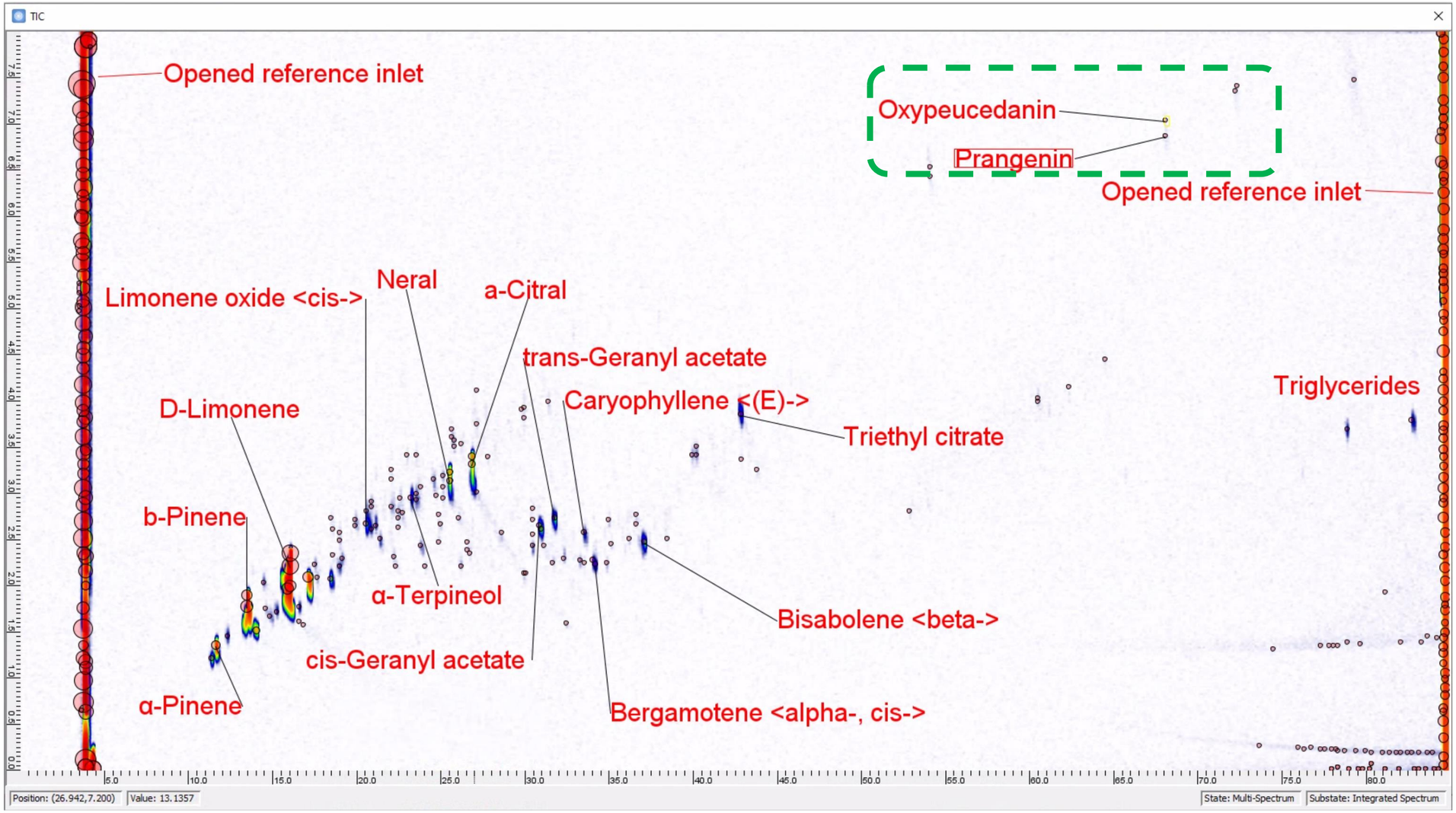
12 Compounds in this RT range

[1] YES after interactive blob deconvolution/unmixing
[2] 3rd hit after interactive blob deconvolution/unmixing

Both GCxGC data analysis programs examined were equally effective in detecting blobs and assigning compounds, although there were slight differences in the detection and ID of minor compounds.

