Philip Marriott

A New Paradigm: Comprehensive two-dimensional GC (GC×GC) for High Resolution Volatile Chemical Analysis - *The Power & The Passion*

… beginning with ‘Cryogenics’ in GC

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Cryogenics & Gas Chromatography?

Hot (GC) & Cold (Cryo-device)

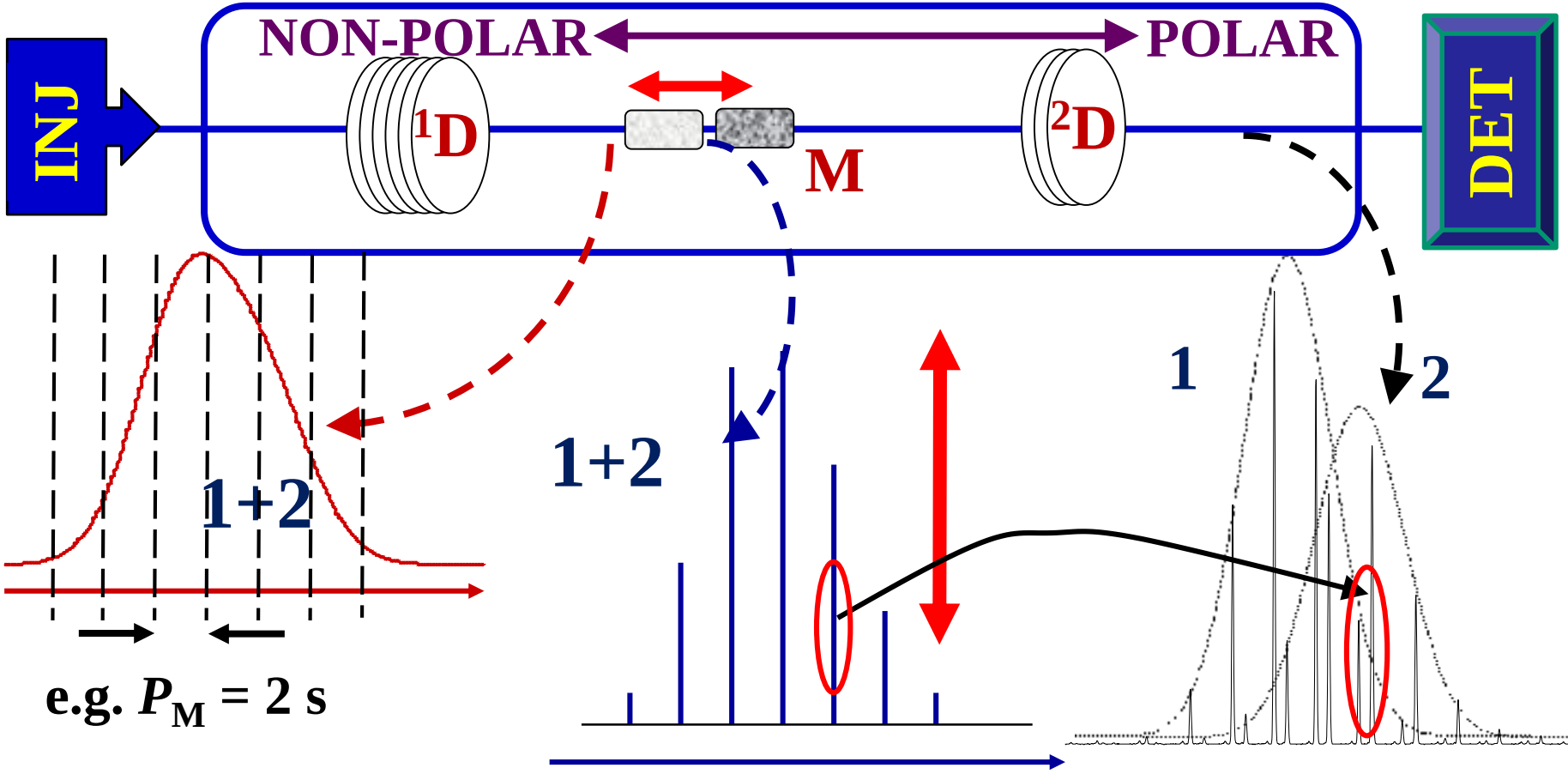
Yin & Yang

A perfect combination ??

GCxGC process ~ Tutorial

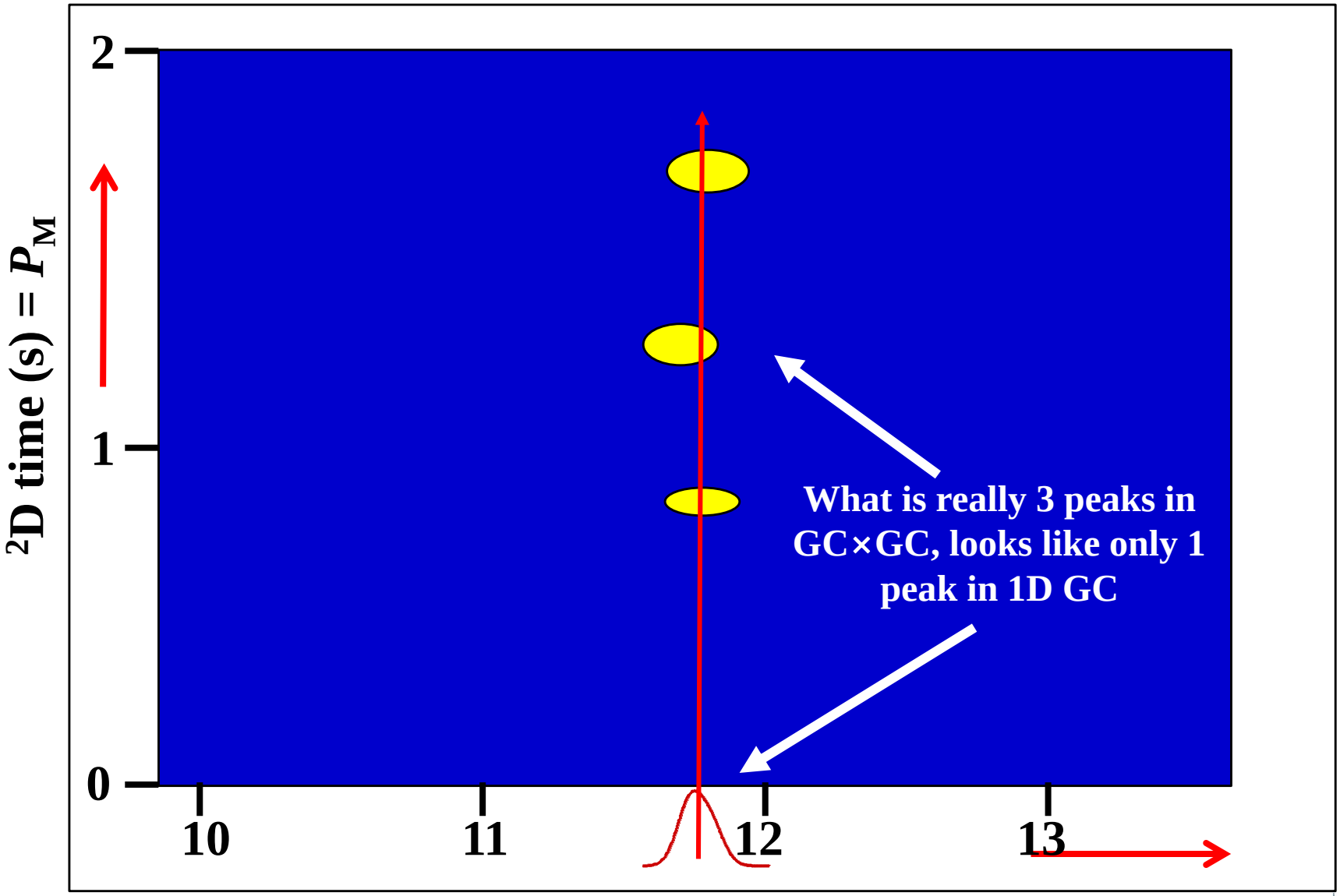
COMMENT..

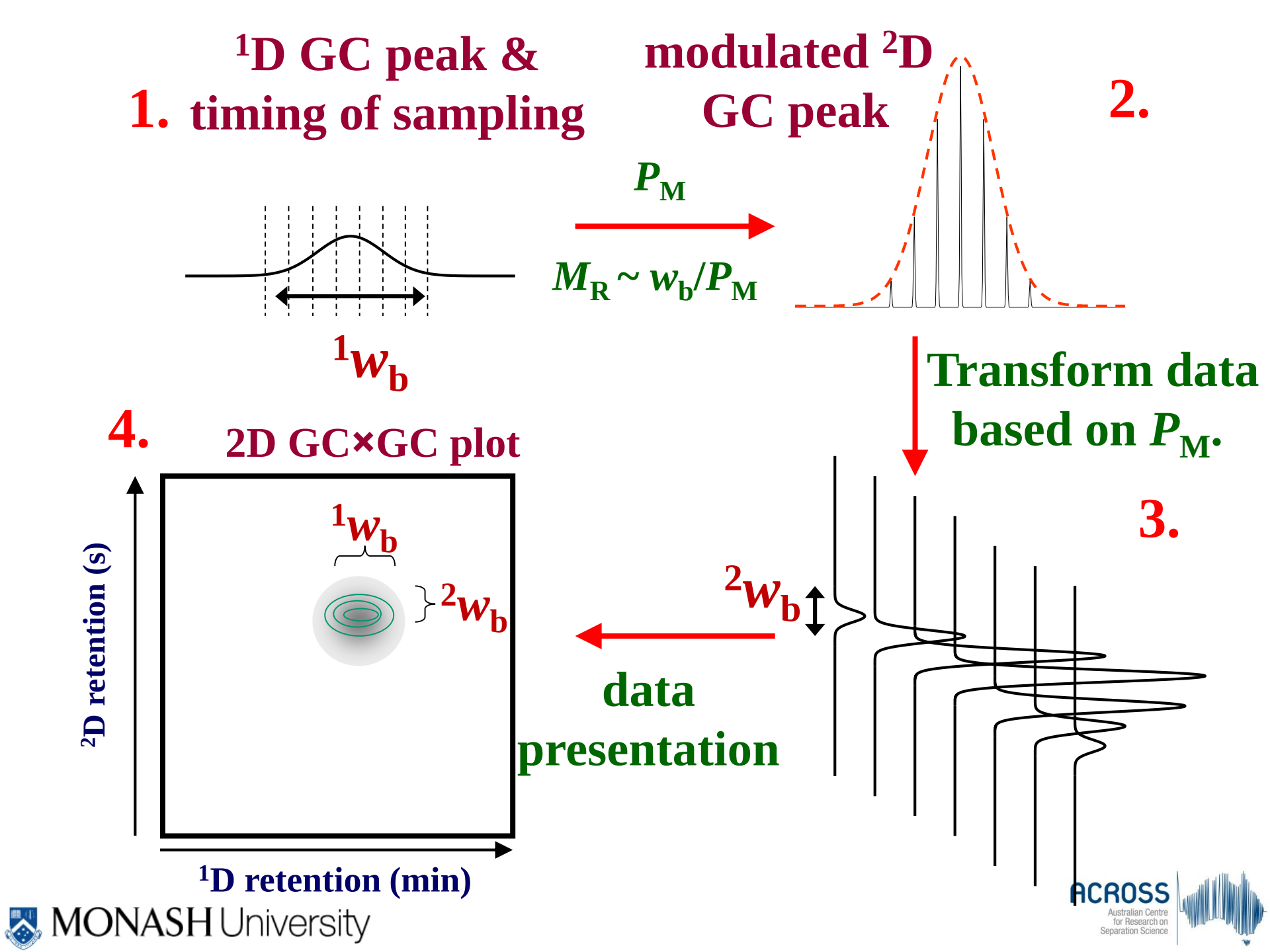
M – CRYOGENIC modulator
^{1}D, ^{2}D – first/second dimension cols
1 + 2 – overlapping compounds on ^{1}D



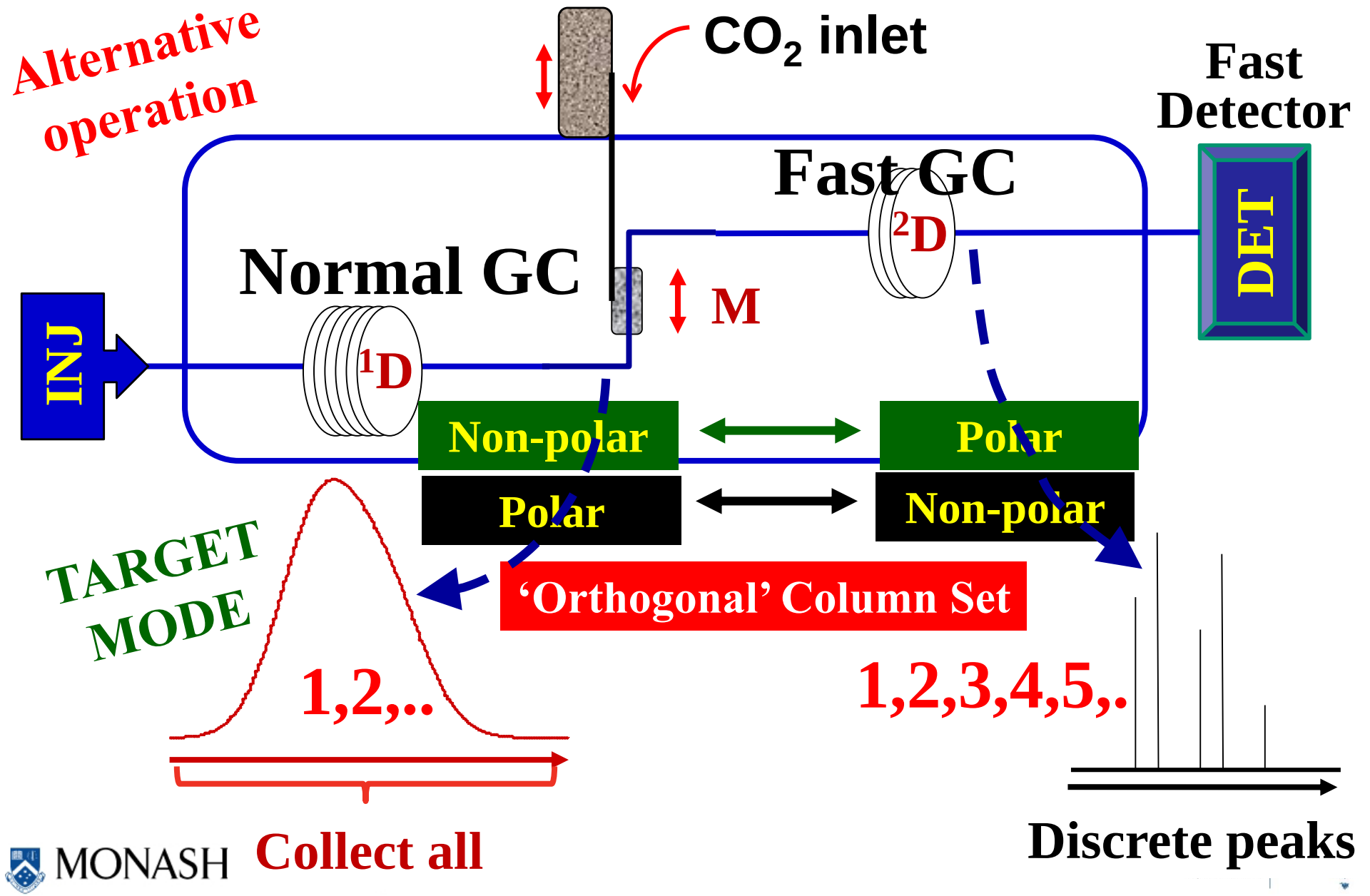
A peak at the end of the ^{1}D column may be unresolved. This is sliced (trapped or modulated); focussed as multiple narrow bands by back-&-forth cryo-modulator movement; these are introduced to a short ^{2}D column and further separation.

