

# Comparing GCxGC-FID and GC-MS for Purity Checks of Fragrance Allergen Reference Materials

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# 7<sup>th</sup> Amendment of the European Cosmetics Directive

- Certain substances are contact allergens for fragrance-sensitive people
  - 26 substances
  - 24 can be gas chromatographed
- Allergens must be in product list of ingredients if they exceed certain levels
  - 0.001% in leave-on products
  - 0.01% in rinse-off products

Allergen Name		Allergen Name
Amyl cinnamal		Lyrall
Benzyl alcohol		Anise alcohol
Cinnamyl alcohol		Benzyl cinnamate
Citral		Farnesol
Hydroxycitronellal		Lilial
Eugenol		Linalool
Isoeugenol		Benzyl benzoate
Amylcinnamyl alcohol		Citronellol
Benzyl salicylate		Hexyl cinnamal
Cinnamal		Limonene
Coumarin		Methyl 2-octynoate
Geraniol		Alpha-isomethyl ionone

# Need for Fragrance Allergen Analytical Reference Materials

- Calibration and quantification for products containing fragrances
  - Individuals available
  - But commercial mixes are wanted/needed
- Quality assurance purposes
  - “Second source” standards
  - Regulating/auditing bodies may not want in-house analysts preparing their own standards

# Challenges for Fragrance Allergen Analytical Reference Materials

- Hard to find materials meeting purity requirements
- Vendor specified purities often in error
  - Even when they claim GC or GC-MS on certificate of analysis!
- Comprehensive mixes are unstable
  - Aldehydes and ketones together acceptable
  - Non-carbonyl compounds in separate mix

# IFRA Allergen Standard Purity Requirements

- Almost all compounds:  $>95\%$
- Alpha-isomethyl ionone:  $>88\%$
- Isomers might be summed for purity calculations
- Method not clear as to how purity is determined
  - Presumed to be area% for this work
  - Does not necessarily specify GC-MS

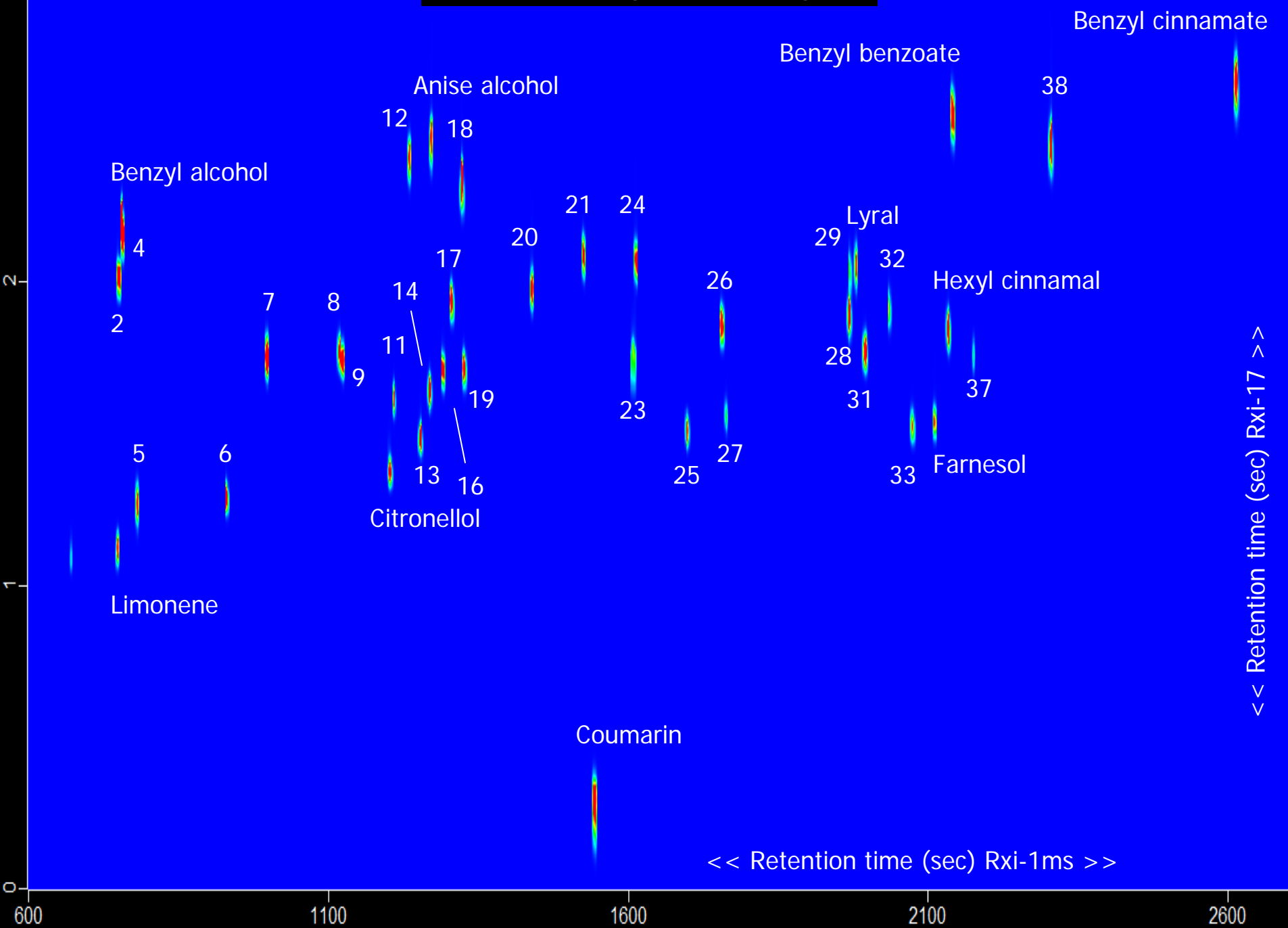
# GC-MS Conditions

- Split injection (~20 mg/mL standards)
- 30m x 0.25mm x 0.25 $\mu$ m Rxi-5ms
  - Constant linear velocity helium, 35 cm/sec
  - 50°C (2 min), 10°C/min to 300°C (5 min)
- Quadrupole mass spectrometer
  - Electron ionization at 70 eV
  - 40 to 400 m/z

# GCxGC-FID

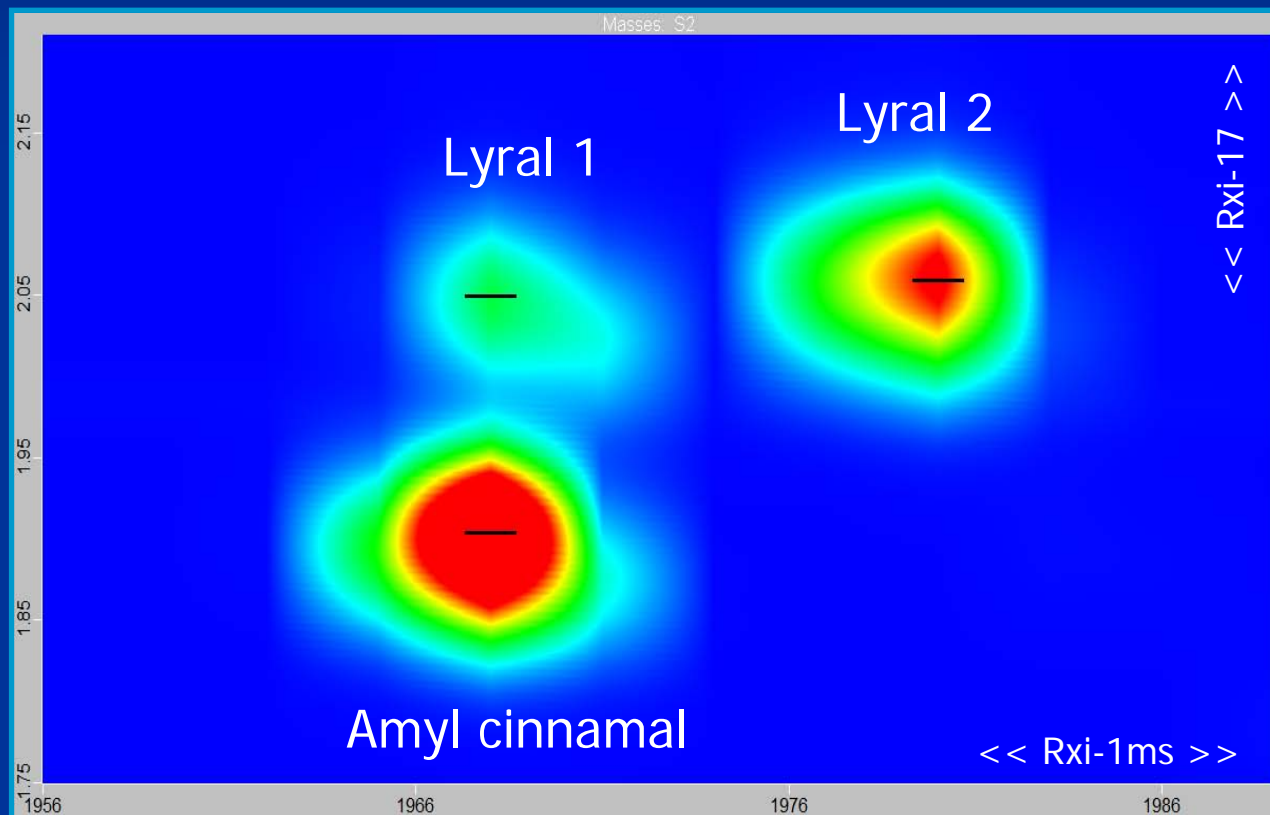
- 30m x 0.25mm x 0.25 $\mu$ m Rxi-1ms
  - 100% dimethyl polysiloxane
- 40°C (1min), 4°C/min to 240°C
- Thermal modulation, 3 sec
- 1m x 0.10mm x 0.10 $\mu$ m Rxi-17
  - 50% diphenyl / 50% dimethyl polysiloxane
- 45°C (1min), 4°C/min to 245°C