- AnalyzerPro XD is easy to use for sample comparisons and uses a 2D deconvolution algorithm by default.
- GC Image has excellent graphics and has powerful tools for general GC x GC analysis
- For 1D GC-MS, msFineAnalysis iQ and AI use chromatographic deconvolution and integrate all available data

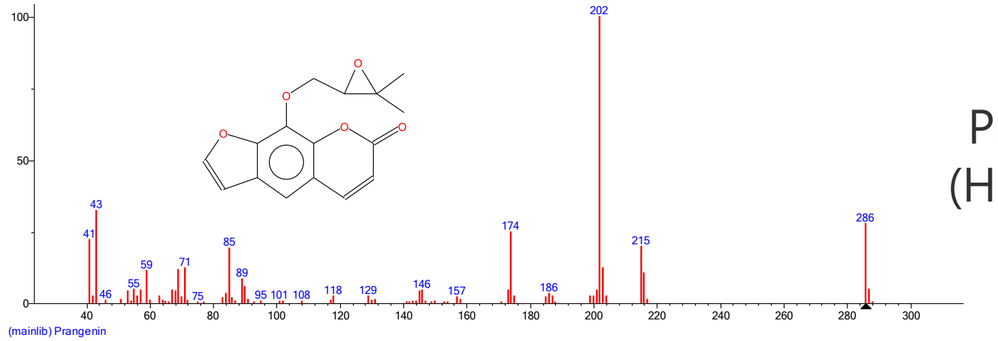
GCxGC-HRTOFMS of lemon oil. Let's look at the circled region.



These furanocoumarins in lemon oil have an identical 1D retention index

C00002490	737-52-0	Oxypeucedanin	C16H14O5	286.08412356
C00037261	2880-49-1	Heraclenin	C16H14O5	286.08412356

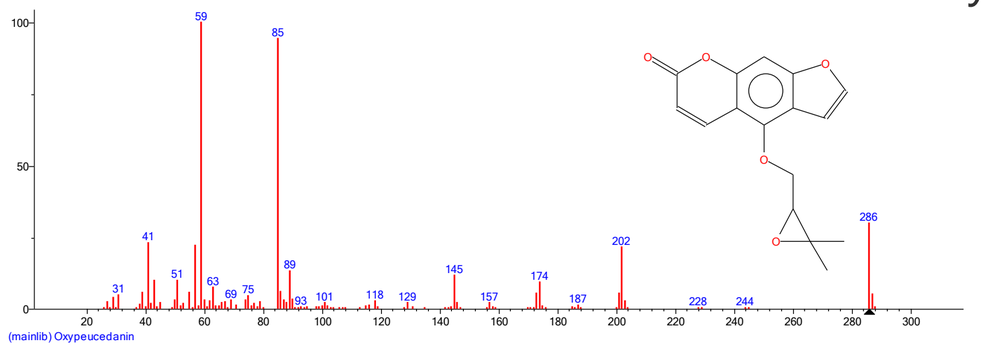
http://www.knapsackfamily.com/knapsack_core/result.php?sname=organism&word=Citrus%20limon



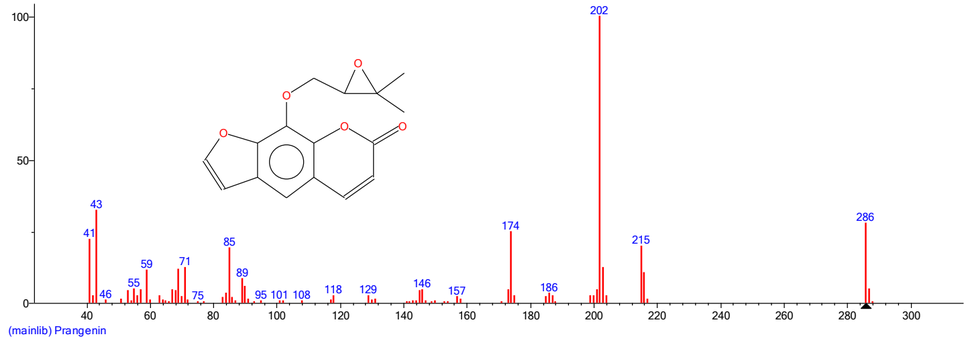
Prangenin
(Heraclenin)