Present result as a 2D plot based on ^{1}D time (^1t_R) and ^{2}D time (^2t_R)




1D GC peak &
1. timing of sampling
modulated 2D
GC peak
2.
P_M
$M_R \sim w_b/P_M$
^1w_b
Transform data
based on P_M.
3.
4.
2D GC×GC plot
^1w_b
^2w_b
^2w_b
data
presentation
2D retention (s)
1D retention (min)

Longitudinally Modulated Cryogenic System: LMCS



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Movies of LMCS Operation

MODULATION DRIVE UNIT

CRYOTRAP MOVEMENT

REAL-TIME DATA ACQUISITION

– as seen on chromatography data system …

Advantages & Attributes of GC×GC

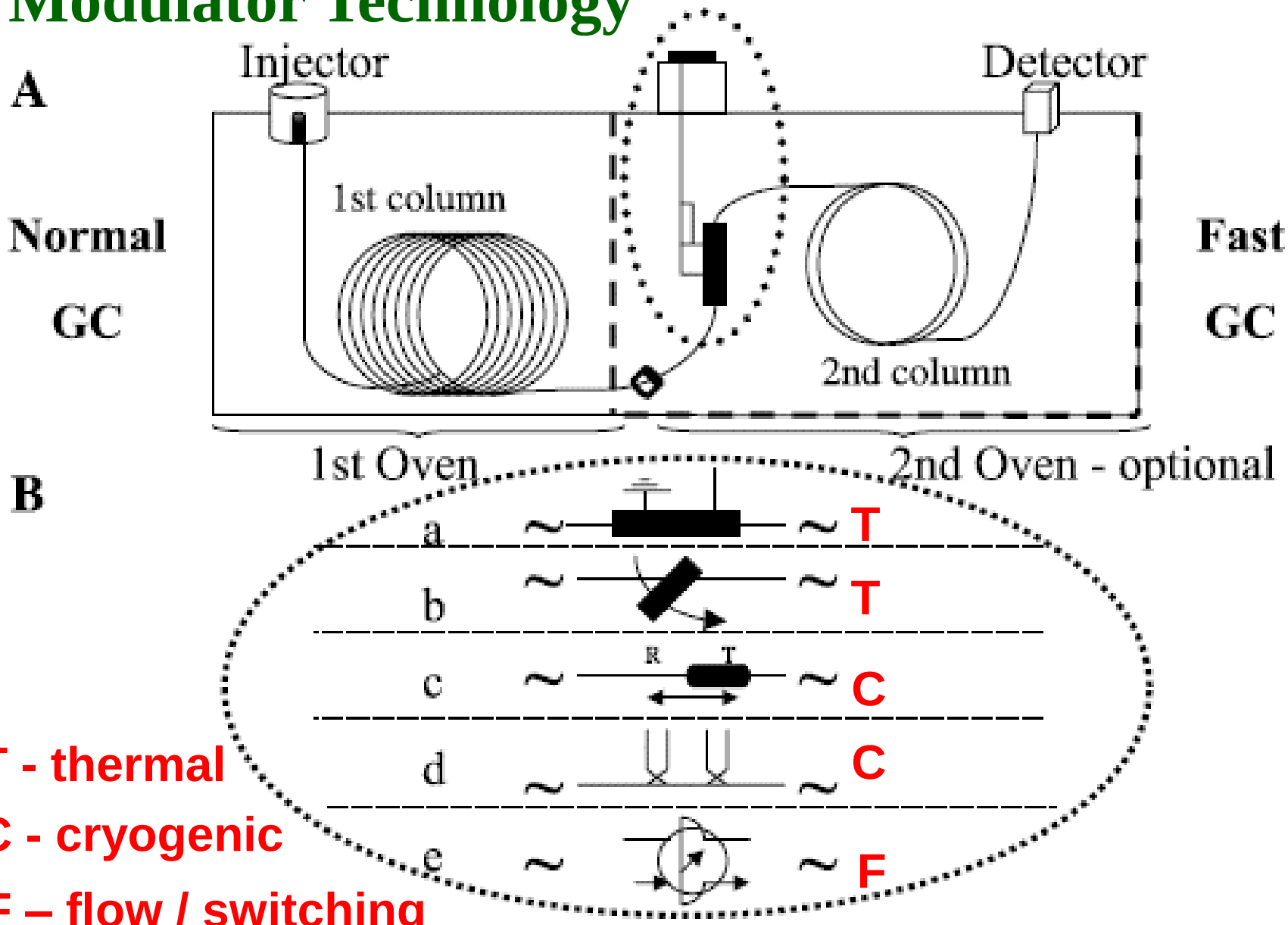
- GC×GC Uses 2 columns; 1st (^{1}D) = long, 2nd (^{2}D) = short
- GC×GC Has a modulator device
- GC×GC Narrow 'cuts' are sampled from ^{1}D, transferred rapidly to ^{2}D
- GC×GC ^{2}D provides additional separation – in v short time
- GC×GC Generates a "2D separation space"
- GC×GC Peak compression e.g. 5 s → 0.2 s; = taller peaks
- GC×GC Better detection sensitivity – improved LOD
- GC×GC Improves MS detection – reduced chemical background from column and matrix
- GC×GC Meets many 'desirables' of GC development – Separation & Sensitivity

"A Disruptive GC Technology" – meaning?

Modulator Technology Examples

a. Electric (painted col) – Phillips (U Illinois, original)
b. Sweeper modulator – Phillips (original)
c. Cryogenic modulator – Ours
d. Dual stage modulator – Various; now LECO Inc
Loop / cryogenic modulator – Zoex Inc
e. Diaphragm modulator – Synovec (U Washington)
Flow modulator – Seeley (U Oakland)
Solid-state (Peltier) mod – J&X Technol, Nanjing

Modulator Technology



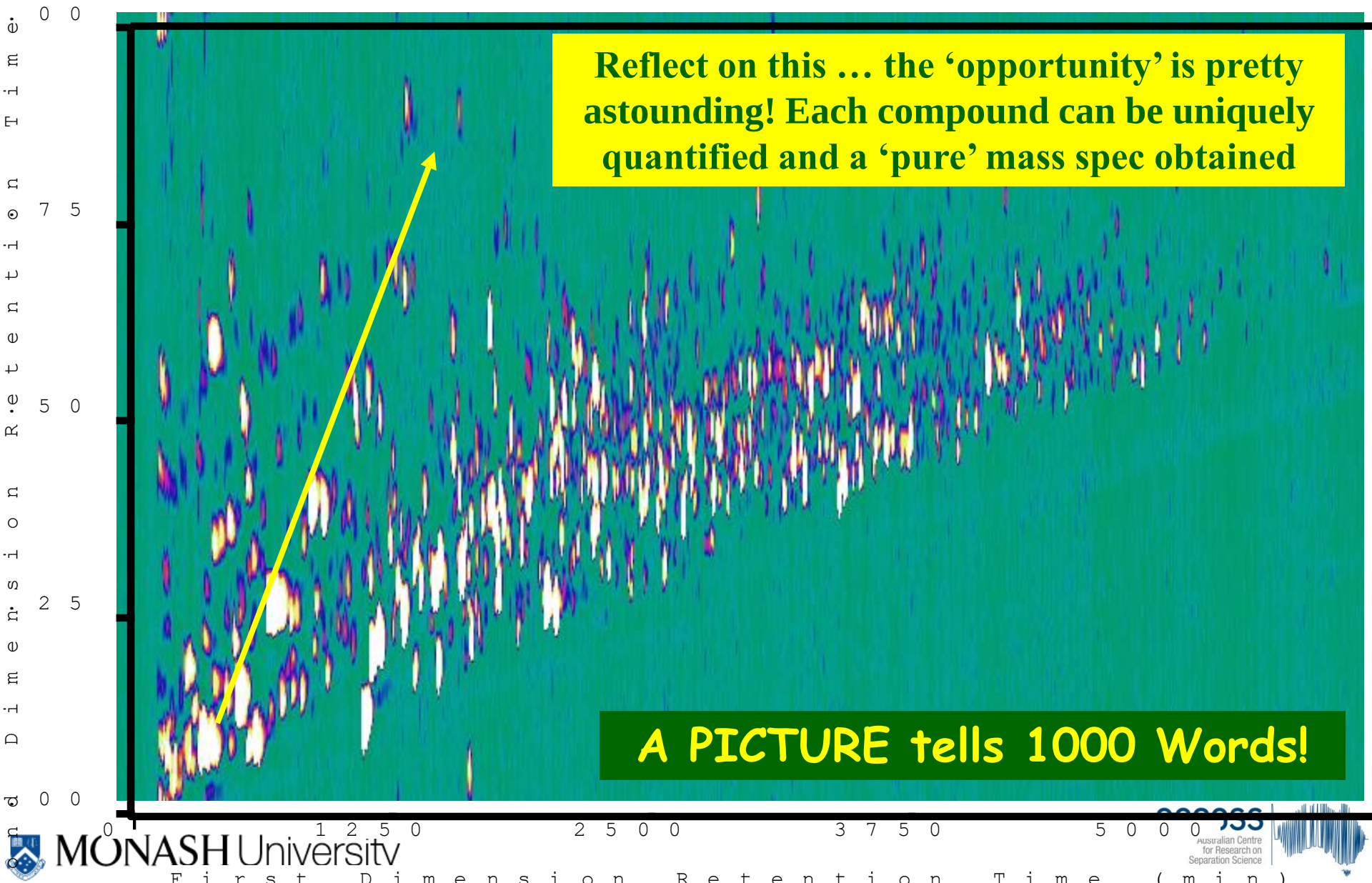
T - thermal

C - cryogenic

F – flow / switching

Arabica Coffee Headspace:

SPME-GC×GC /TOFMS sampling of 1 roasted coffee bean



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* DEANS SWITCH *
Fragrances & Aroma
Metabolom
Doping Co
ECD/F
N & S produ
Essential oils
Strawberry
Petrochemical
Flavonoids
PESTICIDES
PAHs
AMINO ACIDS
GC×GC-qMS
Fungicides
Illicit Drugs
Herbs & Spices
WINE
Allerge
Fatty Acids
TAGs & DAGs
POLLUTANTS
Cryogenic
SPME
FLAVOURS
Biofuels
STEROIDS
Natural Products
Joss Smoke
TCM
PHA
Enantiomers
LMCS

Technical aspects of GC×GC Technology

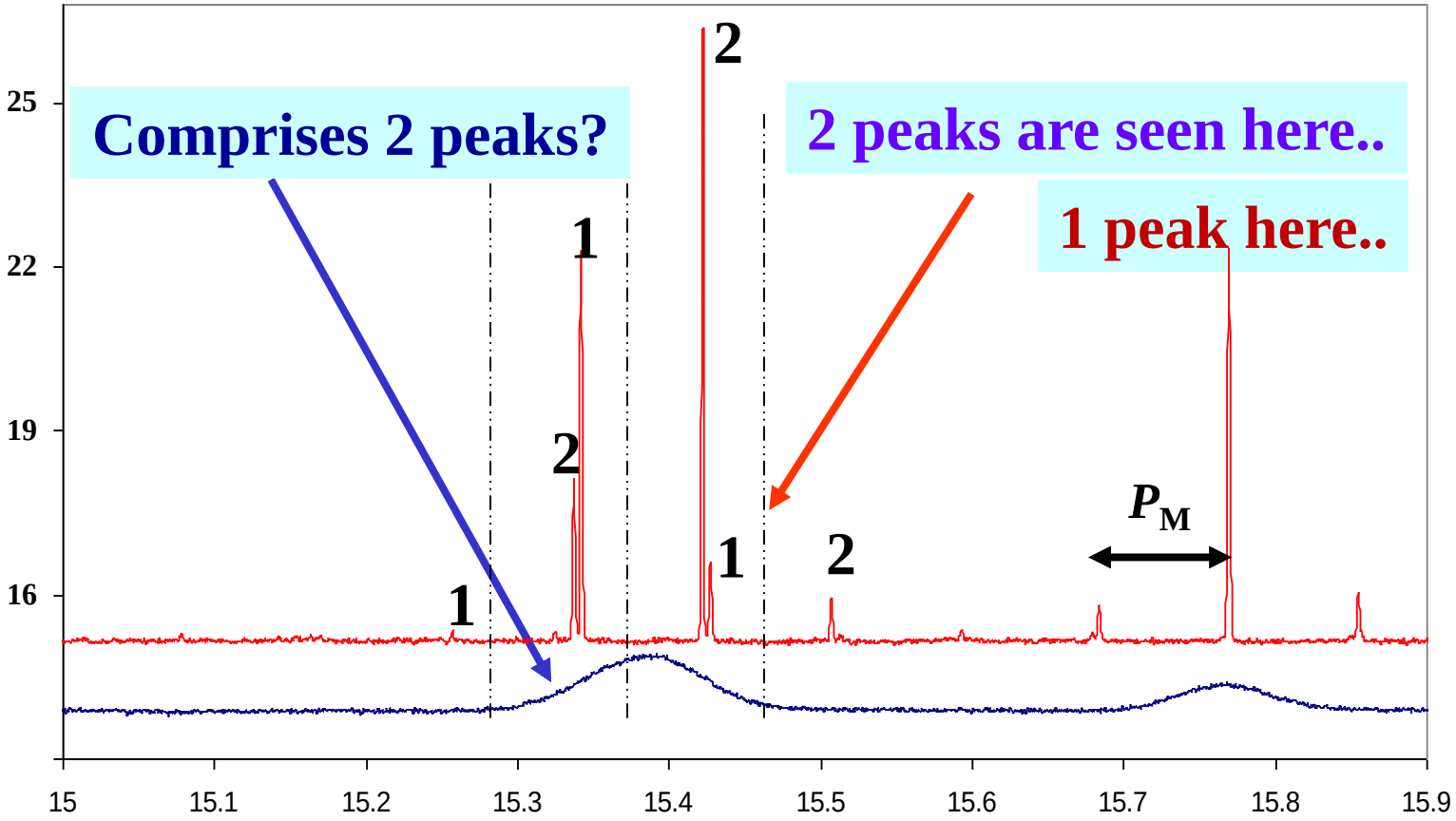
- GC×GC New **nomenclature** needed; define 1st & 2nd dimensions (e.g. ^{1}D, ^1t_R, ^1w_b, $^1w_{b1}$; ^1N; peak capacity, n_c, ^1n_c, $n_{c,tot}$) & operating procedure (modulation period P_M; modulation ratio M_R; modl number n_M??)
- GC×GC **Quantitative analysis** requires all modulated peaks of a compound to be summed. … more accurate than for a 1D GC method??
- GC×GC Choice of ^{1}D and ^{2}D column phase '**polarity**' plays a critical role in presentation of the total sample separation – '**orthogonal**'?; if the phases are the same, expect that there will be no separation in the ^{2}D axis.
- GC×GC Software presents retention of compounds in 2D space as ^1t_R and ^2t_R coords.
- GC×GC Expect that for replicate analyses, a compound should always appear in the **same 2D space position** – a VALUABLE capability
- GC×GC '**Zone compression**' with a cryogenic modulator leads to peak magnitude (response height) increase – but of course not in total peak area.
- GC×GC Underlying **interferences much reduced** e.g. 'chemical noise', less 'phase bleed', and matrix interference. This is important in GC×GC-MS
- GC×GC The **modulated peak distribution** depends on the sampling pattern over the ^{1}D peak. We call it 'in-phase', or 'out-of-phase'.
- GC×GC **Independent elution** on the ^{1}D column & on the ^{2}D column

Selected Nomenclature of GC×GC Technology

Modulator	Interface device between the two columns in a GC×GC separation system that accumulates or samples narrow bands from the eluate of the ^{1}D column for fast re-injection into the ^{2}D column.
Modulation period (P_M)	Duration of a complete cycle of modulation in a GC×GC separation system (equals the data conversion time of each second dimension chromatogram, *i.e.*, the time between two successive injections into the ^{2}D column).
Modulation ratio (M_R)	The ratio of the peak width at baseline ($^{1}w_b$) for the ^{1}D peak to the modulation period (P_M). $M_R = {}^{1}w_b / P_M$
Modulation phase (Φ; F_M) (In-phase; 180 ^{o} out of phase)	The pattern of modulated peaks caused by the time relationship between the shape of the analyte peak and the sampling process of the modulator in a GC×GC separation system.
Contour plot (Colour plot; Apex plot)	Two-dimensional plot representing a comprehensive two-dimensional separation, in which the colour represents the signal intensity of the components in the separation system
Column set	The combination of columns used for a given comprehensive 2D chromatography experiment.

