



# **ESSENTIAL SEPARATIONS:**

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

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### JEOL mass spectrometers used for this study



Q1600GC UltraQuad<sup>™</sup> 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<sup>™</sup> SQ-Zeta





High pumping capacity!

#### 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



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### 2: GC-MS High-Resolution Time-of Flight Mass Spectrometer: AccuTOF<sup>™</sup> 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



#### **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

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#### **Photo Ionization**

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



#### AccuTOF GC-Alpha only



#### **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:
    - 50 °C (0 min)
    - 5°C min<sup>-1</sup> to 220°C (0 min) 2.
    - 20°C to 300 °C 3.
    - Hold 8 minutes 4.

- - Oven:

## 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)

1. 60°C (0 min) 2. 3°C min<sup>-1</sup> to 315°C (0 min)





## Essential oil samples analyzed





• "JEOL" -- unidentified source

• a.k.a. Citrus × aurantium f. aurantium

a.k.a. Citrus × aurantium f. aurantium



### Single Quadrupole: GC-MS comparison of Peppermint Oils with El A Only A > B



					Gen	eral				0	Variance Co	omponent	Analysis Res	ault					T	otal Resu	lt.					
10	)	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]	∆RI (iu)	Formula	DBE	El Base Peak (Lib.)	MW	Molecular Weight Check	H.
	074	8.69	1031	A-EI-2	7.69	22941187	1 48934348	9.46	-	A Only	<-4	0.000	5	0	1,4-Dimethyl-4-vinyl-1-cycloh	1743-61-9	W12main	703	1015	16	C10 H16	3.0	68	136		
	075	8.72	1032	A-EI-5	7.37	73933315	€ 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	~	
	076	8.81	1036	B-EI-4	7.66	14876757	7 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	1	
	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	-	
	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	1	
	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	1	
	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	~	
	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	844	1106	48	C10 H16 O	3.0	71	152	14	
	082	9.46	1059	A-EI-5	8.05	7354641	2864258	0.55	~	A = 8	-0.16	0.035	5	5	y-Terpinene	99-85-4	replib	965	1060	1	C10 H16	3.0	93	136	~	
	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	~	
	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	
	085	9.82	1072	A-EI-5	13.84	49455946	13037052	2.52	~	A > B	-1.14	0.000	5	5	5-lsopropyl-2-methylbicyclo[	546-79-2	replib	944	1075	3	C10 H18 O	2.0	43	154	1	
	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	
	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	
	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	~	
-		10.05	4000			200000	007007		1.31				-	-	lae o teoro	5000 00 0		0.02			C40.040.00		~~	170		
-																										-

Analysis Method Default

Edit

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Rerun

Reset

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



			Spectru	m Info		
ct/	lsotope Matching	IM m/z	IM Ionization	El Base Peak	SI Base Peak	
ł	0.99	155	SI	43	137	0
		314	SI	111	141	
-14	N/A	176	SI	57	159	
1	0.91	137	SI	93	137	
-14	N/A	158	SI	98	158	
	-	427	SI	71	159	
£.	0.89	137	SI	93	137	
ł	N/A	131	SI	83	176	
		-		41	-	
ł	0.57	153	SI	43	137	
			-	28		
	-	-	-	29	-	
ł	0.70	137	SI	93	137	
			**			2
			-	Rerun	Res	set



#### $\gamma$ -terpinene Equal in both samples

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# Compare peppermint oil samples with msFineAnalysis iQ Single quadrupole: El and Cl data