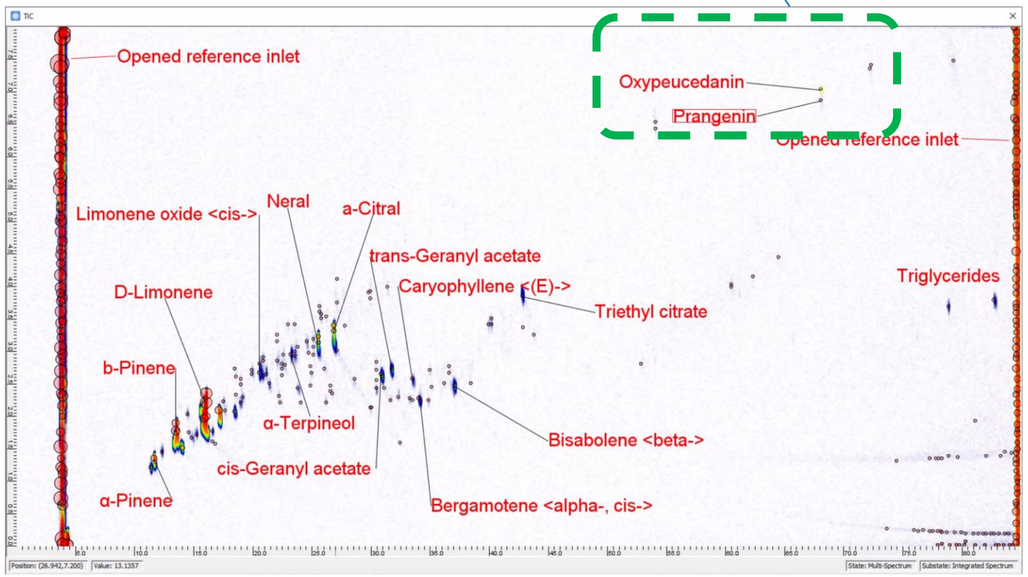
Calculated RI for semi-nonpolar column is
2324 IU for both compounds

Oxypeucedanin

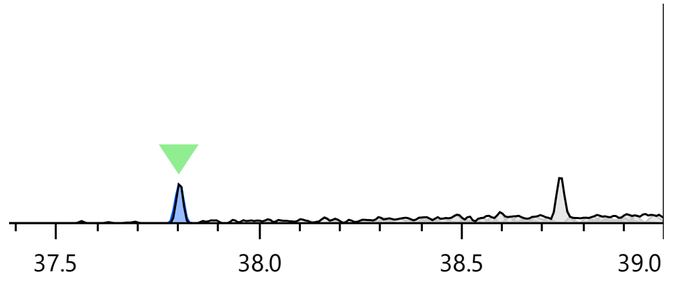


1D GC-MS does not see both compounds





1D GC-HRTOFMS only detects one of these compounds

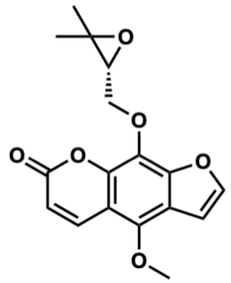


- GCxGC-HRTOFMS library search gave the correct ID as the first match.
- 1D GC-HRTOFMS gave the correct composition from the FI data. Needed to choose among DB hits.
- KNapSacK search supports the presence of this furanocoumarin compound in lemon.

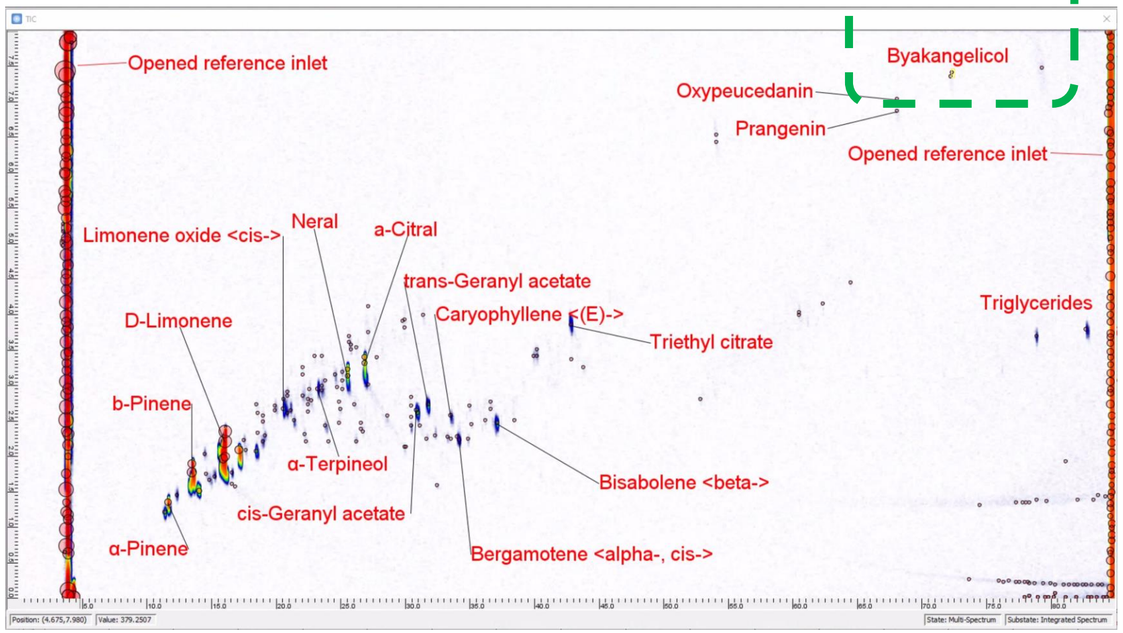
Metabolite Information	
Name	Byakangelicol
Formula	C17H16O6
Mw	316.09468824
CAS RN	26091-79-2
C_ID	C00053053
InChIKey	
InChICode	
SMILES	
Start Substs in Alk. Biosynthesis (Prediction)	