COMPARISON: GC×GC vs. 1D GC

GC×GC OFFERS BETTER SEPARATION



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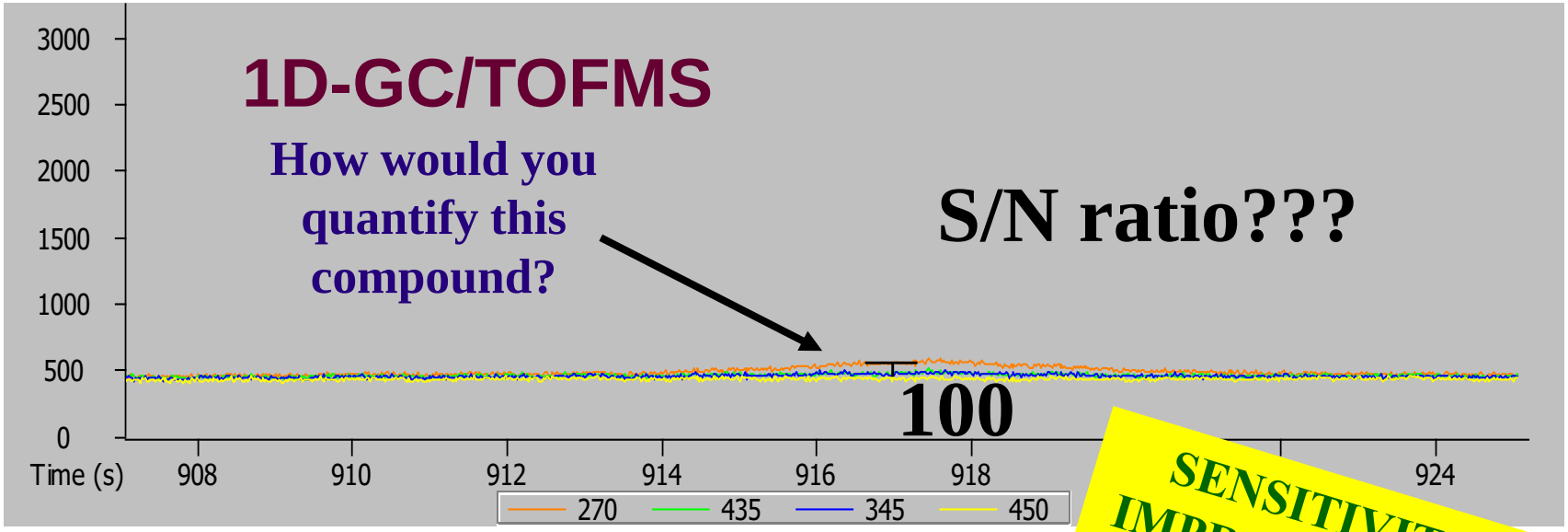
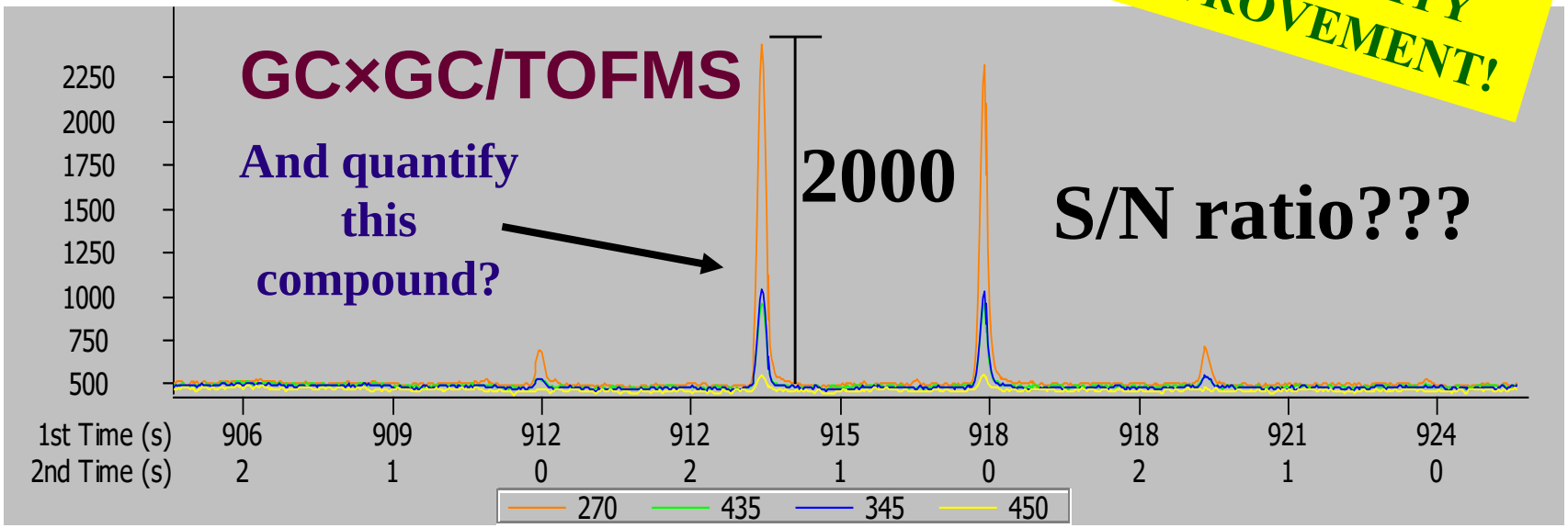
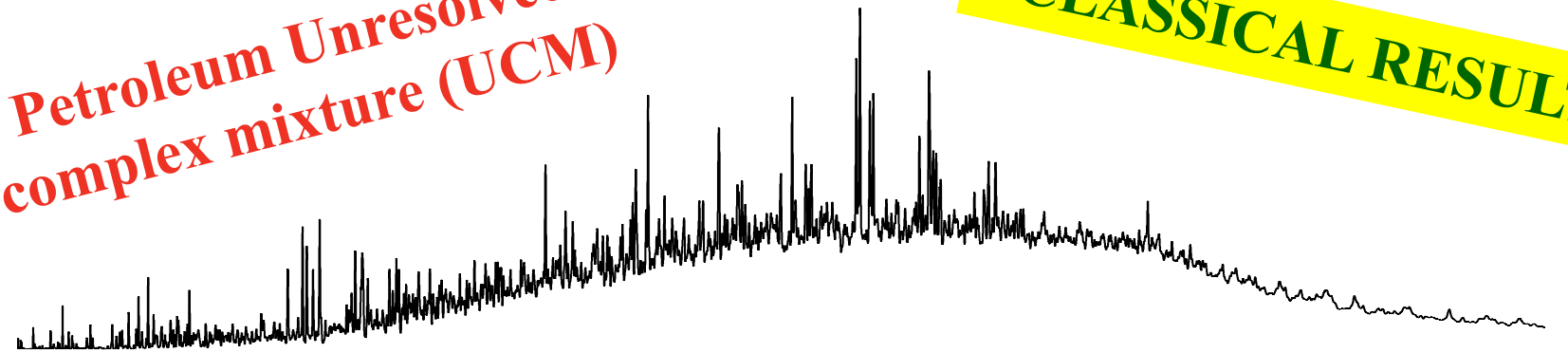

1D-GC/TOFMS
How would you quantify this compound?
S/N ratio???
100
SENSITIVITY IMPROVEMENT!
3000
2500
2000
1500
1000
500
0
Time (s)
908
910
912
914
916
918
924
270
435
345
450

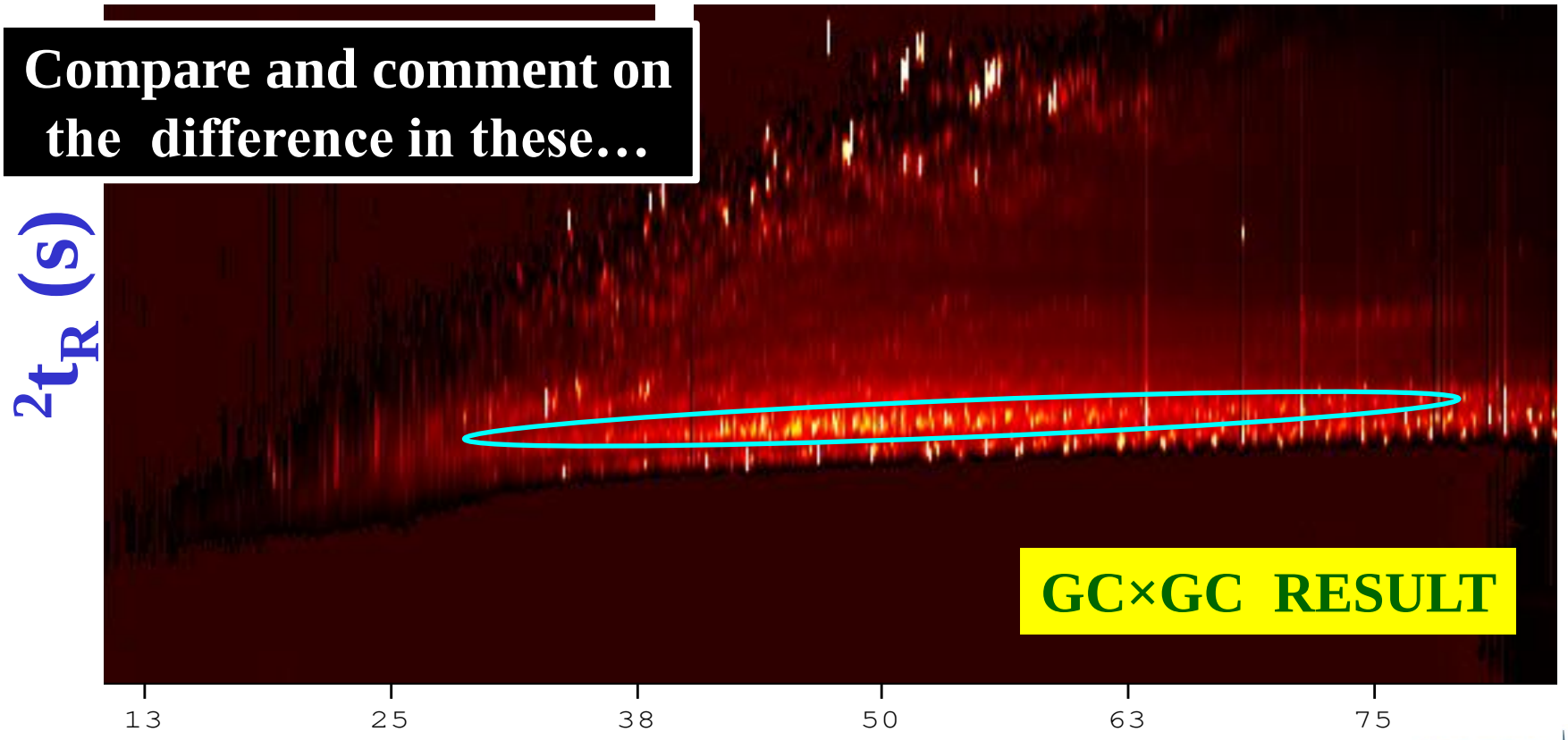

GC×GC/TOFMS
And quantify this compound?
2000
S/N ratio???
2250
2000
1750
1500
1250
1000
750
500
1st Time (s)
2nd Time (s)
906 2
909 1
912 0
912 2
915 1
918 0
918 2
921 1
924 0
270
435
345
450

Petroleum Unresolved complex mixture (UCM)

CLASSICAL RESULT

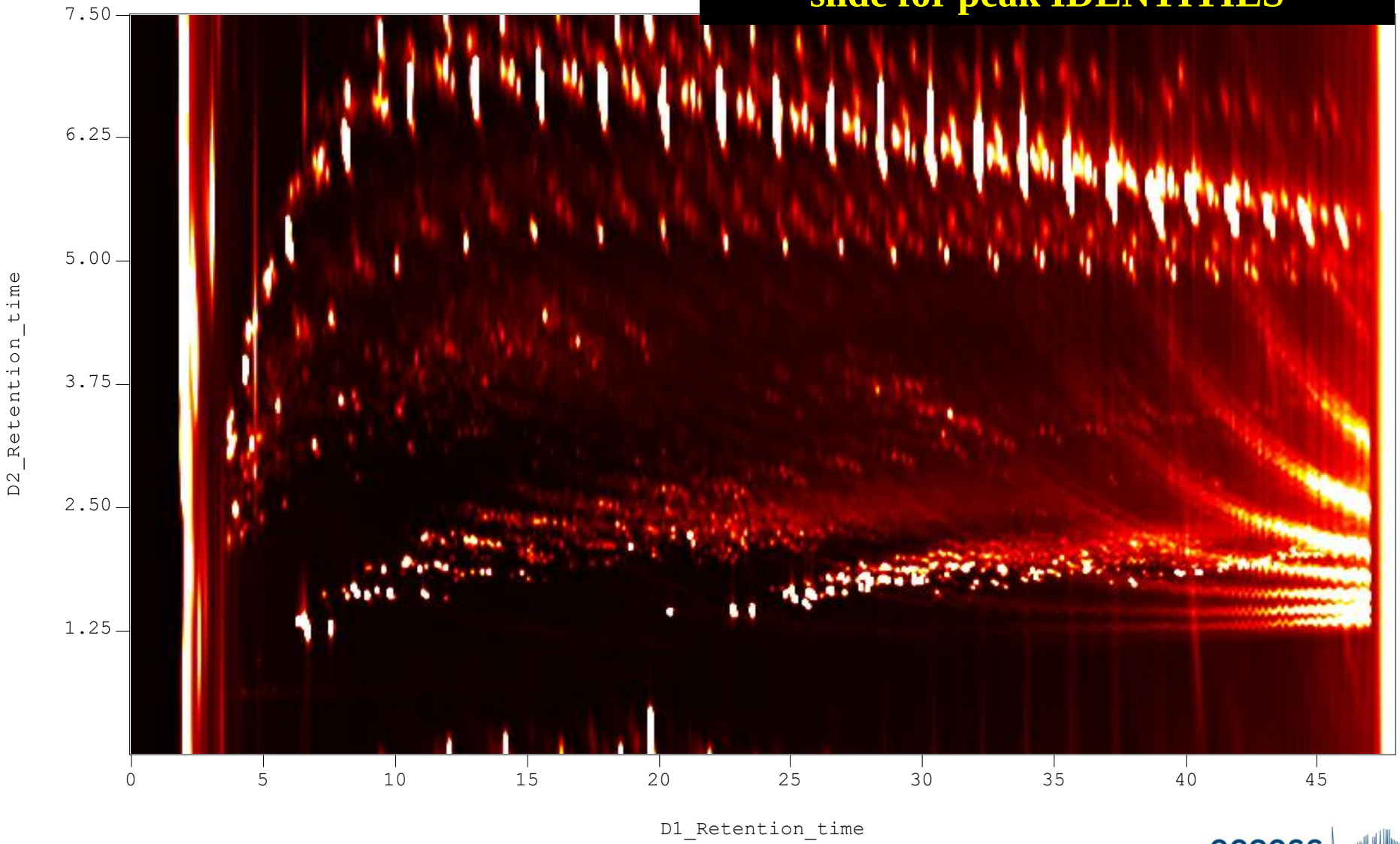


Compare and comment on the difference in these…



Alternative GC×GC RESULT for the same sample..