A	1		A < B		A = B	A	4 > B																						
					Gen	eral					Variance Co	omponent	Analysis Res	ult					1	lotal Resul	lt								
	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]	∆RI (iu)	Formula	DBE Po (L	Base eak ib.)	MW	Molecular Weight Check	Adduct/ Loss	lsotope Matching	IM m/z	H F
	067	8.15	1012	A-EI-3	7.13	317601	1 145653	0.03	-	A Only	< -4	0.071	3	0	Propanoic acid, 2-methyl-, 3-	2050-01-3	replib	696	1014	2	C9 H18 O2	1.0	43	158	-	•	1	468	
	06	8.25	1015	A-EI-1	6.43	874320	399918	0.08	~	A > B	-1.24	0.000	5	5	Propanoic acid, 2-methyl-, 2-	2445-69-4	replib	890	1016	1	C9 H18 O2	1.0	71	158	1	+NH4	N/A	176	1
	06	8.34	1019	B-EI-4	9.35	6280175	5 2779584	0.54	~	A = B	0.98	0.000	5	5	1,3-Cyclohexadiene, 1-methy	99-86-5	replib	951	1017	1	C10 H16	3.0	121	136	1	+H	0.71	137	
	070	8.52	1025	B-EI-4	9.26	1533783	469182	0.09		B Only	> 4	0.022	0	4	3-Oxatricyclo[5.1.0.02,4]octan	20053-58-1	W12main	647	961	64	C10 H16 O	3.0	119	152	N/A	-	-	-	
	071	8.56	1026	A-EI-5	8.01	70785250	22907979	4.43	-	A = B	-0.64	0.000	5	5	o-Cymene	527-84-4	mainlib	952	1022	4	C10 H14	4.0	119	134	1	none	0.30	134	ŧ.
	072	8.59	1028	A-EI-3	8.65	1393728	588268	0.11	~	A Only	< -4	0.000	5	0	3-Octanol, 2-methyl-	26533-34-6	replib	553	1092	64	C9 H20 O	0.0	55	144	-	-	-	464	į.
	073	8.60	1028	B-EI-5	8.01	346623	3 135917	0.03		B Only	> 4	0.000	0	5	Octane, 1-methoxy-	929-56-6	mainlib	606	1026	2	C9 H20 O	0.0	45	144	N/A	-		-	
	074	8.69	1031	A-EI-2	7.69	22941187	1 48934348	9.46	· 🗸	A Only	< -4	0.000	5	0	1,4-Dimethyl-4-vinyl-1-cyclob	1743-61-9	W12main	703	1015	16	C10 H16	3.0	68	136	-	-	-	425	1
	07:	8.72	1032	A-EI-5	7.37	73933315	€ 23221410	44.87	-	A = B	-0.94	0.000	5	5	Limonene	138-86-3	W12main	821	1018	14	C10 H16	3.0	68	136	1	+NH4	0.65	154	1
	07	8.81	1036	B-EI-4	7.66	14876757	7 40298650	( 77.87	<ul> <li>Image: A state</li> </ul>	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	1
	077	8.84	1036	A-EI-2	4.81	1935072	2 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	ł.
	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	1	+NH4	N/A	176	1
	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	1
-	<		40.17						-			0.070	-	^		10000 07 0				^		~ ~			•	•••••			



#### Eucalyptol More abundant in Fluka

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137

57 159



# Chromatographic deconvolution for small Eugenol peak



	D R	T min] F	RI [iu] Da	ata	Width [s]	Area	Height	Height [%]	Link	Class	Log2(B/A)	p-value	Count (A)	Count (B)	Library Name	CAS#	Lib.	Similarity	Lib. RI (iu)	∆RI [iu]	Formula	DBE	El Base Peak (Lib.)	MW	Molecular Weight Check	Adduct/ Loss	Isotope Matching	IM m/z	IM Ionizat
	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
	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
	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
	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
	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
	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
	007	1.76	642 A	A-EI-1	5.79	3076091	2411445	0.47	1	A > B	-3.46	0.001	5	4	Acetic acid	64-19-7	replib	910	610	32	C2 H4 O2	1.0	43	60	1	none	0.95	60	EI
	800	1.85	650 B	8-EI-1	6.09	541481106	336917836	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	1	none	0.95	86	EI
	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	1	none	N/A	74	EI
	010	2.00	663 B	8-EI-1	3.53	133491928	115692976	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	-	-	-	-
	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
	012	2.20	681 A	-EI-2	3.53	1735661	1425185	0.28	1	A Only	< -4	0.000	5	0	Butanal, 2-methyl-	96-17-3	mainlib	762	662	18	C5 H10 O	1.0	41	86	1	+H	N/A	87	SI
	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
-							******		ান		0.00	0.014					7. 874			-									~ *