# GCxGC of Fragrance Allergens



**Table 1.** GC conditions

Column	Oven program	Time (min)	Initial gas velocity (cm/sec)	Co-elutions
DB1, 60m × 0.25mm × 0.25µm	100°-2min; 10°/min; 280°C	25	50	Amylcinnamic ald./1st peak of HMPCC*



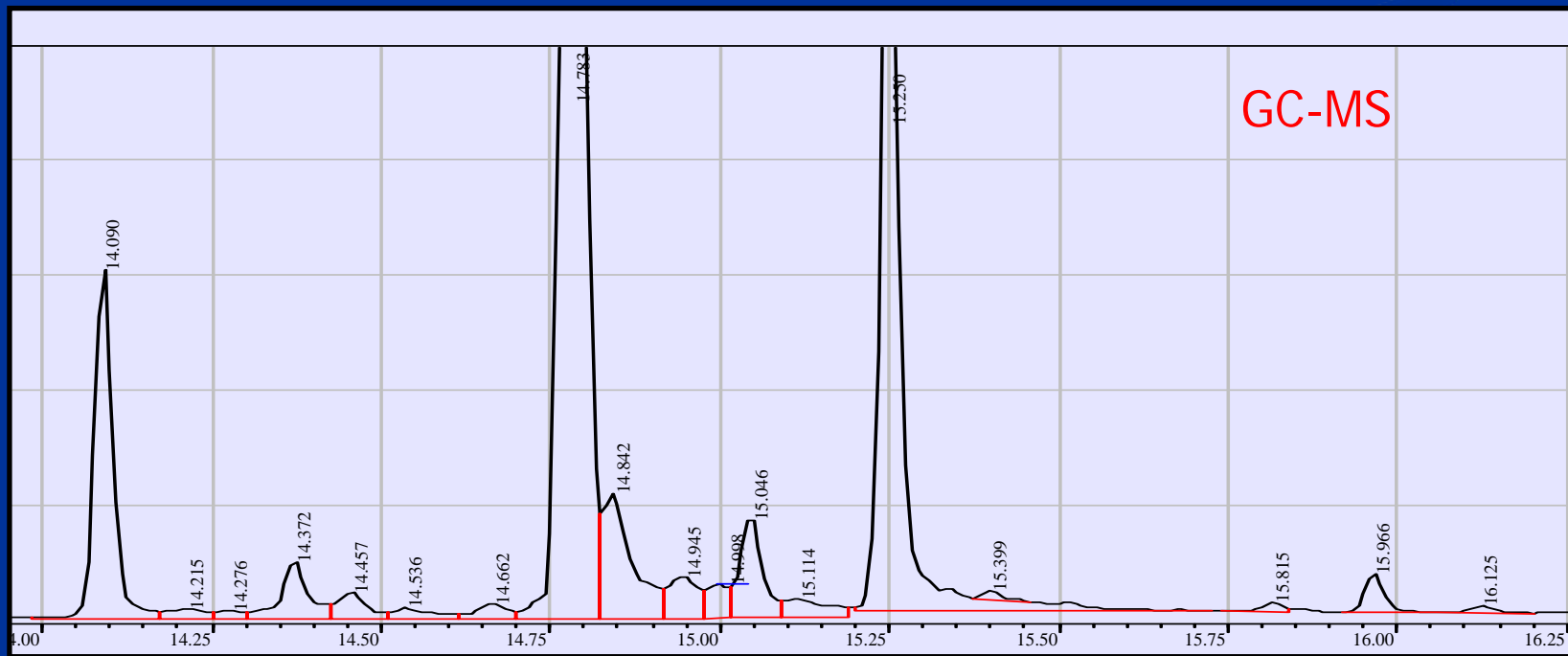
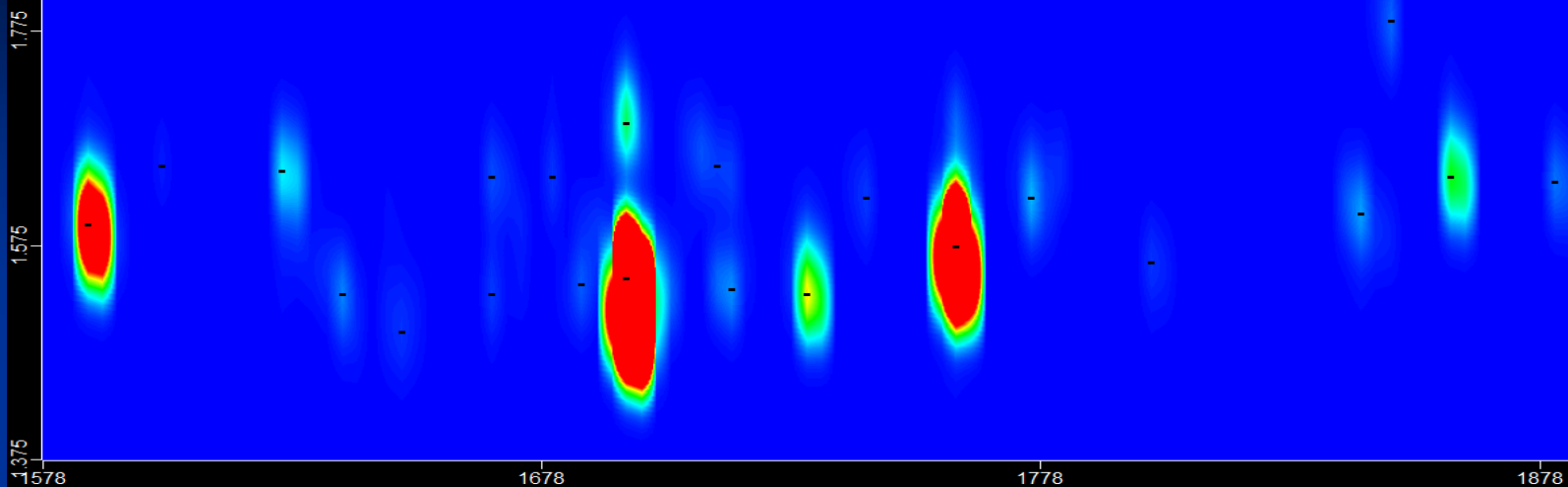
↑  
Lyrals

Allergen Name	GCxGC-FID	GC-MS
Amyl cinnamal	98.2	97.1
Benzyl alcohol	100	100
Cinnamyl alcohol	98.5	98.8
<b>Citral</b>	<b>93.0</b>	98.4
<b>Hydroxycitronellal</b>	<b>41.2</b>	<b>92.3</b>
Eugenol	100	99.5
Isoeugenol	98.8	98.4
<b>Amylcinnamyl alcohol</b>	<b>68.9</b>	<b>74.3</b>
Benzyl salicylate	99.8	100
<b>Cinnamal</b>	<b>85.0</b>	<b>87.5</b>
Coumarin	100	99.9
Geraniol	96.8	97.0

Allergen Name	GCxGC-FID	GC-MS
Lyrar	98.5	98.0
Anise alcohol	99.7	99.4
Benzyl cinnamate	99.9	99.7
Farnesol	95.7	95.1
<b>Lilial</b>	<b>94.1</b>	96.4
Linalool	97.5	97.5
Benzyl benzoate	99.8	100
Citronellol	96.4	97.5
<b>Hexyl cinnamal</b>	95.7	<b>94.4</b>
Limonene	99.7	100
Methyl 2-octynoate	99.2	99.1
<b>Alpha-isomethyl ionone</b>	<b>59.2</b>	<b>58.1</b>