**** Why is there a difference between the two presentations? See the next slide for peak IDENTITIES**



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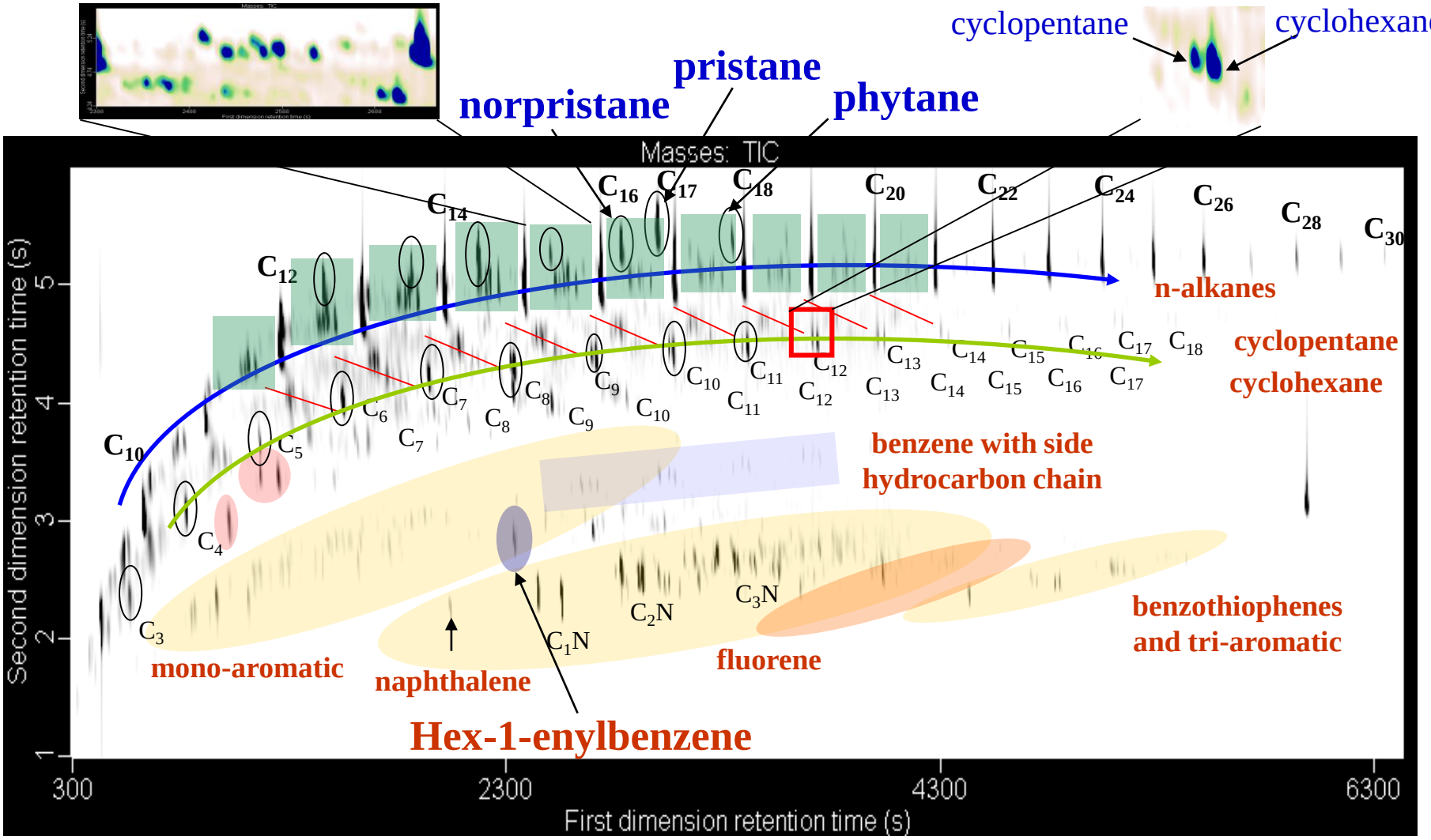


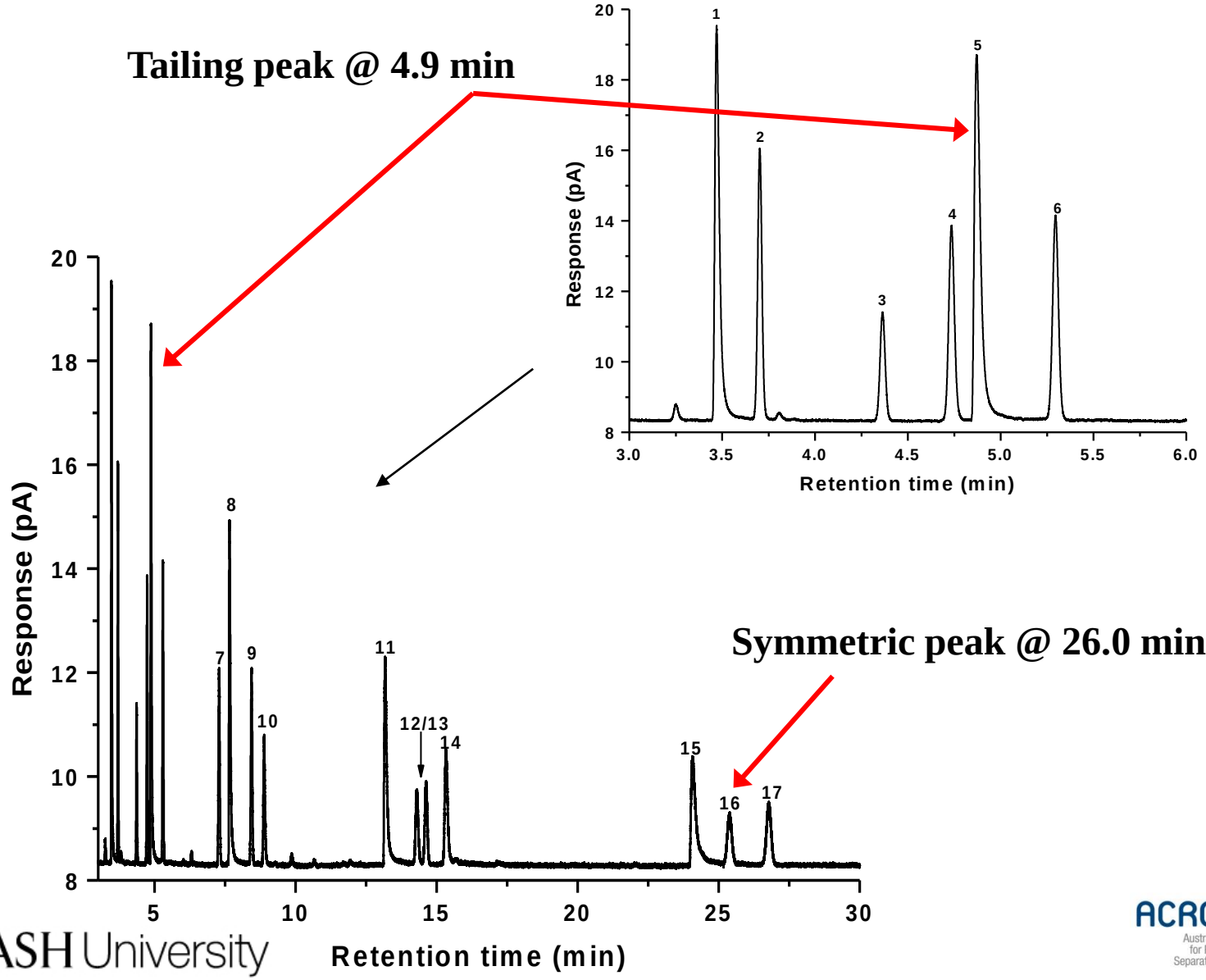
Branched-alkane

Branched-cyclopentane/cyclohexane

Marine; Early Cretaceous; limited degradation

REAL DATA – isothermal; alcohols and non-polar compounds

Check modulation performance for this sample, P_M; M_R



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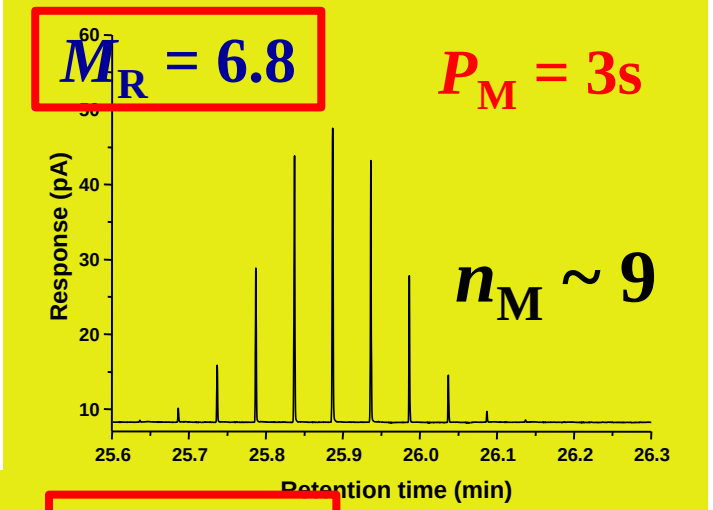
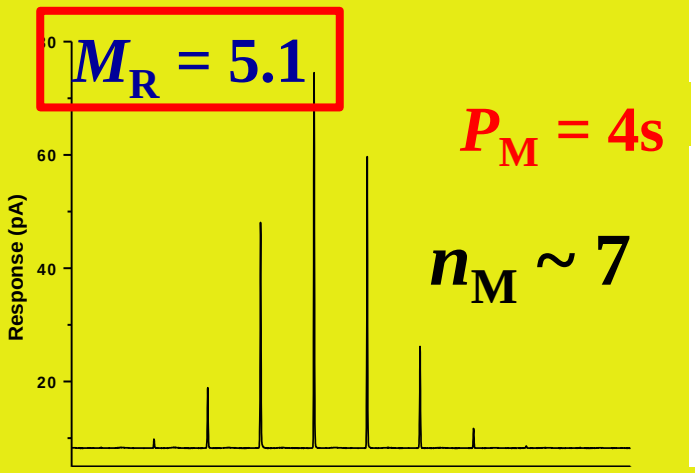
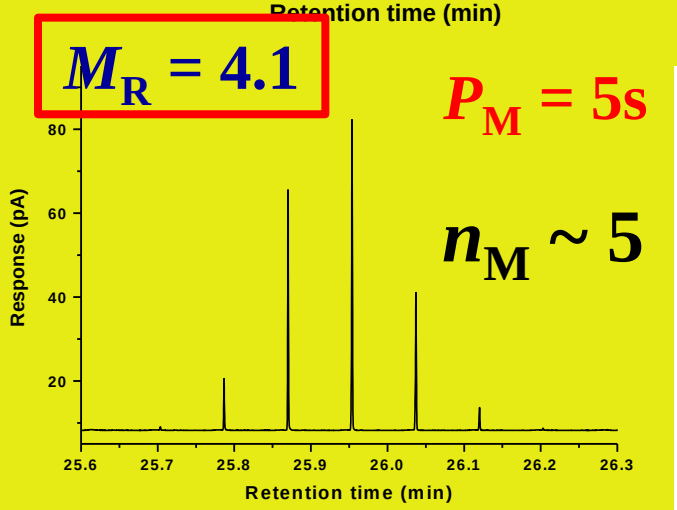
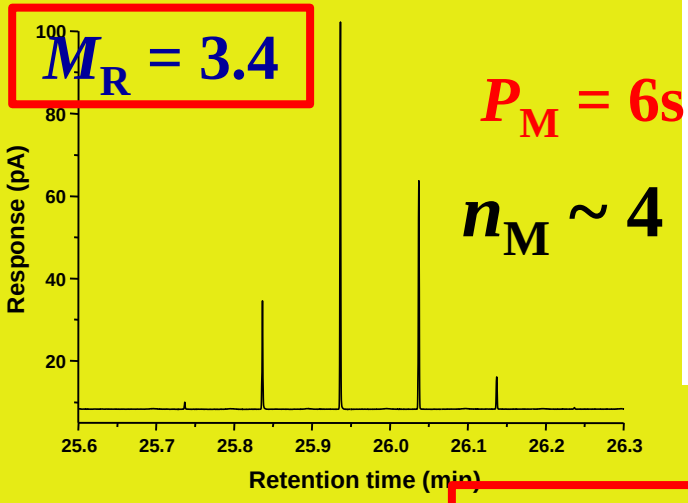




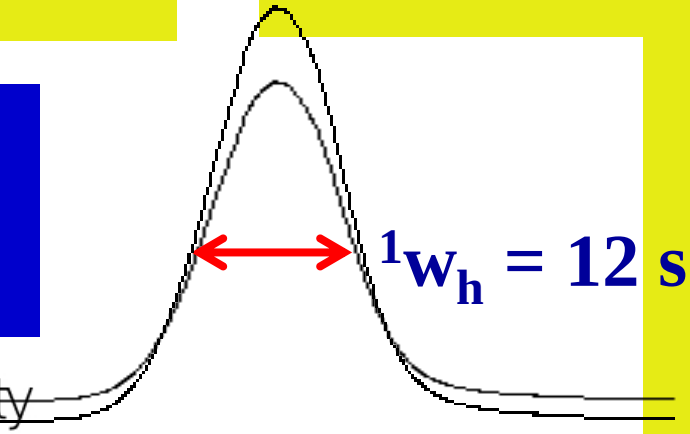


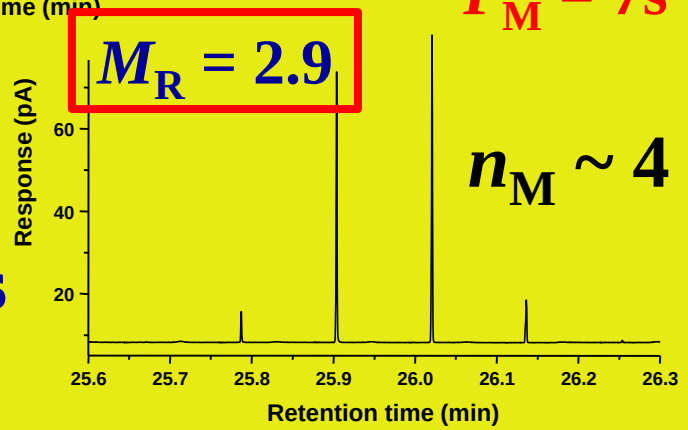


Symmetric REAL ^{2}D PEAKS Isothermal GC … Alter P_M

n_M = modulation number!!