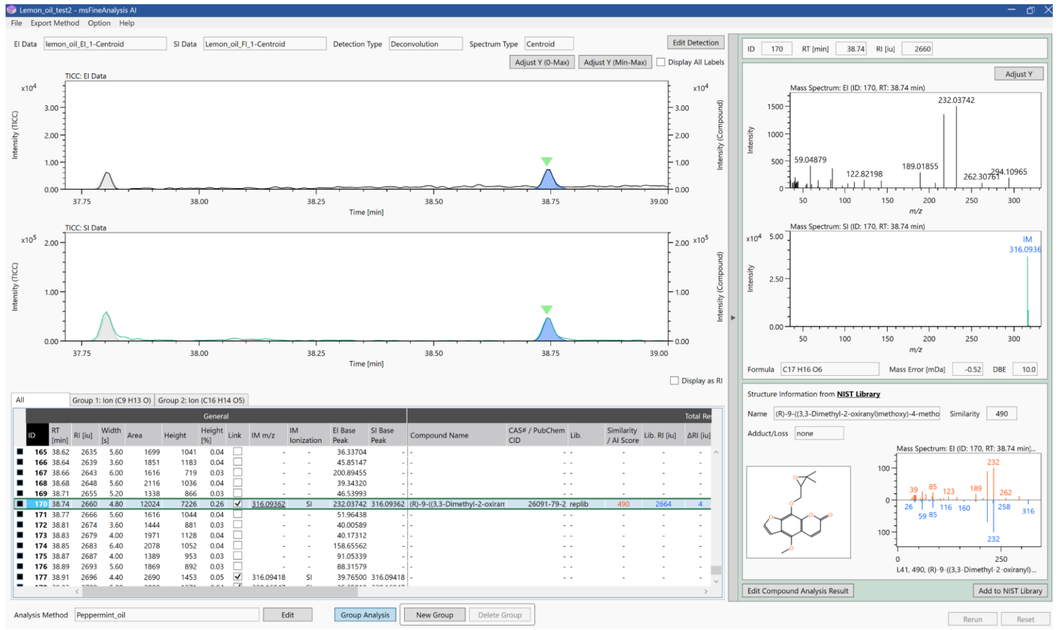
Organism	Kingdom	Family	Species	Reference
	Plantae	Rutaceae	Citrus lemon	Ref.
	Plantae	Rutaceae	Citrus limon	Ref.