# Alpha-isomethyl ionone

GCxGC-FID

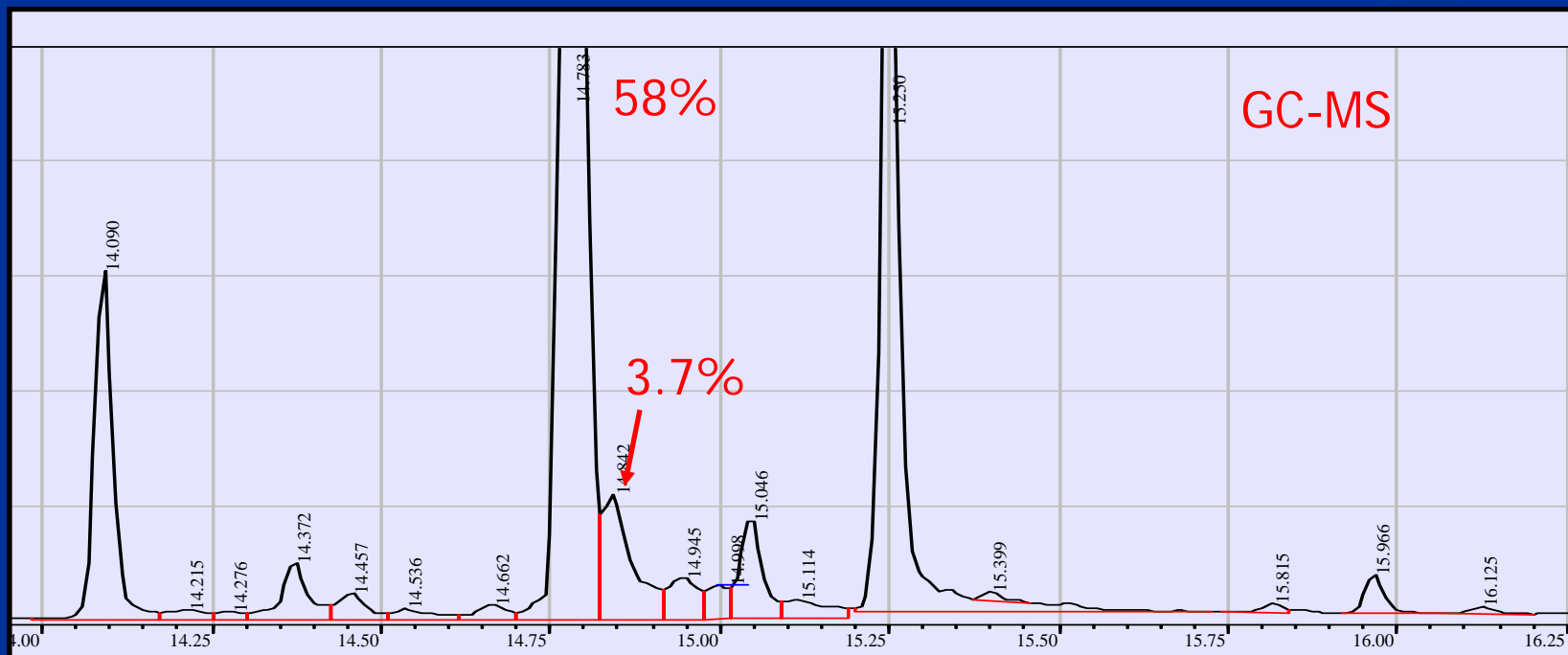
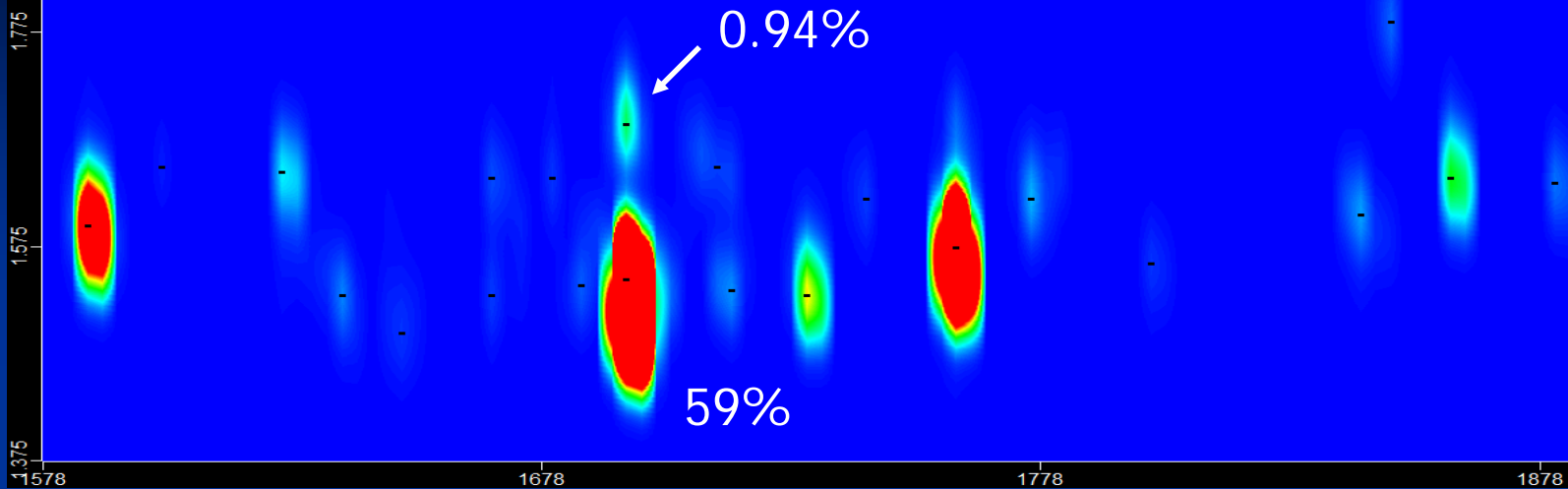


RT (min)	Area%		RT1 (sec)	RT2 (sec)	Area%
14.09	7.3		1587	1.60	7.2
14.22	0.39		1602	1.65	0.052
14.28	0.24				
14.37	1.7		1626	1.65	0.86
14.46	0.78		1638	1.53	0.40
14.54	0.43		1650	1.50	0.28
			1668	1.53	0.10
14.66	0.49		1668	1.64	0.31
			1680	1.64	0.18
			1686	1.54	0.20
14.78	58		1695	1.55	59
14.84	3.7		1695	1.69	0.94

RT (min)	Area%		RT1 (sec)	RT2 (sec)	Area%
14.09	7.3		1587	1.60	7.2
14.22	0.39		1602	1.65	0.052
14.28	0.24				
14.37	1.7		1626	1.65	0.86
14.46	0.78		1638	1.53	0.40
14.54	0.43		1650	1.50	0.28
			1668	1.53	0.10
14.66	0.49		1668	1.64	0.31
			1680	1.64	0.18
			1686	1.54	0.20
14.78	58		1695	1.55	59
14.84	3.7		1695	1.69	0.94

# Alpha-isomethyl ionone

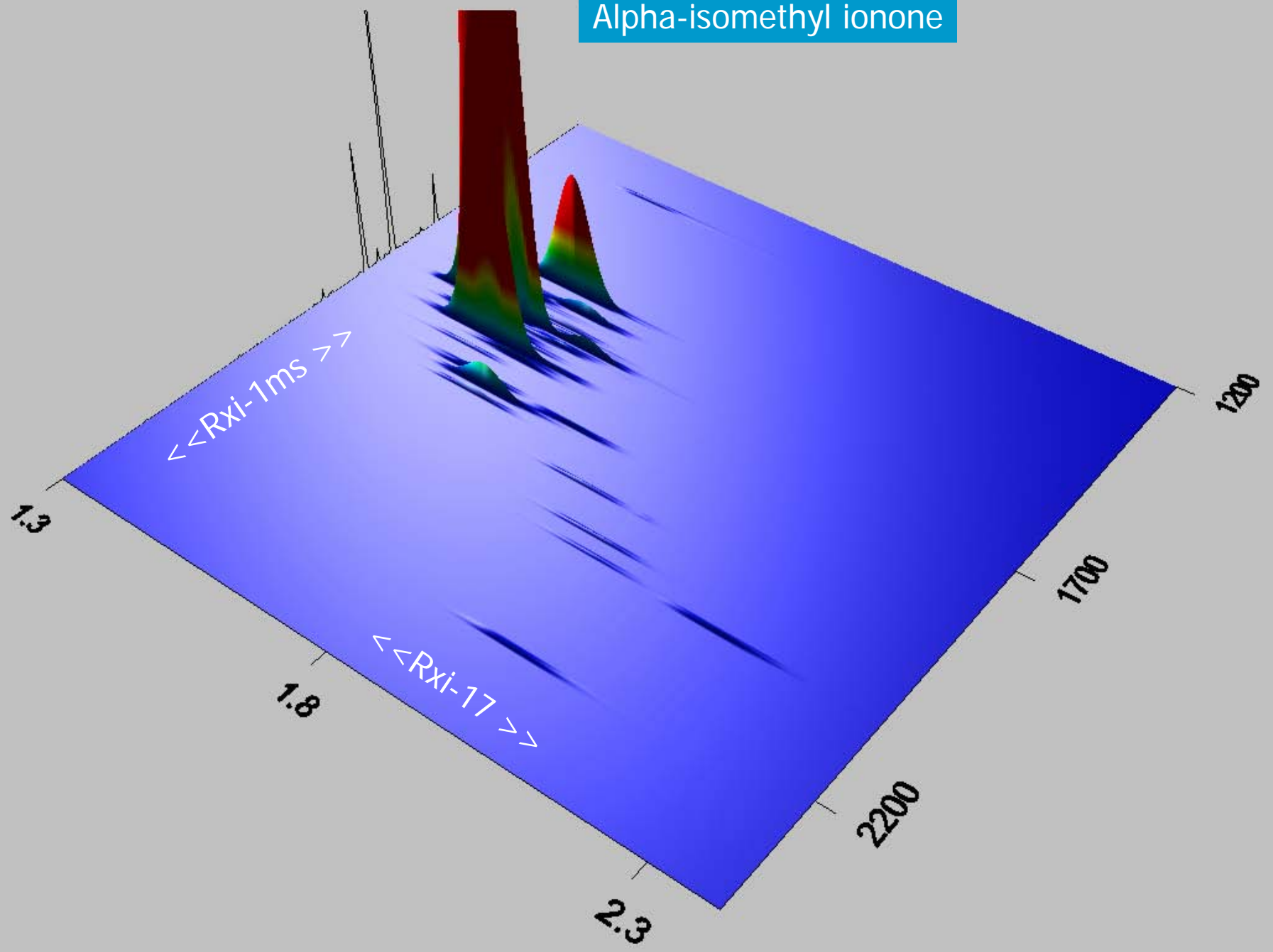
GCxGC-FID



# GCxGC-FID Repeatability

- Alpha-isomethyl ionone
  - Has wide range of area percents
- Three vials analyzed
  - Autosampler injections, 0.2 $\mu$ L
  - Triplicate injections of each, back-to-back
- All data processing done automatically through GCxGC software
  - No manual review or integration adjustments