HOW MANY modulations do we want??



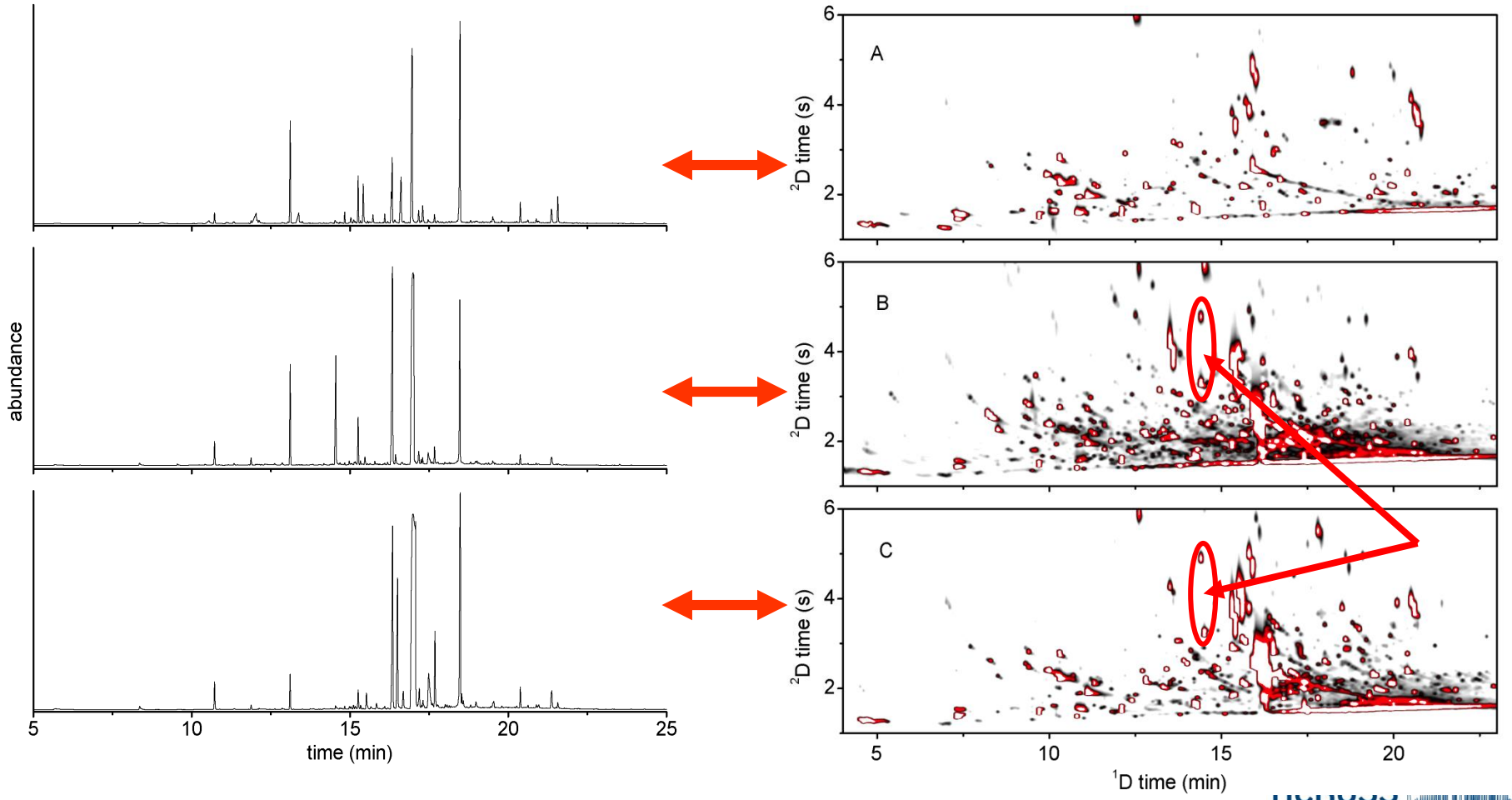


Illicit Drugs - GC×GC

REPRODUCIBLE; HIGH DATA DENSITY COMPARED WITH 1D GC

GC-qMS

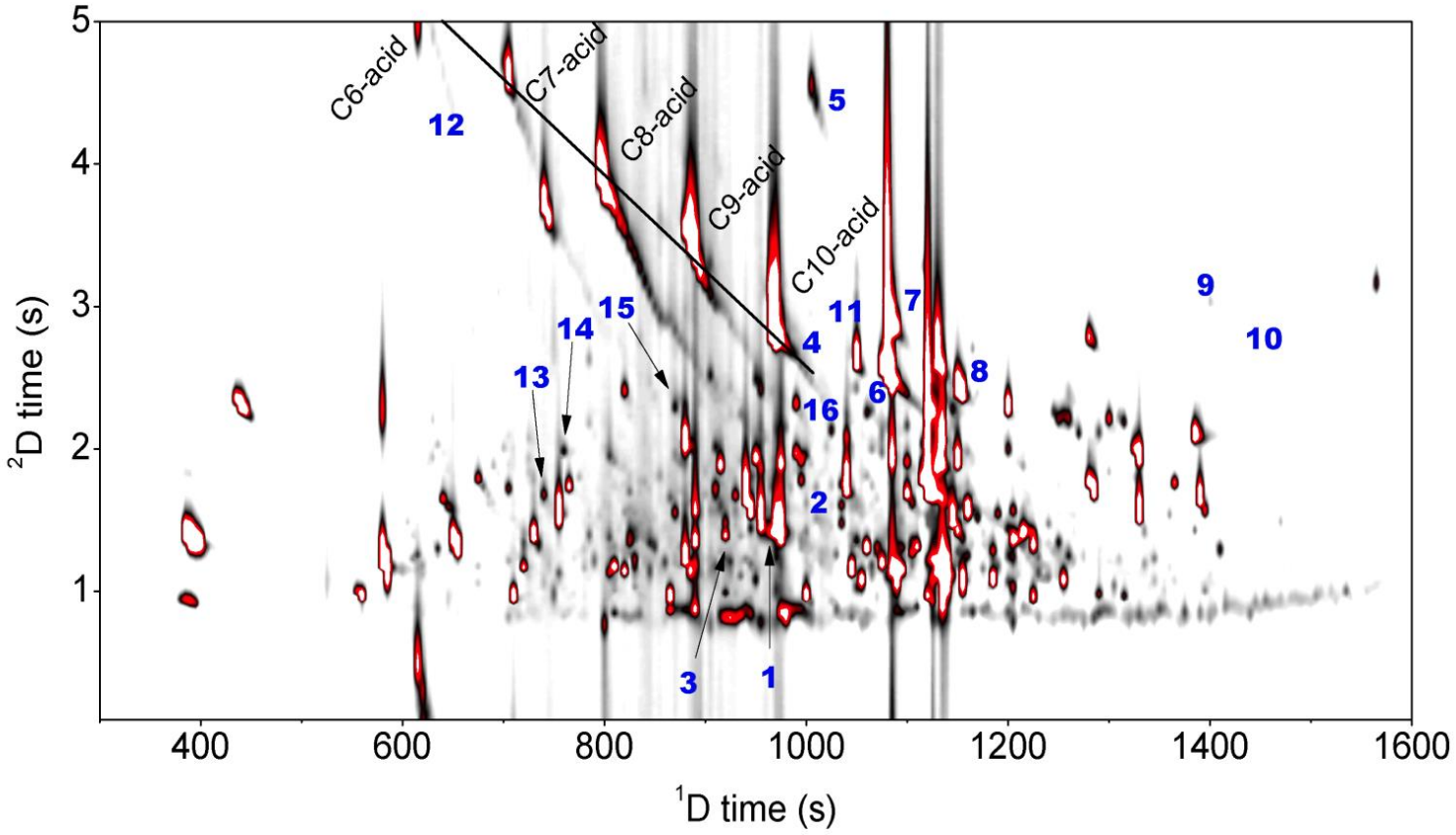
GC×GC-FID



Ecstasy profiling using GC×GC-TOFMS

450 out of 1200 identified
- S/N > 100
- MAM > 800

BPX5 / BP20 column set
= non-polar / polar



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Ecstasy profiling by GC×GC-TOFMS

Table of selected 16 components for profiling

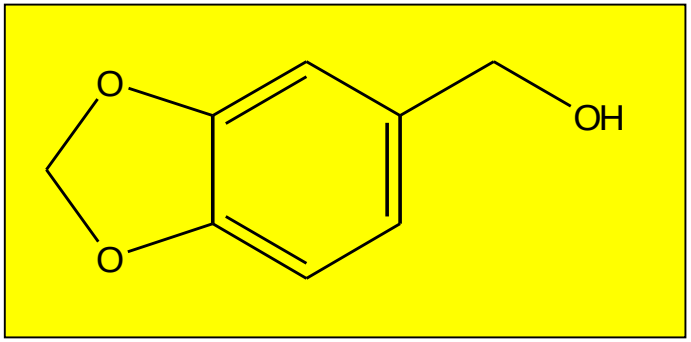
Much wider choice for component selection in GC×GC

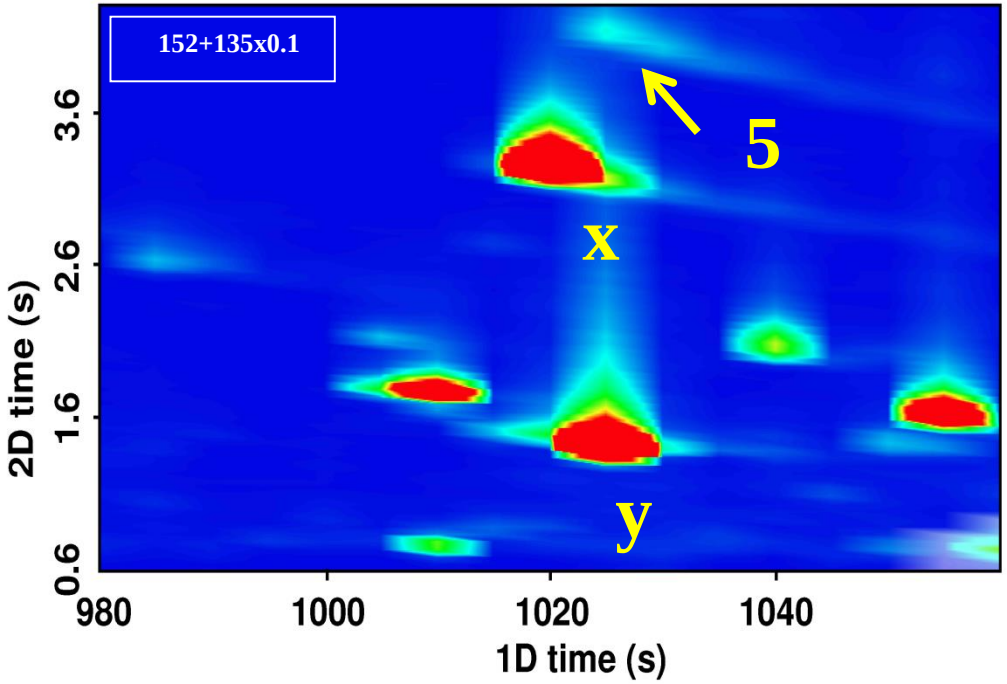
- ↑ sensitivity
- ↑ separation

Nº	Compound	^{1}D time (s)	^{2}D time (s)	Quant ion (m/z)
1	safrole	**960**	**1.58**	**162**
2	isosafrole-2	**995**	**1.78**	**162**
3	3,4-methylenedioxyphenylpropane (3,4-MD propane)	**920**	**1.48**	**164**
4	Piperonal	**970**	**2.86**	**149**
5	1,3-benzodioxole-5-MeOH (benzodioxole-5-MeOH)	**1005**	**4.54**	**152**
6	3,4-methylenedioxyphenyl-2-propanone (Piperonylmethylketone, PMK)	**1080**	**2.58**	**178**
7	3,4-methylenedioxyphenyl-2-propanol (PMK-OH)	**1085**	**2.88**	**180**
8	unknown 147	**1150**	**2.420**	**147**
9	N-formyl-MDMA	**1400**	**3.080**	**162**
10	N-acetyl-MDMA	**1415**	**2.78**	**162**
11	3,4-methylenedioxyacetophenone (3,4-MDAcPh)	**1050**	**2.62**	**164**
12	Benzylalcohol	**670**	**4.24**	**108**
13	3,4-methylenedioxytoluene (3,4-MD toluene)	**740**	**1.68**	**135**
14	benzylmethylketone (BMK)	**760**	**1.98**	**134**
15	Benzothiazole	**870**	**2.28**	**135**
16	3,4-methylenedioxybenzylchloride (3,4-MD-benz-Cl)	**990**	**2.30**	**170**

Example of Resolution benefits of GC×GC-MS

Example: Comp. 5





- Production specific
- Polar component
- Present in trace amount
- Co-elutes with x and y

Comp. x – gives 135 m/z ion

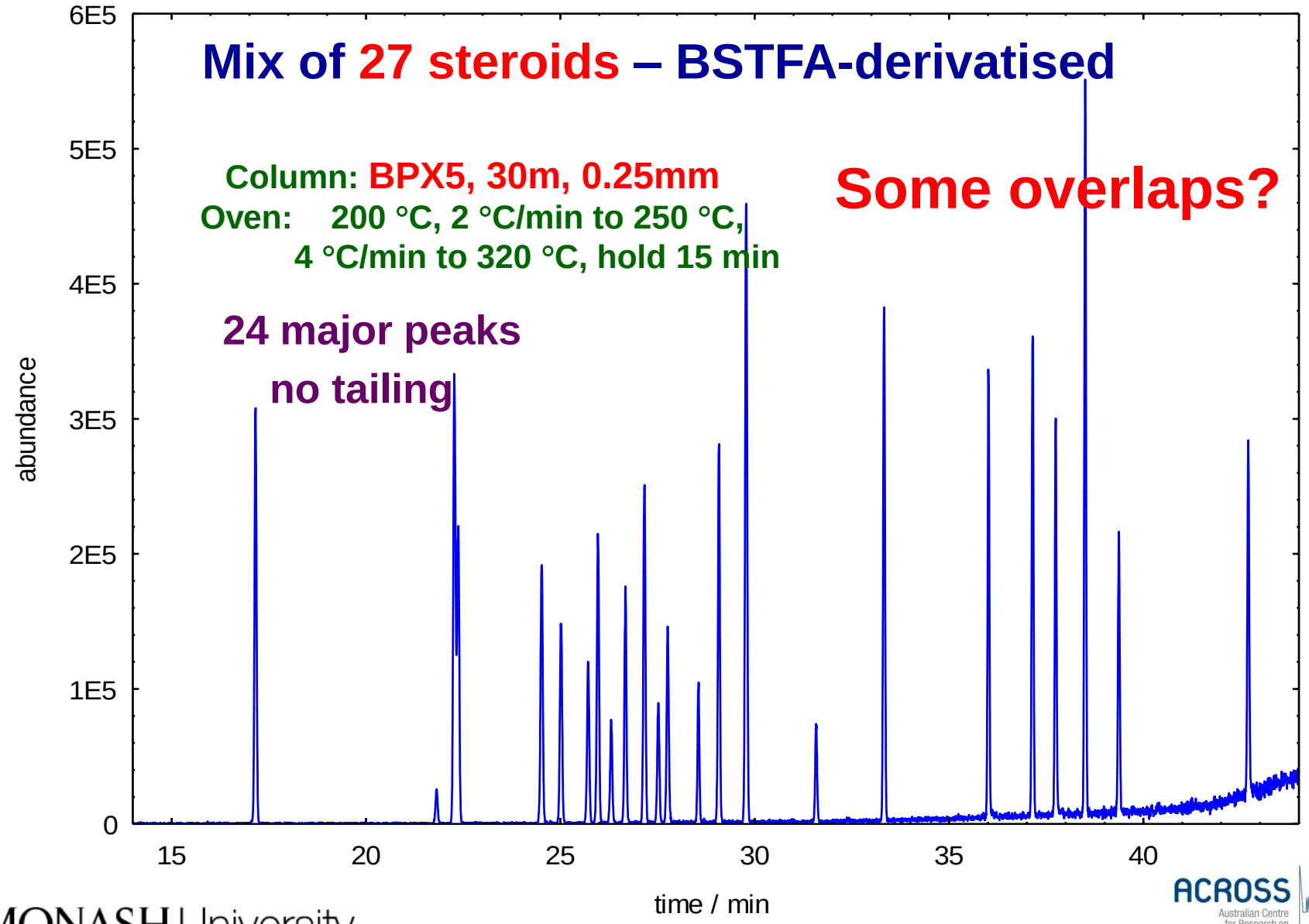
Comp. y – gives 152 m/z ion

135 m/z and 152 m/z are most characteristic for comp. 5

Thus '5' is desirable for profiling studies

In 1D GC, 5 is hard to measure!