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



Co	mponent Matrix using Area from Component, nor	malized to TIC		o 🗠 O Tr	▼, ■, 📋 ≓ 🚓 🖬 📴 ≕+	Eucalyptol, P	eppermint-oil-
	Components	Target Library	Expected RT	[1] Peppermint-oil-OLD1-10_GCxGC_EI+(eiFi)_01-Centroid.cdf Sakae	[2] Peppermint-oil-OLD1-10_GCxGC_El Sakae	Expected RT 15	.9255 minutes, Al
31	Bicyclo[4.1.0]hept-2-ene, 3,7,7-trimethyl-, (1S-cis)-	Unknown 20240101_115549	15.2525	0.2480		100.00	
32	6-Undecanol	Unknown 20240101_115549	15.5193	0.0579			
33	o-Cymene	Unknown 20240101_115549	15.5243	6.3039		80.00	55.000
34	Formic acid, octyl ester	Unknown 20240101_115549	15.6487	0.0209		\$0.00 \$	
35	1,3-Dioxolane, 2-butyl-2-ethyl-	Unknown 20240101_115549	15.6523	0.0073		) j	
36	Limonene	Unknown 20240101_115549	15.7890	10.3369		<b>b</b> 60.00	
37	Eucalyptol	Unknown 20240101_115549	15.9255	42.4702		Abu	
38	1,3,6-Octatriene, 3,7-dimethyl-, (Z)-	Unknown 20240101_115549	16.0527	0.7276		tive	
39	Component_0251	Unknown 20240101_115549	16.1859	0.0474		<b>a</b> 40.00 -	
40	N-Acrylonitrylaziridine	Unknown 20240101_115549	16.4505	0.0074		-	
41	Pentanoic acid, butyl ester	Unknown 20240101_115549	16.4542	0.0534		20.00 -	
42	β-Ocimene	Unknown 20240101_115549	16.5869	0.6445			
43	2,4-Pentanedione, 1,1,1,5,5,5-hexafluoro-	Unknown 20240101_115549	16.9074	0.0062		0.00	
44	Pentane, 3-ethyl-	Unknown 20240101_115549	16.9882	0.0126			60 60
45	Cyclohexanone, 5-methyl-2-(1-methylethyl)-, trans-	Unknown 20240101_115549	17.0367	0.0506	*		00



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

#### Sequence Results

#### 🗅 🐾

#### PCA Volcano Plot Spectra Chromatograms QC Samples



Comp	onent Matrix using Area from Component, normalized to TIC				·∽ ⊙ T• T <sub>%</sub> T• 📋 ≓ ♣ 🖬 12 =+
	Components	Ŷ	Target Library	Expected RT	[1] Peppermint-oil-OLD1-10_GCxGC_EI+(eiFi)_01-Cen Sakae
887	Ethyl diazoacetate		Unknown 20240101_115549	40.0662	
869	Ethyl di-N-butylamine		Unknown 20240101_115549	38.6111	
2212	Ethylene phosphite		Unknown 20240101_115549	48.2308	
8610	Ethyleniminoacetonitryl		Unknown 20240101_115549	8.7144	
31	Ethyleniminoacetonitryl		Unknown 20240101_115549	14.5900	
251	Ethyleniminoacetonitryl		Unknown 20240101_115549	40.0314	
512	Ethylidenecyclooctane		Unknown 20240101_115549	19.8004	
7	Eucalyptol		Unknown 20240101_115549	15.9255	
189	Eugenol		Unknown 20240101_115549	30.7525	
57	Evodone		Unknown 20240101_115549	30.2282	
544	exo-Norborneol, trifluoroacetate		Unknown 20240101_115549	42.4781	
017	exo-Tricyclo[5.2.1.0(2,6)]decane		Unknown 20240101_115549	27.6680	_
583	Formaldehyde, (2-butenyl)methylhydrazone		Unknown 20240101_115549	27.1468	
382	Formaldehyde, methyl(2-propenyl)hydrazone		Unknown 20240101_115549	24.3124	
367	Formaldehyde, methyl(2-propynyl)hydrazone	_	Unknown 20240101_115549	33.2375	

RT 30.7525 minutes, All Channels





#### Eugenol More abundant in Fluka Height = 0.57%

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# Peppermint Oil and Spearmint Oil: very different compositions!