Alpha-isomethyl ionone

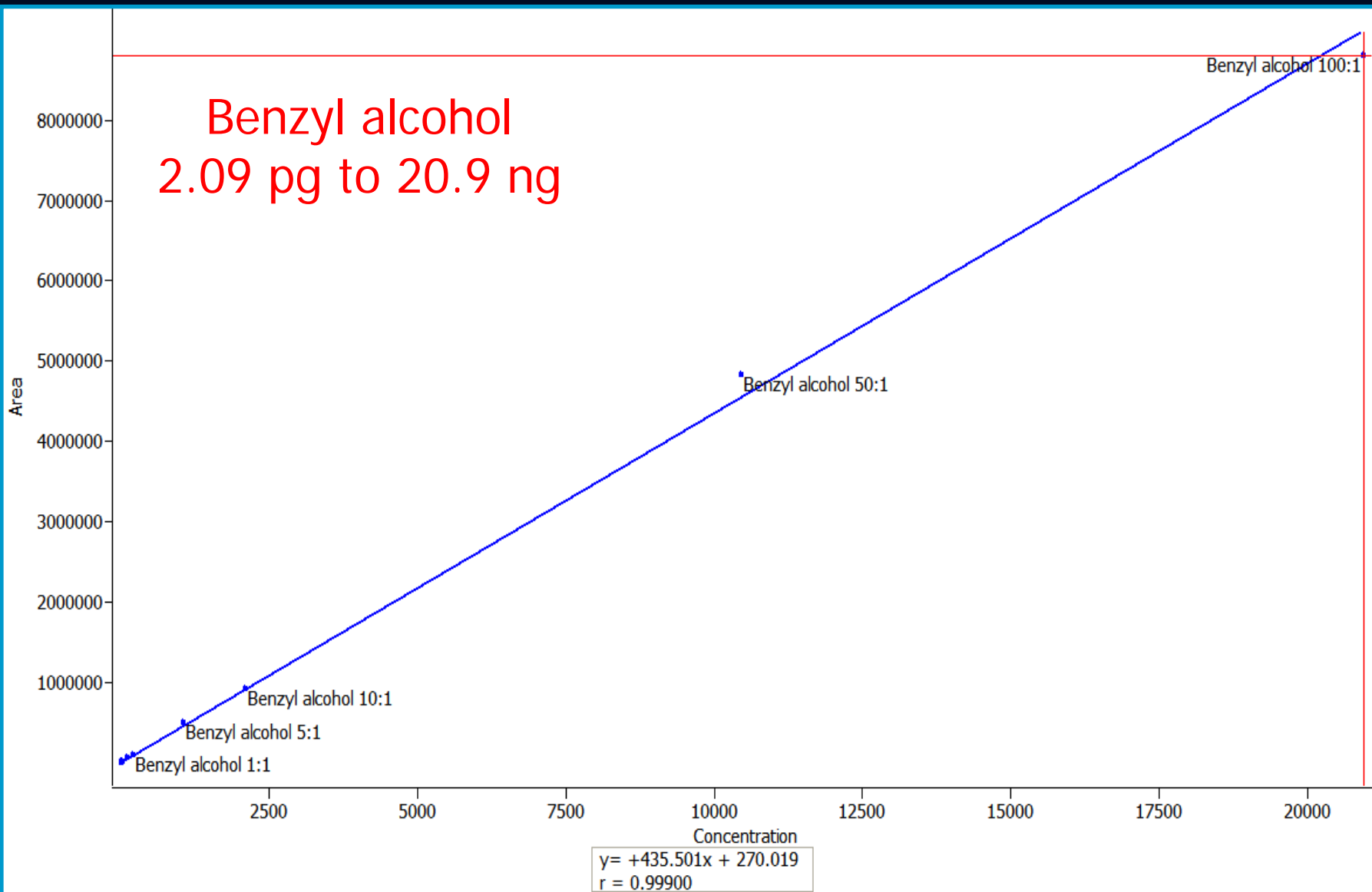


RT1 (sec)	RT2 (sec)	Area% Avg	RSD%	ng OC
1695	1.55	59	0.71	11
1761	1.57	23	0.51	4.2
1587	1.59	7.2	0.45	1.3
1860	1.63	1.6	0.64	0.29
1626	1.64	0.94	3.4	0.17
1695	1.69	0.90	0.57	0.17
1800	1.55	0.11	3.3	0.020
1668	1.53	0.11	41	0.020
1311	1.54	0.098	4.1	0.018
2040	1.97	0.081	27	0.015

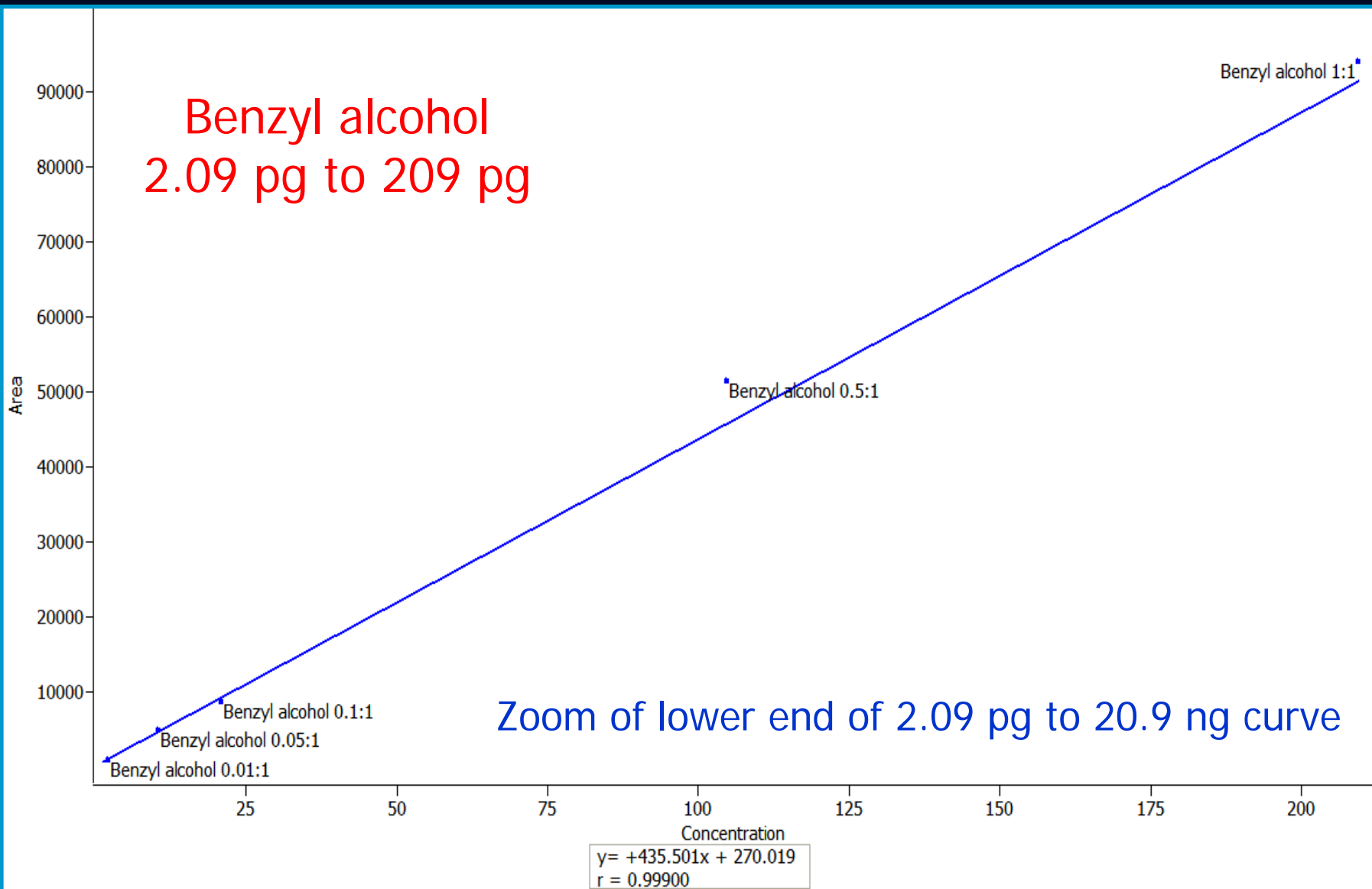
# GCxGC-FID Linearity

- Benzyl alcohol
  - 100% purity
  - 20.94 mg/mL stock
- 2.09 pg to 20.9 ng on column
  - Four orders-of-magnitude
- Calibration curve
  - External standard
  - 1/conc weighting