BSTFA-DERIVATISED STEROIDS ON 1D GC-MS



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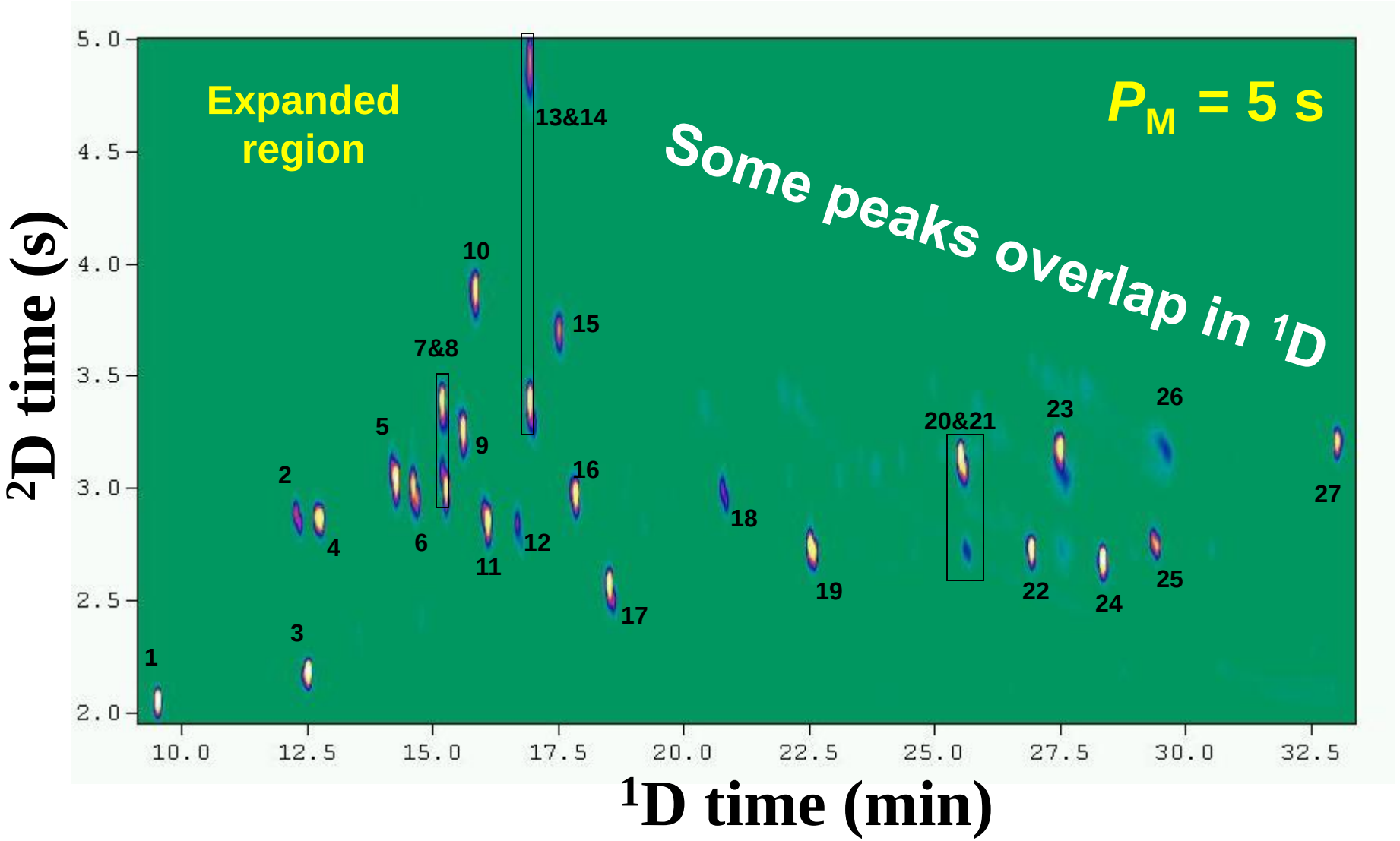
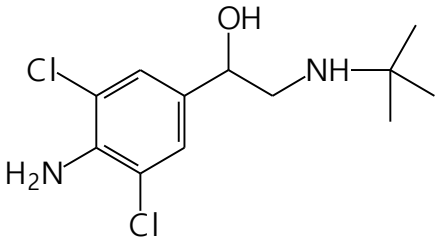
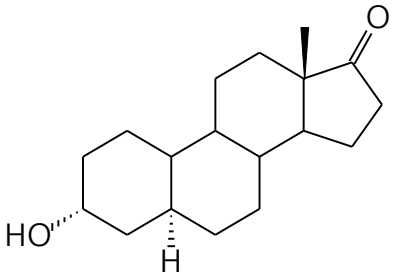
BSTFA-DERIVATISED STEROIDS: GC×GC-FID



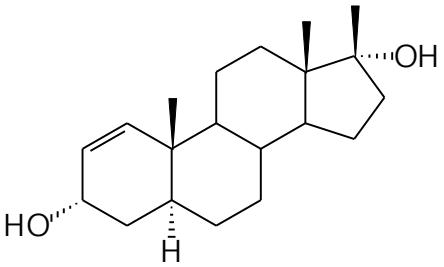
The substances: WADA (World Anti-Doping Agency) key anabolic agents – TEST analytes for LAB performance



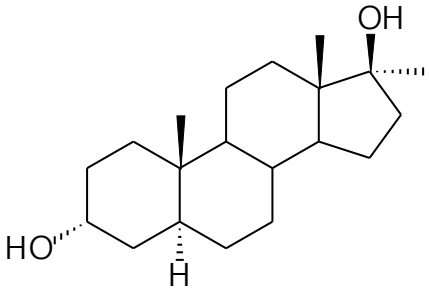
1. Clenbuterol



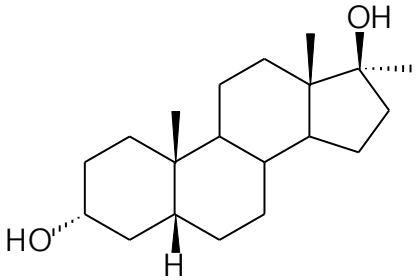
2. 19-norandrosterone



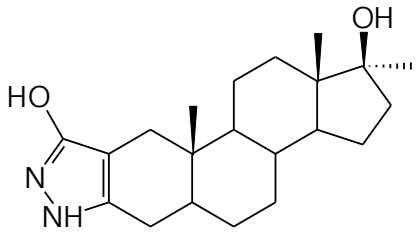
3. Epimethendiol



4. Methyltestosterone metabolite M1



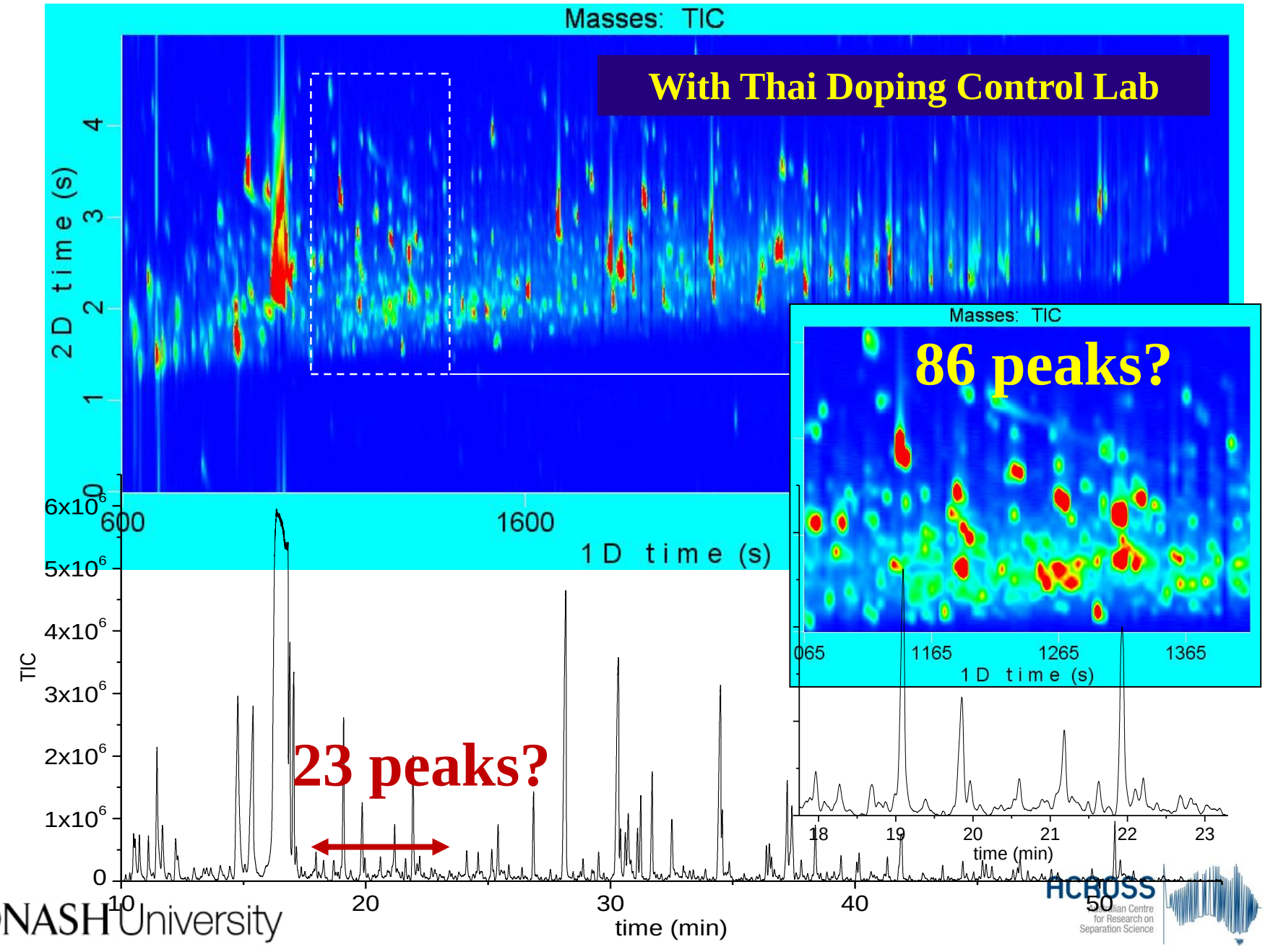
5. Methyltestosterone metabolite M2



6. 3'hydroxystanozolol

Labs must be able to correctly ID all these in matrix, and meet various analysis criteria to retain their accreditation with WADA.
GC×GC was able to meet these criteria.

WADA Urine Positive Control (UPC), spiked at 5 ng/mL



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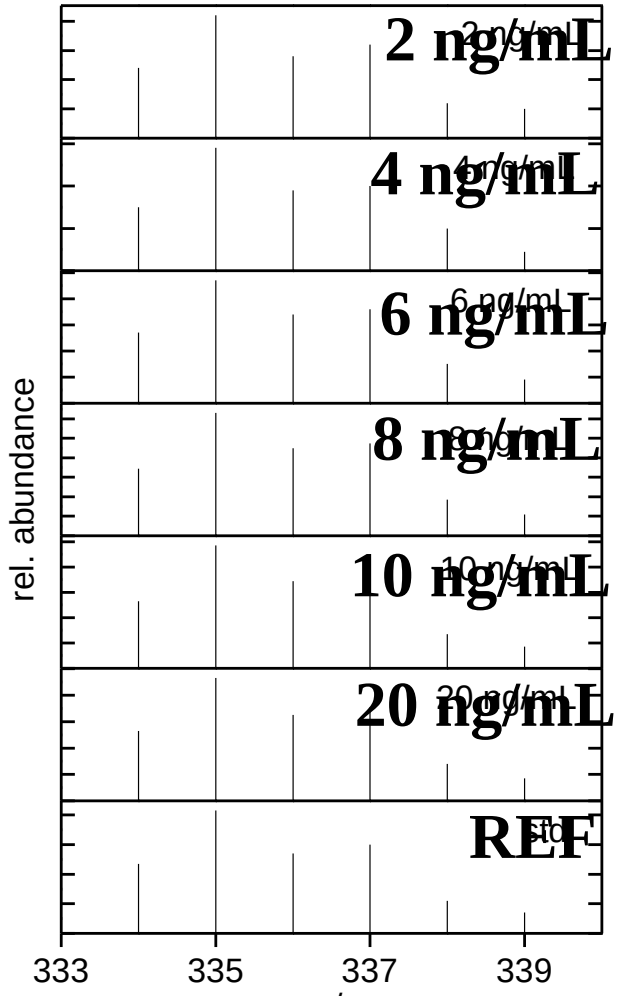
WADA ANTI-DOPING RULE VIOLATIONS

1. PRESENCE OF A PROHIBITED SUBSTANCE OR ITS METABOLITES OR MARKERS IN AN ATHLETE'S SAMPLE.
2. USE OR ATTEMPTED USE OF A PROHIBITED SUBSTANCE OR PROHIBITED METHOD.
3. EVADING, REFUSING OR FAILING TO SUBMIT TO SAMPLE COLLECTION.
4. WHEREABOUTS FAILURES—THREE STRIKES RULE.
5. TAMPERING OR ATTEMPTED TAMPERING.
6. POSSESSION OF PROHIBITED SUBSTANCES AND PROHIBITED METHODS.
7. TRAFFICKING OR ATTEMPTED TRAFFICKING.
8. ADMINISTRATION OR ATTEMPTED ADMINISTRATION.
9. COMPLICITY.
10. PROHIBITED ASSOCIATION.
11. ACTS BY AN ATHLETE OR OTHER PERSON TO DISCOURAGE OR RETALIATE AGAINST REPORTING TO AUTHORITIES.