### Peppermint Oil









#### Spearmint Oil



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### 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



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# AnalyzerPro XD results for the selected region



Jame	Confidence %	Forward	Reverse	<b>CAS</b> #	Library
Varine	confidence /0		Neverse		Library
5-Cyclohexadie	97.30	972	972	59034-54-7	w12main
-Pinene	92.14	919	924	80-56-8	w12rep
amphene	85.40	851	858	79-92-5	ffnsc3
(10)-Pinene	91.08	900	933	127-91-3	w12rep
-Octen-3-ol	86.79	867	867	339 <mark>1-86-4</mark>	w12rep
-Octanone	93.89	938	938	106-68-3	w12rep
Octanone	90.35	896	918	106-68-3	replib
6-Octadiene, 7-	89.09	890	890	123-35-3	w12rep
-Octanone	92.19	921	921	111-13-7	w12rep
-Hydroxyoctanc	77.97	718	921	n/a	w12main
-Heptanol, <mark>6-</mark> me	82.32	775	933	18720-66-6	w12main
E,6Z)-2,6-Dime	82.18	821	821	7216-56-0	w12main
cetic acid, hexyl	87.69	873	883	142-92-7	w12rep

	e	<b>Ξ</b> =+	6 🖬
Reverse	CAS #	Library	
972	59034-54-7	w12main	-
924	80- <mark>56-</mark> 8	w12rep	
858	79-92-5	ffnsc3	
933	127-91-3	w12rep	
867	3391-86-4	w12rep	
938	106-68-3	w12rep	
918	106-68-3	replib	
890	123-35-3	w12rep	
921	111-13-7	w12rep	
921	n/a	w12main	
933	18720-66-6	w12main	
821	7216-56-0	w12main	
883	142-92-7	w12rep	-



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



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# msFineAnalysis iQ results for GC-single quadrupole MS

#### 🖗 Lavender\_oil - msFineAnalysis iQ (El + SI)

File Export Method Option Help



					General								Total R	Result								Spectru	n Info		
	ID	RT [min]	RI (iu)	Width [s]	Area	Height	Height [%]	Link	Library Name	CAS#	Lib.	Similarity	Lib. RI (iu)	∆RI [iu]	Formula	DBE	El Base Peak (Lib.)	MW	Molecular Weight Check	Adduct/ Loss	IM m/z	IM Ionization	El Base Peak	SI Base Peak	
	001	6.00	926	0.99	80688	28887	0.18	~	3-Thujene	2807-05-2	w12rep	780	902	24	C10 H16	3.0	93	130	~	+H	137	SI	93	137	
	002	6.21	934	6.29	494570	246610	1.52	-	2-Pinene	80-56-8	W12rep	869	948	14	C10 H16	3.0	93	136	1	+H	137	SI	93	137	
	003	6.62	951	8.04	327790	153464	0.95	-	Camphene	79-92-5	FFNSC3	858	953	2	C10 H16	3.0	93	136	1	+H	137	SI	93	137	
	004	7.17	974	5.59	98825	44952	0.28	~	1,3-Cyclohexadiene, 2-methyl	99-83-2	W12main	763	969	5	C10 H16	3.0	93	136	1	+H	137	SI	93	137	-
	005	7.31	979	5.59	963660	454072	2.80	-	2(10)-Pinene	127-91-3	W12rep	846	972	7	C10 H16	3.0	93	136	1	+H	137	SI	93	137	
	006	7.45	985	5.94	936307	470276	2.90	-	3-Octanone	106-68-3	mainlib	851	986	1	C8 H16 O	1.0	43	128	1	+NH4	146	SI	99	129	
	007	7.56	989	5.94	783429	384821	2.37	~	2(10)-Pinene	127-91-3	W12rep	805	972	17	C10 H16	3.0	93	136	-	-	146	SI	93	137	
	008	7.75	997	6.99	65470	31802	0.20		3-Octanol	589-98-0	replib	667	993	4	C8 H18 O	0.0	59	130	-	-	101	EI	83	-	
	009	8.11	1010	4.89	65221	31488	0.19	~	β-Ocimene	13877-91-3	replib	678	1037	27	C10 H16	3.0	93	136	~	+H	137	SI	93	137	
	010	814	1011	6.99	246649	119249	0.74	J	Acetic acid, hexyl ester	142-92-7	mainlib	858	1011	0	C8 H16 O2	1.0	43	144	1	+NH4	162	SI	43	162	
	011	8.40	1021	7.69	65160	30403	0.19		Benzene, 1-methyl-4-(1-meth	99-87-6	W12main	855	1042	21	C10 H14	4.0	119	134	1	none	134	EI	119	-	
	012	8.54	1026	5.94	465094	221008	1.36	-	Benzene, 1-methyl-3-(1-meth	535-77-3	replib	887	1022	4	C10 H14	4.0	119	134	1	none	134	SI	119	119	
	013	8.67	1031	6.99	3015246	1391678	8.58	-	Limonene	138-86-3	FFNSC3	889	1030	1	C10 H16	3.0	68	136	1	+NH4	154	SI	93	137	
	014	8.77	1034	6.29	2188305	1042778	6.43	~	Eucalyptol	470-82-6	mainlib	840	1032	2	C10 H18 O	2.0	43	154	1	+H	155	SI	108	137	
	015	8.80	1035	6.99	921491	343842	2.12	~	(4E,6Z)-2,6-Dimethyl-2,4,6-oc	7216-56-0	W12rep	861	993	42	C10 H16	3.0	93	136	-	-	135	SI	93	135	
	016	9.10	1046	6.64	697133	332453	2.05	1	3-Carene	13466-78-9	mainlib	876	1011	35	C10 H16	3.0	93	136	1	+H	137	SI	93	137	
	5																								
An	alysis N	lethod	Essen	tial_oils					Edit																