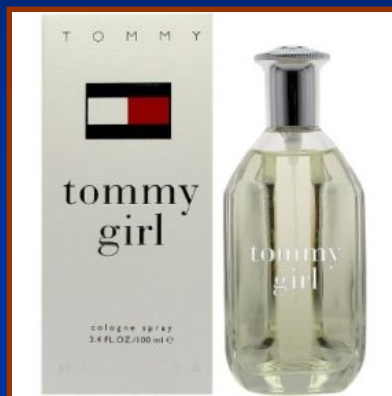
**Benzyl alcohol**  
**2.09 pg to 20.9 ng**



**Benzyl alcohol**  
**2.09 pg to 209 pg**

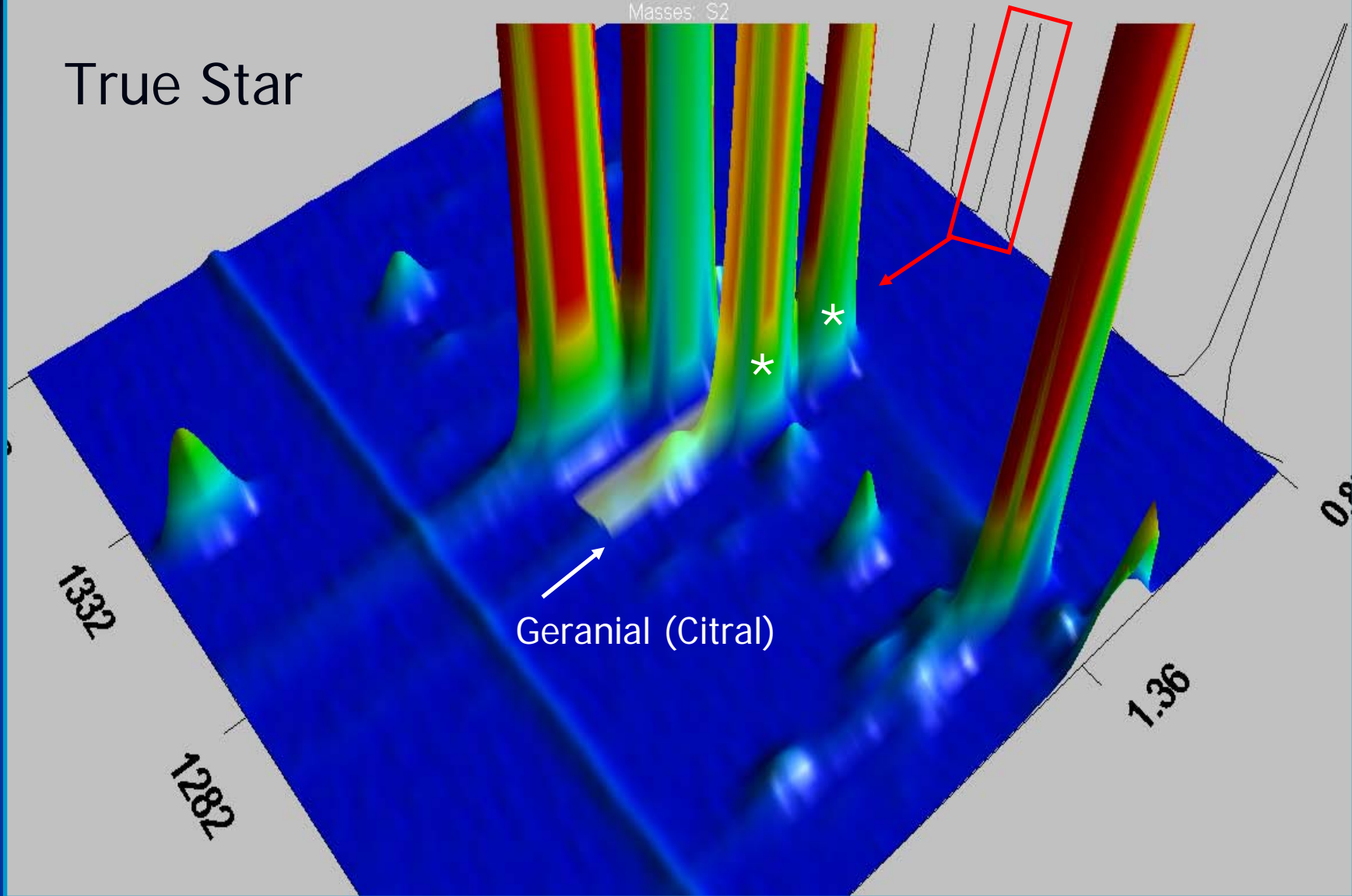


# GCxGC-FID of Perfumes



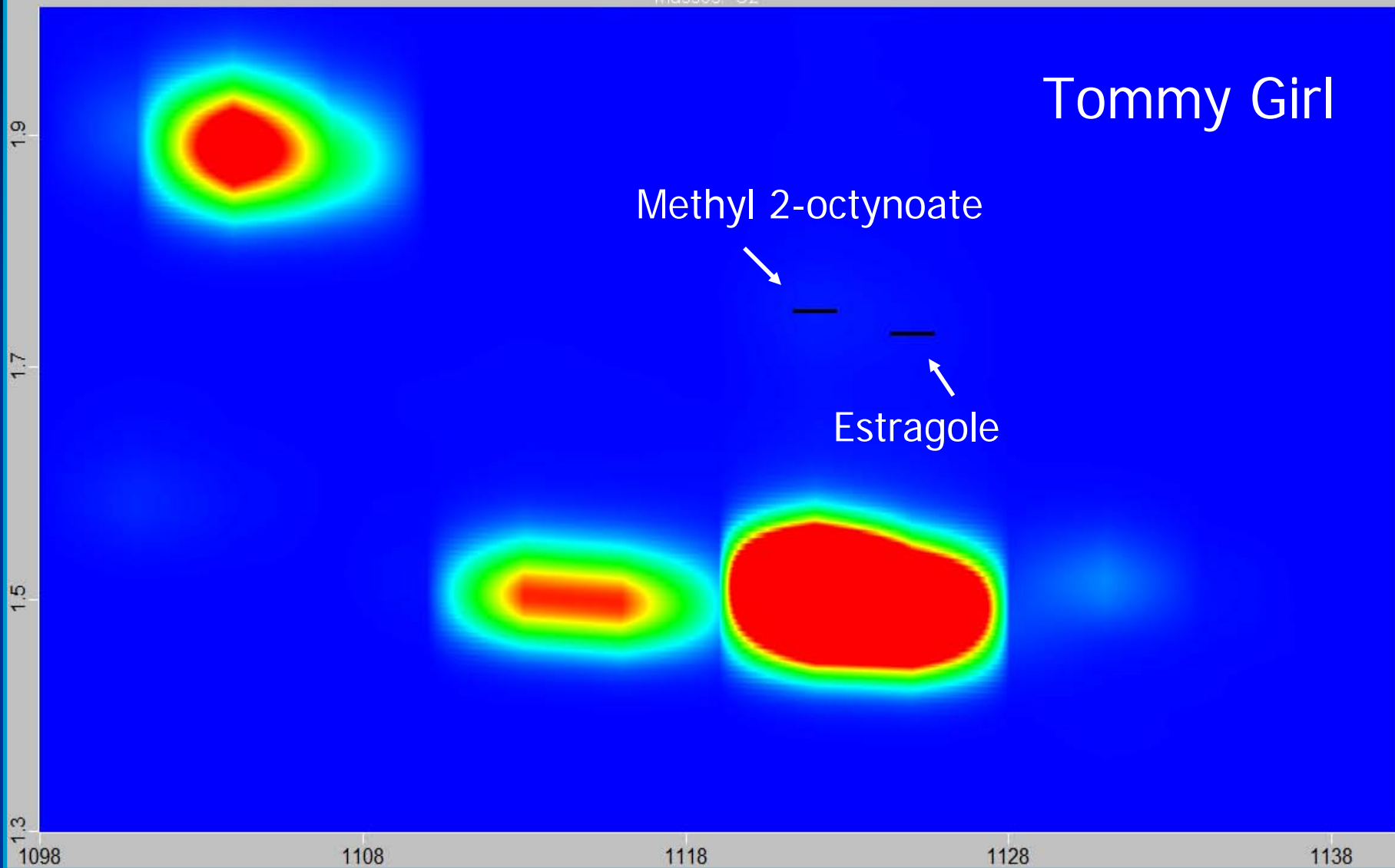
Masses: S2

True Star



Masses: S2

Tommy Girl



Masses: S2

Methyl 2-octynoate

Tommy Girl

1.795

1.695

1.595

—

—

Estragole

1119

1121

1123

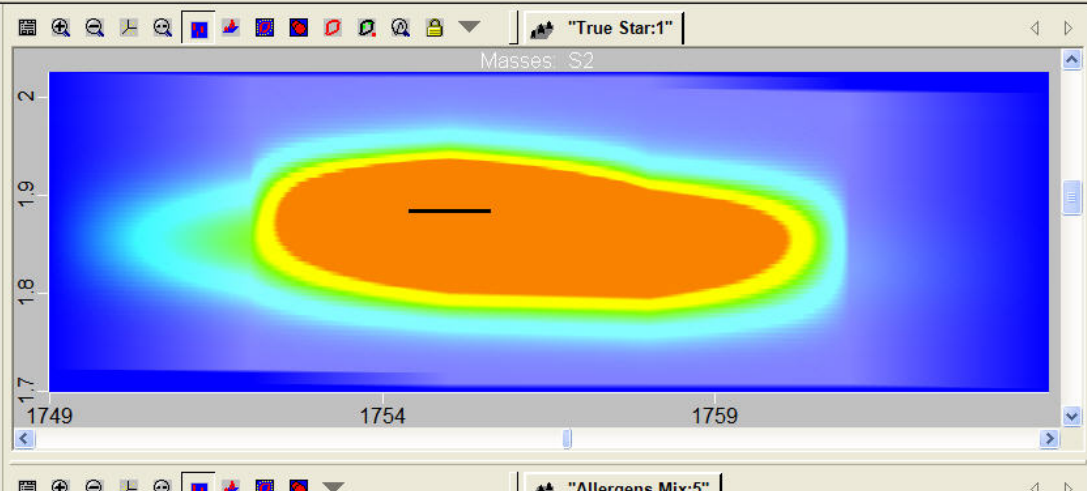
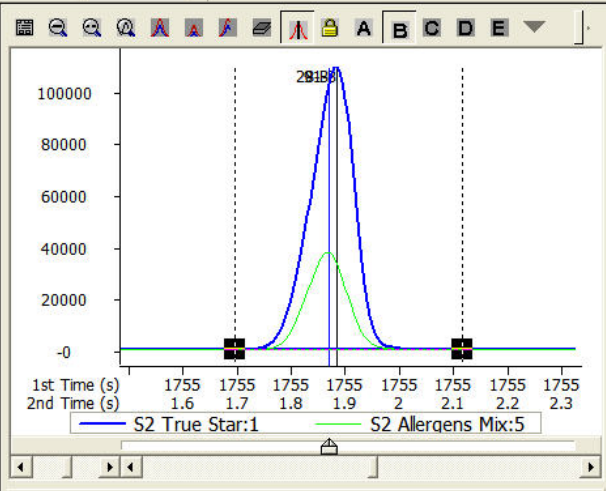
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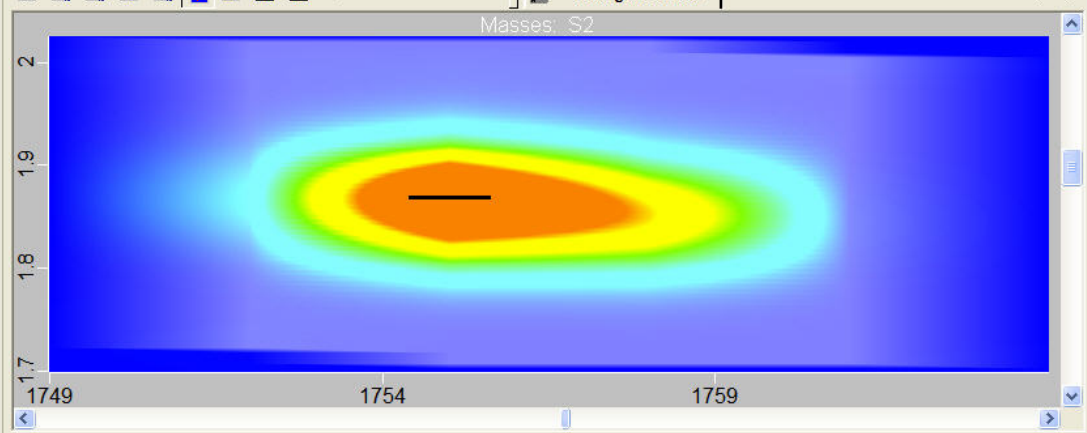
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- Allergens Mix:5
- True Star:1
- Light Blue:1
- Tommy Girl:1
- Curve:1
- Romance:1
- Lovely:1
- Lovely:2

Peak Table (1)

Peak #	1st Dimension	2nd Dimension	Quantification	Area %
33	1995	1.780	Amylcinnamyl alcohol	2.9187
32	1980	2.060	Lyril 2	2.2079
108	1980	2.055	Lyril 2	4.8985
31	1968	2.050	Lyril 1	0.91142
106	1971	2.010	Amyl cinnamal	37.371
105	1968	2.050	Lyril 1	1.2429
30	1968	1.905	Amyl cinnamal	3.4570
29	1764	1.560	Alpha-isomethylionon	0.70911
93	1764	1.545	Alpha-isomethylionon	0.56858
91	1755	1.885	Lilial	16.339
28*	1755	1.870	Lilial	2.8551
27	1698	1.515	Alpha-isomethyl iono	1.8858
84	1698	1.515	Alpha-isomethyl iono	9.7882
26	1614	2.075	Isoeugenol	2.5413
			Isoeugenol	



X=1752, 1.950  
Y=1362  
Workstation



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1st Time (s) 783 783 783 783 783 783  
 2nd Time (s) 0.9 1 1.1 1.2 1.3 1.4 1.5 1.6

— S2 True Star:1 — S2 Allergens Mix:5

6-B 16-3

Peak Table (1)

Peak #	1st Dimension	2nd Dimension	Quantification	Area %
38	1209	1.605	Citral 1	0.012799
37	1203	1.370	Citronellol	3.5015
11	1203	1.380	Citronellol	1.8618
			Estragole	
10	1125	1.745	Estragole	1.6206
9	1119	1.775	Methyl-2-octynoate	3.2351
			Methyl-2-octynoate	
			Camphor	
8	999	1.760	Camphor	3.7006
7	930	1.295	Linalool	2.1625