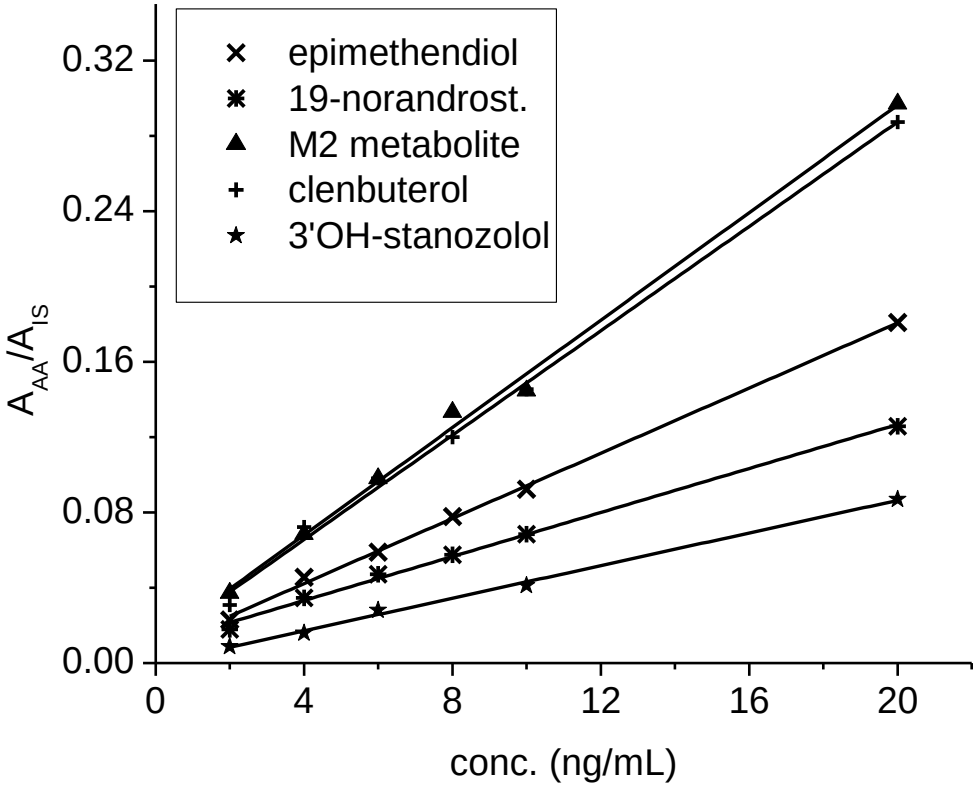
Only 1 rule is about direct doping in a sample – an **"Adverse Analytical Finding"**

VALIDATION OF RESULTS, WADA CRITERIA

Check Isotope pattern for clenbuterol (2x Cl atoms)



Calibr Linearity



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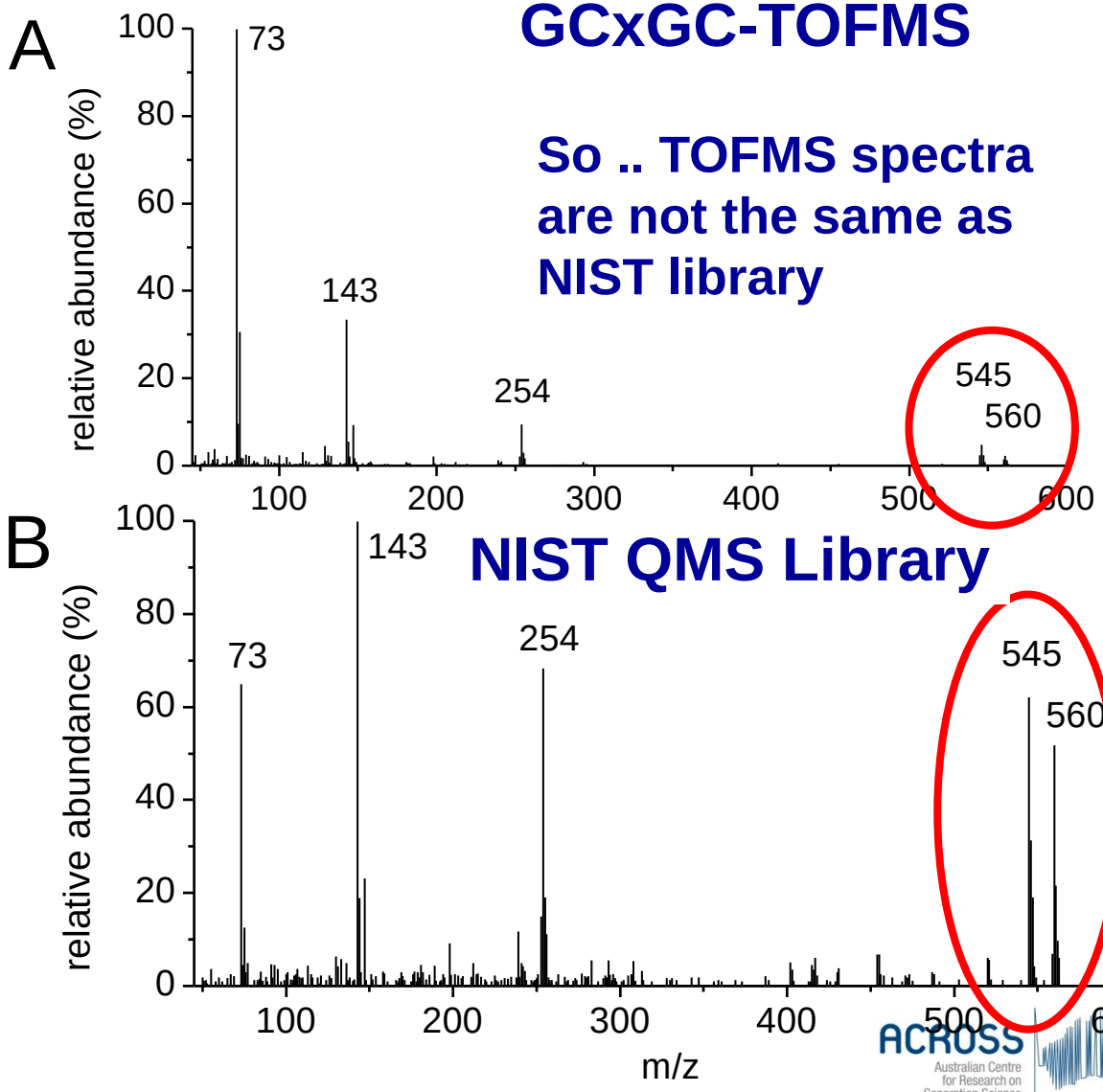
VALIDATION OF RESULTS, WADA CRITERIA

MS Similarity

19-norandrosterone

Conc. (ng/mL)	Match
Std. soln.	945
20	926
10	867
8	891
6	888
4	799
2	784

Stanozolol (Ben Johnstone)



And…
High Resolution MDGC

… supported by ‘Cryogenic’ modulation in GC

Tobacco Essential Oil

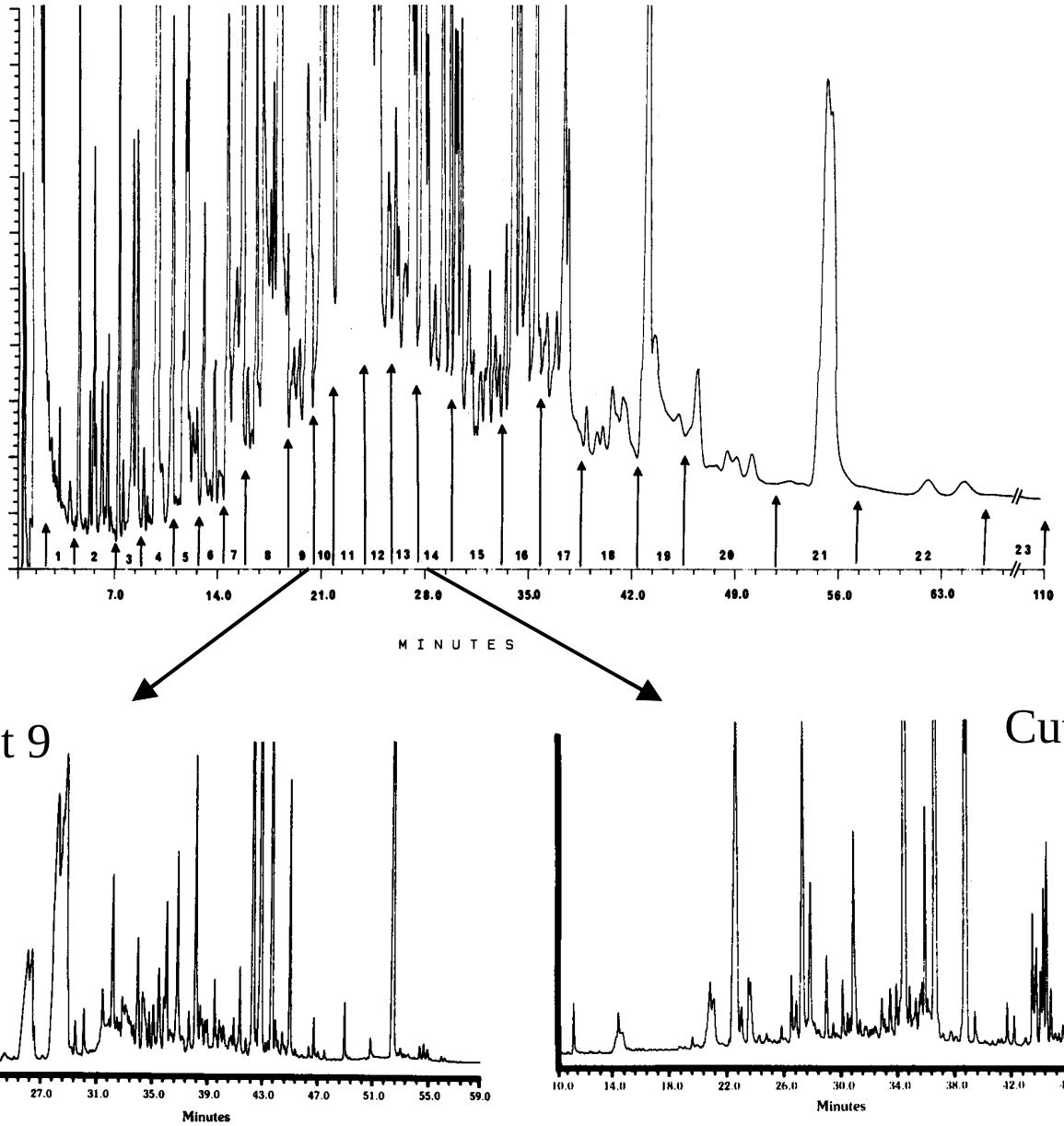
GORDON's Conventional MDGC

24 Heart cuts; 24 injections

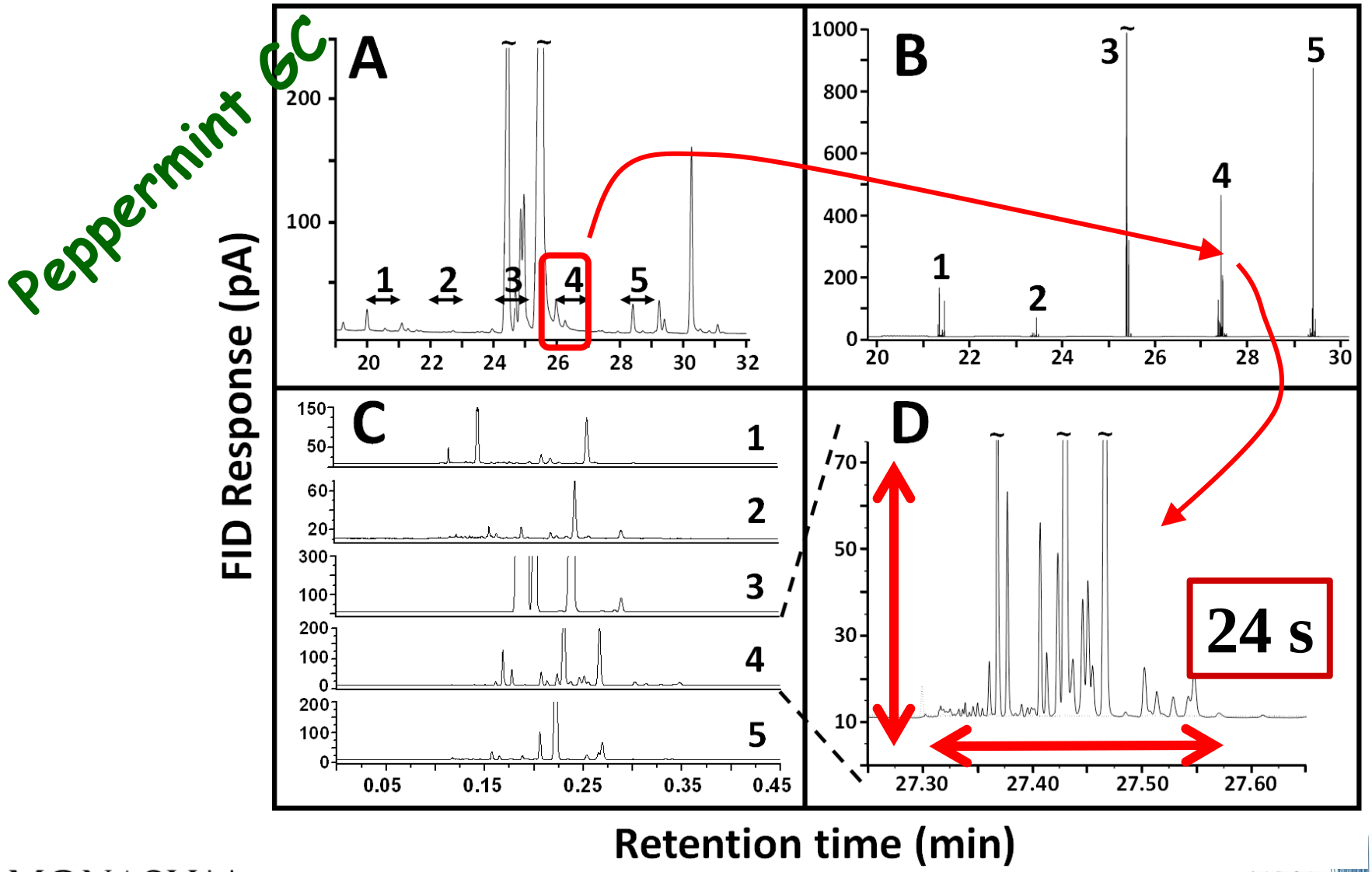
Each H/C passed to a long ^{2}D col, with full GC analysis

48 h Analysis!

Can we do it in 1 h



A: Full GC analysis; B: Transfer heart-cuts shown to a fast elution ^{2}D column, (7 m long; narrow bore) using cryogenic trapping & modulation; C: expand each region 1-5. D: Region 4 shows ~ 30-40 compounds, but A. suggested only 3 or 4!