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# 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	YES	NO
3-Hydroxyoctanoic Acid (Butyl Butanoate is Hit #6)	NO <sup>2</sup>	NO	Butyl butanoate
3-Octanol	3-Octanol	NO	3-Octanol
b-Ocimene	YES	YES	YES
Acetic acid, hexyl ester	YES	YES	YES
<sup>1</sup> YES after in <sup>2</sup> 3 <sup>rd</sup> hit afte	nteractive blob deconvol r interactive blob deconv	ution/unmixing /olution/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

12 Compounds in this RT range

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



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

<u>C00002490</u>	737-52-0	Oxypeucedanin	C16H14O5 286.08412356
<u>C00037261</u>	2880-49-1	Heraclenin	C16H14O5 286.08412356

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



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



Prangenin (Heraclenin)

Oxypeucedanin





# 1D GC-MS does not see both compounds





#### 1D GC-HRTOFMS only detects one of these compounds



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- 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.



		Metabo
Name	Byakangelicol	
Formula	C17H16O6	
Mw	316.09468824	
CAS RN	26091-79-2	
C_ID	C00053053	
InChlKey		
InChICode		
SMILES		
Start Substs in Alk. Biosynthesis (Prediction)		
Organism	Kingdom Plantae Plantae	Fam Rutaceae Rutaceae



All			Group 1	: Ion (CS	) H13 O)	Group 2: Ion	(C16 H1	I4 O5)			
			General								
	ID	RT [min]	RI (iu)	Width [s]	Area	Height	Height [%]	Link	IM m/z	IM Ionization	El Pe
	165	38.62	2635	5.60	1699	1041	0.04		-	-	3
	166	38.64	2639	3.60	1851	1183	0.04		-		4
ł.	167	38.66	2643	6.00	1616	719	0.03		-	-	20
	168	38.68	2648	5.60	2116	1036	0.04		-	-	3
ł.	169	38.71	2655	5.20	1338	866	0.03		-	-	4
ī	170	38.74	2660	4.80	12024	7226	0.26	$\checkmark$	316.09362	SI	23
Ī	171	38.77	2666	5.60	1616	1044	0.04				5
I	172	38.81	2674	3.60	1444	881	0.03		-	-	4
	173	38.83	2679	4.00	1971	1128	0.04		-		4
	174	38.85	2683	6.40	2078	1052	0.04		-	-	15
	175	38.87	2687	4.00	1389	953	0.03		-		9
	176	38.89	2693	5.60	1869	892	0.03		-	-	8
	177	38.91	2696	4.40	2690	1453	0.05	$\checkmark$	316.09418	SI	3
			< 7700	5.00	2000		0.01	-	200 4 60 47	0	
			_								
Analysis Method			Peppermint_oil						Edit		



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# 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.

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### 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

- configuration

### **1D GC**

• Shorter run times and simpler hardware

• 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: El 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.