25	930	1.305	Linalool	12.321
			Eucalyptol	
6*	783	1.275	Eucalyptol	2.4668
12	759	2.085	Phenyl acetaldehyde	0.0043071
5	759	2.165	Phenyl acetaldehyde	2.5837

Masses: S2  
1.42  
1.32  
1.22  
1.12  
774 779 784

Masses: S2  
1.42  
1.32  
1.22  
1.12  
774 779 784

X=777.1350  
Y=1054  
Workstation



# Perfume Analysis - Lovely

Allergen	GCxGC-FID	GC-MS
Linalool	0.71	0.84
Geraniol	0.76	3.0
Hydroxycitronellal	0.53	0.63
Alpha-isomethyl ionone	0.017	ND
Lilial	8.5	7.4
<b>Amyl cinnamal</b>	<b>8.0</b>	<b>ND</b>
Hexyl cinnamal	0.85	0.66
Benzyl benzoate	0.017	0.020
Benzyl salicylate	8.3	6.6

Values in area%

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Calibrations

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60000  
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40000  
30000  
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1st Time (s) 1.6 1.7 1.8 1.9 2 2.1  
2nd Time (s)

S2 Lovely:1 S2 Allergens Mix:5

Peak Table (1)

Peak #	1st Dimen	2nd Dimer	Quantification	Area %	Area	Quant Mas	Cc
			Isoeugenol			S2	
26	1614	2.075	Isoeugenol	2.5413	910828	S2	10
27	1698	1.515	Alpha-isomethyl ionone 1	1.8858	675894	S2	10
41	1695	1.500	Alpha-isomethyl ionone 1	0.061990	6035.0	S2	0.
43	1752	1.885	Lilial	30.718	2990514	S2	27
28	1755	1.870	Lilial	2.8551	1023293	S2	10
29	1764	1.560	Alpha-isomethylionone 2	0.70911	254151	S2	10
45	1761	1.550	Alpha-isomethylionone 2	0.029805	2901.6	S2	1.
			Lyril 1			S2	
30*	1968	1.905	Amyl cinnamal	3.4570	1239026	S2	10
54	1968	1.935	Amyl cinnamal	28.865	2810123	S2	22
31	1968	2.050	Lyril 1	0.91142	326661	S2	10
32	1980	2.060	Lyril 2	2.2079	791328	S2	10
			Lyril 2			S2	
			Amylcinnamyl alcohol 1			S2	

"Lovely:1" Masses: S2

2.02  
1.92  
1.82  
1.72

1959 1964 1969 1974

"Allergens Mix:5" Masses: S2

2.02  
1.92  
1.82  
1.72

1959 1964 1969 1974

X=1959, 1.995  
Y=74  
Workstation

Amyl cinnamal?