Structural formula

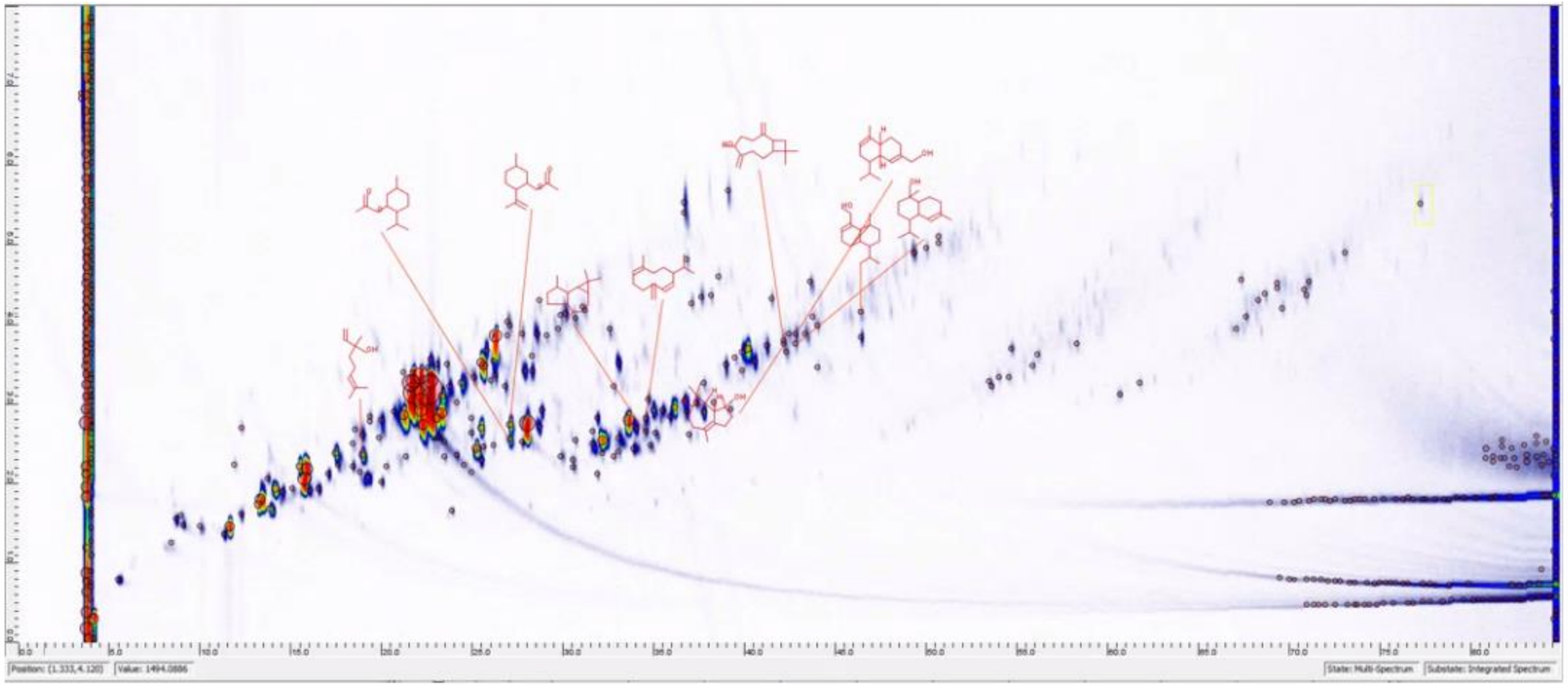


zoom in





What 1D GC-MS does NOT detect in the Sakae peppermint oil



Structures for some compounds in peppermint oil that coelute with 1D GC.
GC x GC separates these nicely.

Summary

GCxGC

- More complex hardware setup, but stable once setup and optimized
- Longer run times – *one late-eluting compound detected in 1D GC for peppermint oil was missed in GC x GC data. (Data omitted – time constraints)*
- Most complete separation: a few compounds detected that were not detected by 1D GC. Clearly, this will be more evident for highly complex mixtures such as petrochemicals!
- Fast blob detection by GC Image and fast 2D deconvolution by AnalyzerPro XD
- Fast database search by both programs

1D GC

- Shorter run times and simpler hardware configuration
- Single quadrupole missed a few minor lavender compounds that were detected by the other methods
- *However, the high dynamic range of the Q1600 single quadrupole MS easily revealed trace differences between orange oils. (Data omitted – time constraints)*
- Chromatographic deconvolution on the HRTOF was surprisingly effective, especially when soft ionization and accurate-*m/z* data is incorporated.
- Chromatographic deconvolution and integrated data analysis a bit slower than GC x GC data processing

Conclusions

- Each method has its own benefits and disadvantages
- Running a 1D separation first can help to decide on optimal 2D separation conditions
- The most complete analysis came from cross-examining data from multiple methods
- Context aids in data analysis! – use information from natural products databases like KNapSacK and Lotus.
- Use all the available information that you have: EI database search, soft ionization, accurate-*m/z* for fragments and molecular species, retention index values
- Structure tools like NIST Hybrid Search and msFineAnalysis AI structure tab can help with compounds that are not in databases.
- The >30-year-old peppermint oil showed little evidence of oxidation or degradation when compared to the fresh oil.