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GCxGC Wine  
GCxGC POP:  
Calibrations

30-54-B 31-B

1st Time (s) 1.6 1.7 1.8 1.9 2.0 2.1

2nd Time (s)

S2 Lovely:1 S2 Allergens Mix:5

Peak Table (1)

Peak #	1st Dimen	2nd Dimer	Quantification	Area %	Area	Quant Mas	Co
			Isoeugenol			S2	
26	1614	2.075	Isoeugenol	2.5413	910828	S2	10
27	1698	1.515	Alpha-isomethyl ionone 1	1.8858	675894	S2	10
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29	1764	1.560	Alpha-isomethylionone 2	0.70911	254151	S2	10
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			Lyril 1			S2	
30*	1968	1.905	Amyl cinnamal	3.4570	1239026	S2	10
54	1968	1.935	Amyl cinnamal	28.865	2810123	S2	22
31	1968	2.050	Lyril 1	0.91142	326661	S2	10
32	1980	2.060	Lyril 2	2.2079	791328	S2	10
			Lyril 2			S2	
			Amylcinnamyl alcohol 1			S2	

Methyldihydrojasmonate

Masses: S2

1.72 1.92 2.02

1959 1964 1969 1974

"Lovely:1"

"Allergens Mix:5"

Masses: S2

1.72 1.92 2.02

1959 1964 1969 1974

X=1959, 1.995 Y=74 Workstation

For Help, press F1, 3/22/2008 1:32:05 PM: Structured Exception - EXCEPTION\_FLT\_INVALID\_OPERATION at address 006EC298.



# Perfume Analysis - Lovely

Allergen	Rxi-17	Stabilwax	GC-MS
Linalool	0.71	1.1	0.84
Geraniol	0.76	0.73	3.0
Hydroxycitronellal	0.53	0.54	0.63
Alpha-isomethyl ionone	0.017	0.0077	ND
Lilial	8.5	8.3	7.4
<b>Amyl cinnamal</b>	<b>8.0</b>	<b>ND</b>	<b>ND</b>
Hexyl cinnamal	0.85	0.81	0.66
Benzyl benzoate	0.017	0.020	0.020
Benzyl salicylate	8.3	8.4	6.6

Values in area%

True Star

0  
1  
2

699 1199 1699 2199 2699

<< Rxi-1ms >>

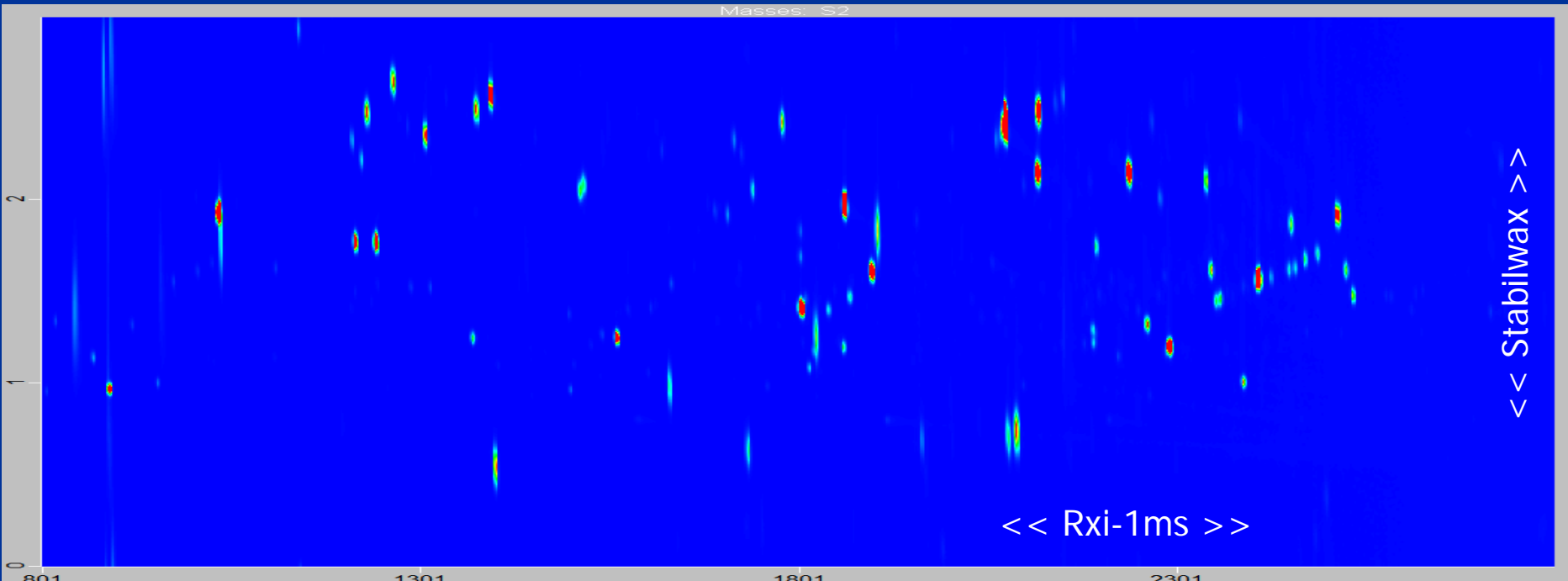
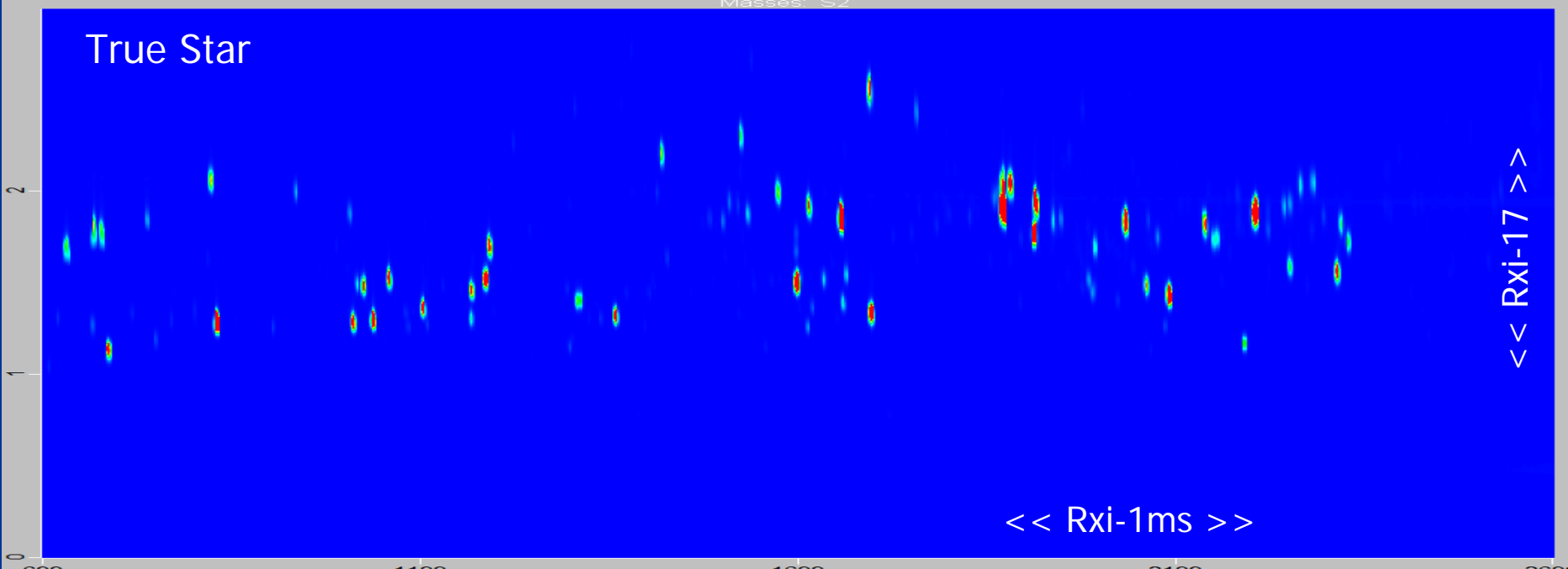
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1  
2

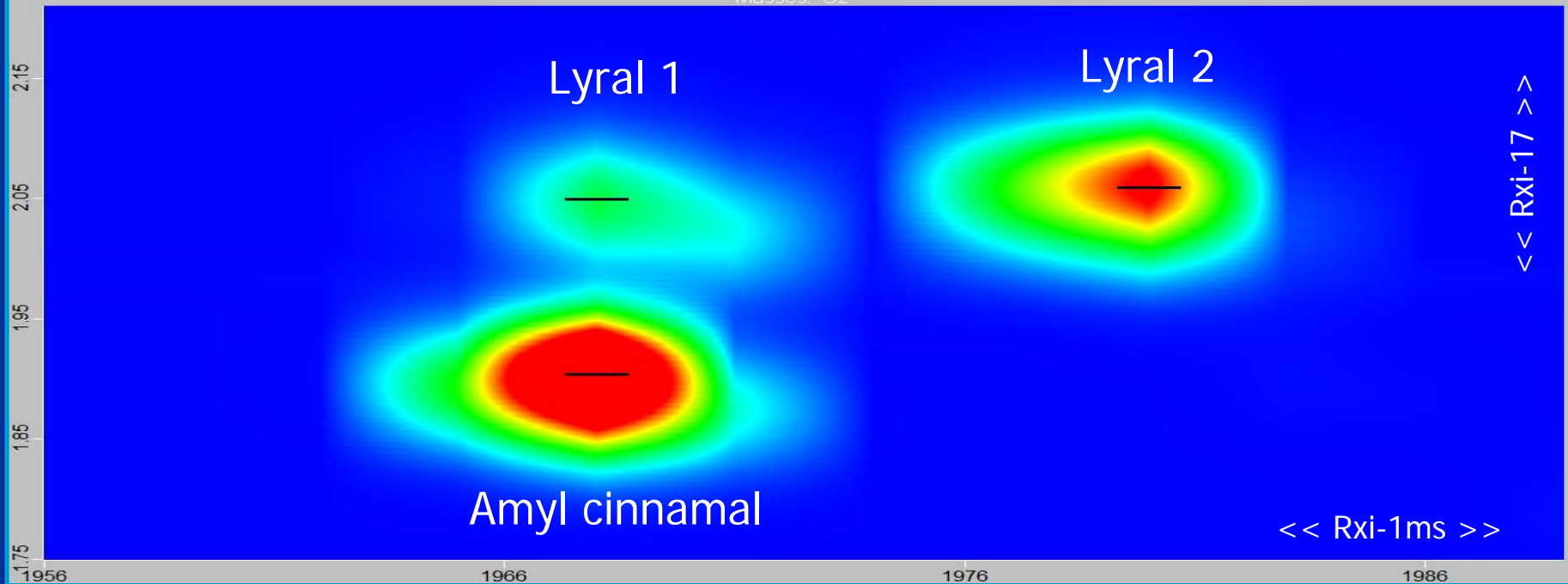
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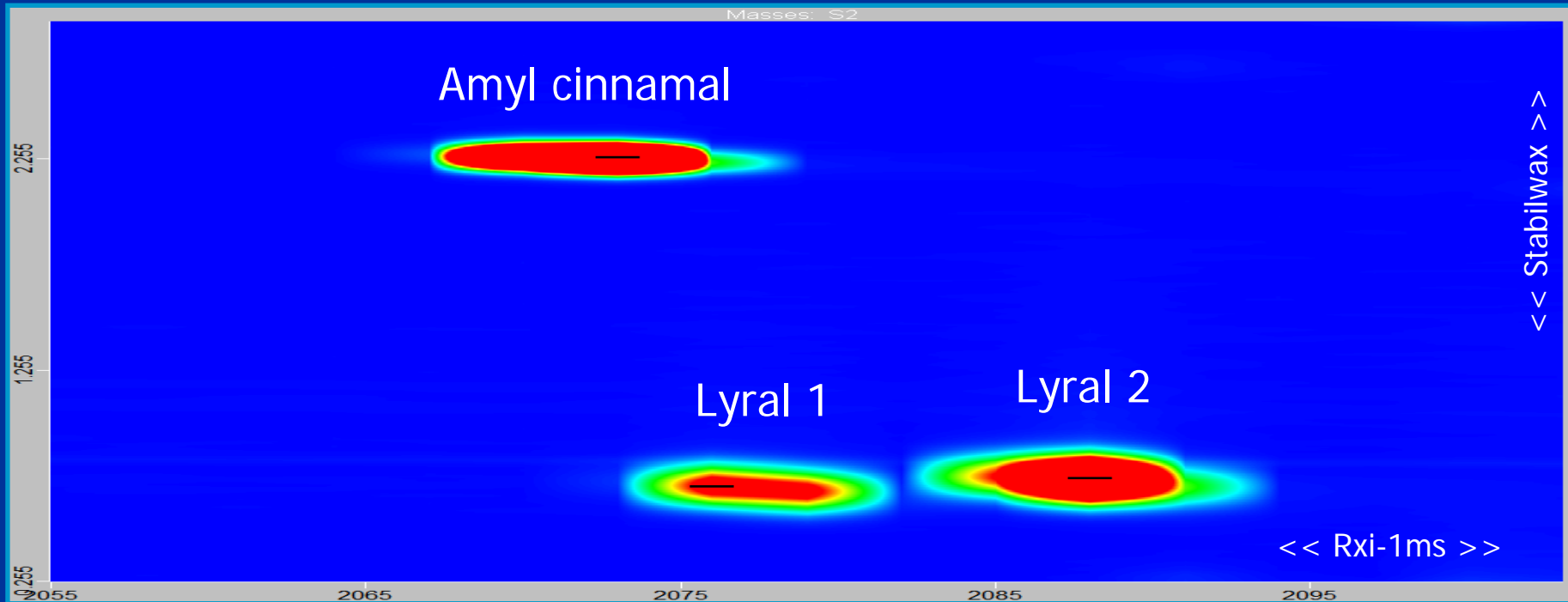
<< Stabilwax >>



Masses: S2

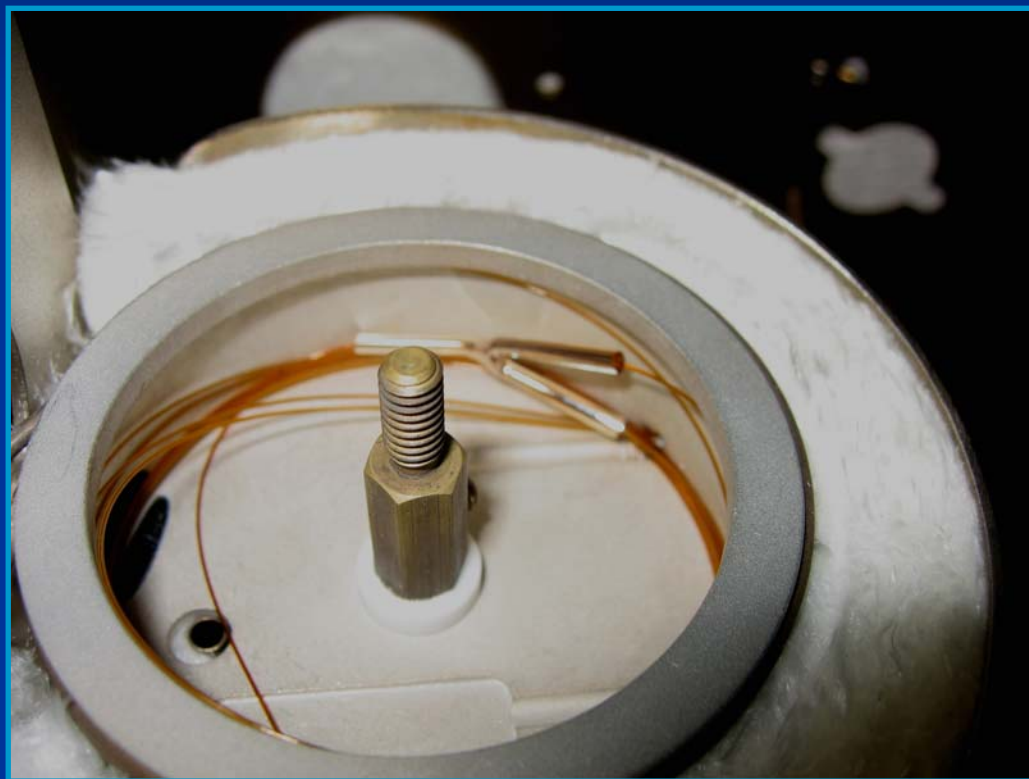
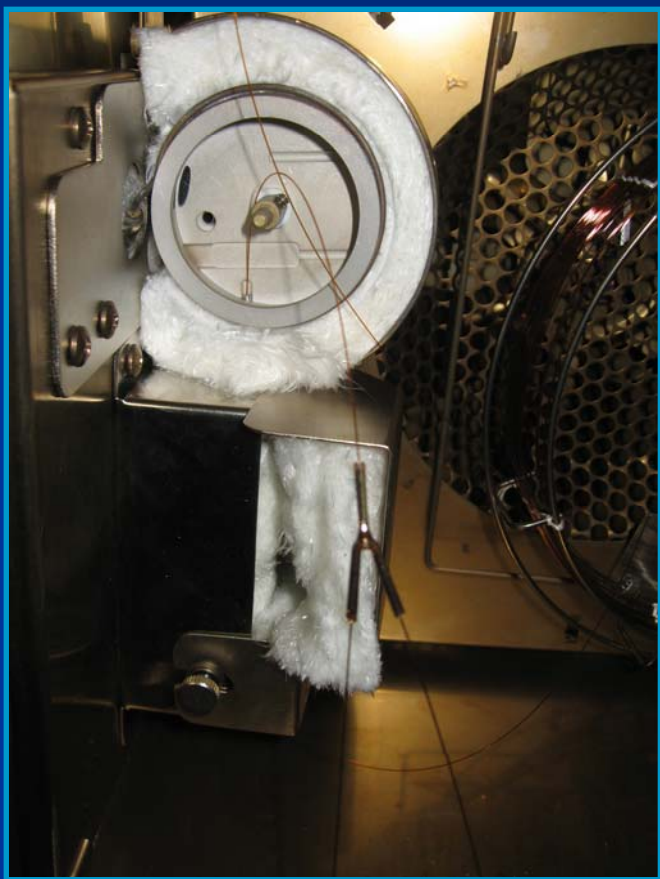


Masses: S2



# GC x Parallel Dual-Column GC-FID

Rxi-17 and Stabilwax secondary columns



# GC x Parallel Dual-Column GC-FID

- Use Gerstel MACH as primary column
- Modulate on 0.10mm uncoated fused silica
- Y split to two FIDS
  - Rxi-17 and Stabilwax
  - Use Agilent oven as secondary oven



# Acknowledgments

- LECO, for GCxGC-FID
- You, for your comprehensive attention
- The organizing committee for the opportunity to speak