GC×GC of Fatty Acid Methyl Esters

- **Samples:**
  - 37 component FAME standard (Supelco)
  - FAME from cod liver oil
- **GC×GC conditions:**
  - ^{1}D 30m x 0.25mm x 0.25µm BPX5 (nonpolar)
  - ^{2}D 1.5m x 0.10mm x 0.10µm BP20 (Polar)
  - Oven: 100°C (5min) / 3°C/min to 260°C (12 min)
  - H_2 1.5mL/min constant flow
  - P_M 3s, start @4.98min; FID 100Hz

Or other options…

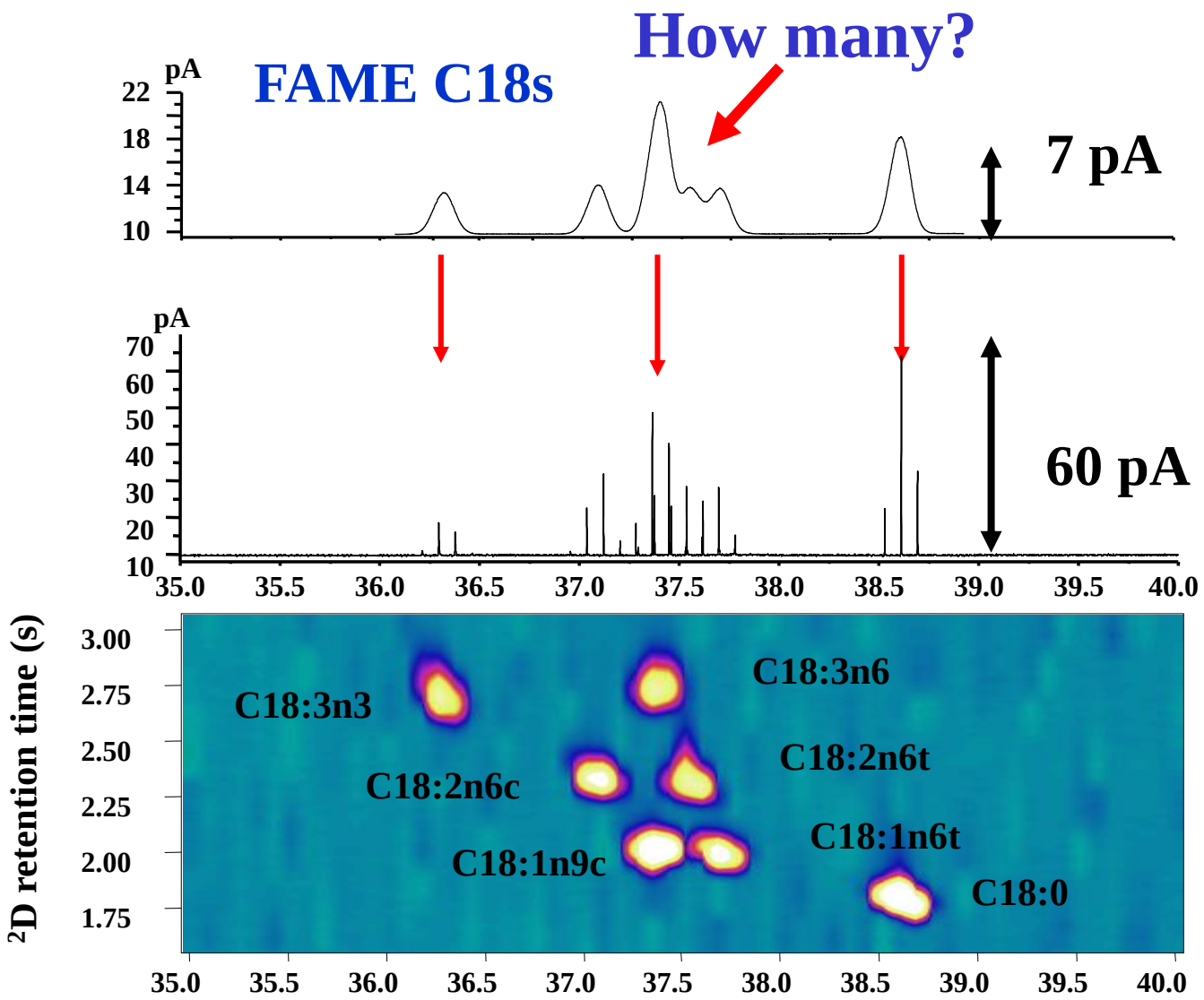
Saponify triglycerides to their fatty acids, derivatise to methyl esters. Analyse by GC, GC×GC.

R-C(O)OH + MeOH/KOH → R-C(O)OCH_3

- **Alkyl Group R varies in Chain Length (but odd C_n not common)**
- **Saturated or unsaturated / polyunsaturated; bad fats – good fats**
- **ω3 / ω6 etc indicate first unsaturated carbon in the FAME**
- **1, 2, 3, 4, 5, 6 etc unsaturated bonds**
- **And other structural variations / branching, cyclics**

Hence separation may be difficult

GC×GC gives STRUCTURE in the 2D space



1D-GC

GC×GC

Improved peak separation

- Higher responses

More information!

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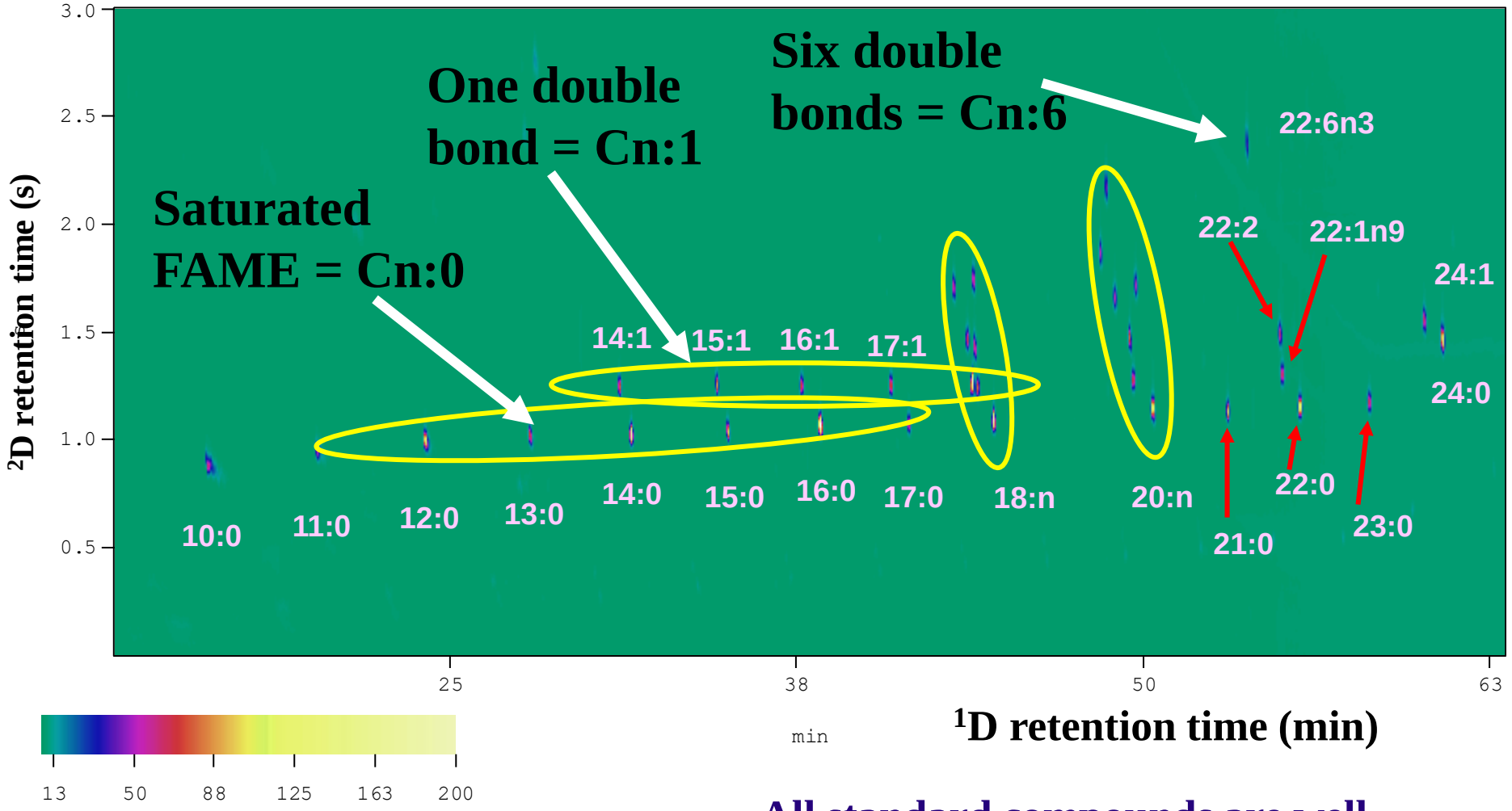
FAME Standard Mixture



Response scale (pA). Baseline is green.

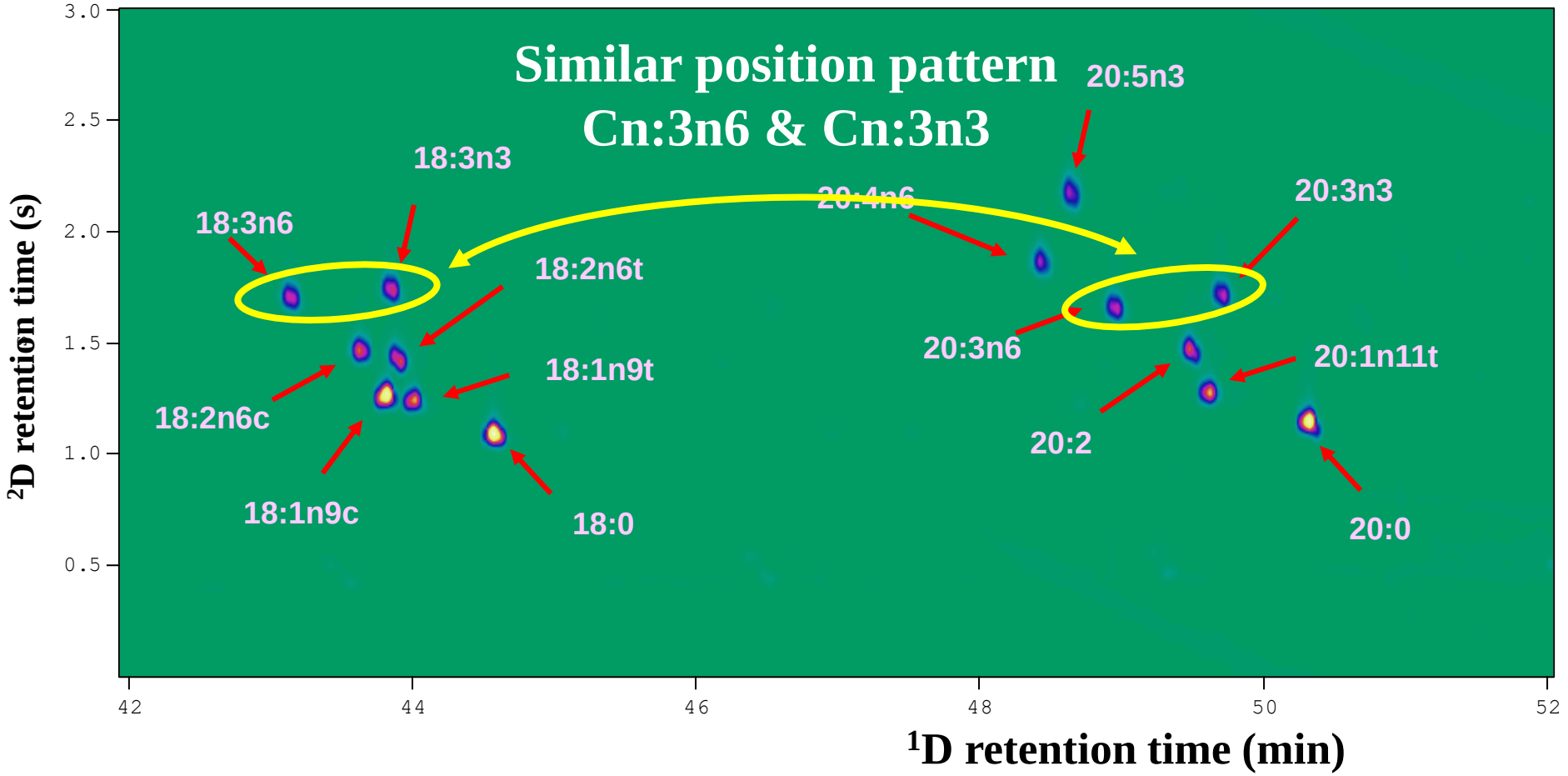
Large peaks are pale/white colour.

Blue ~ 50 pA

All standard compounds are well resolved in the 2D space.

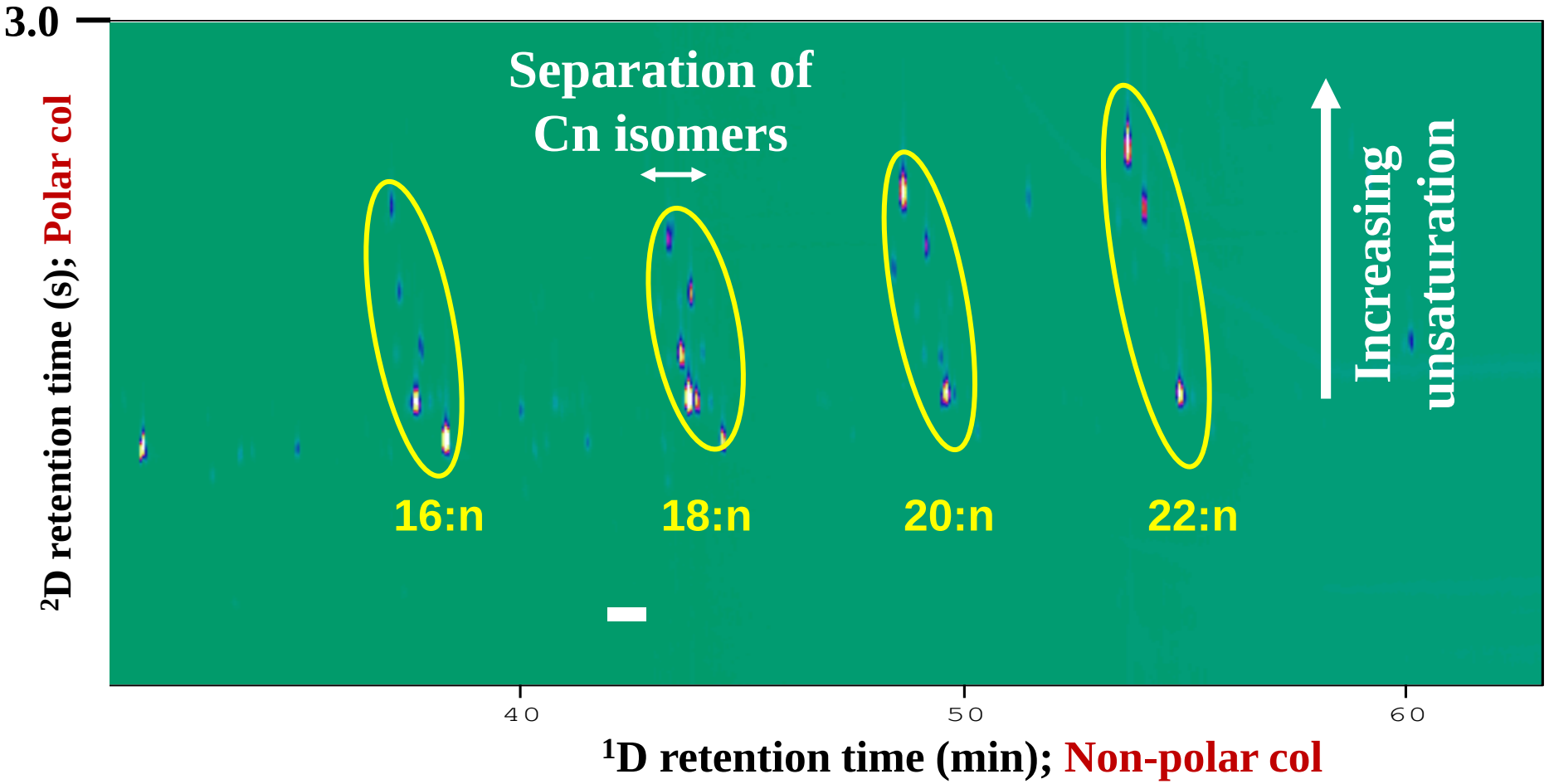
FAME with more double bonds are more polar – so elute later on ^{2}D column

FAME Standard Mixture

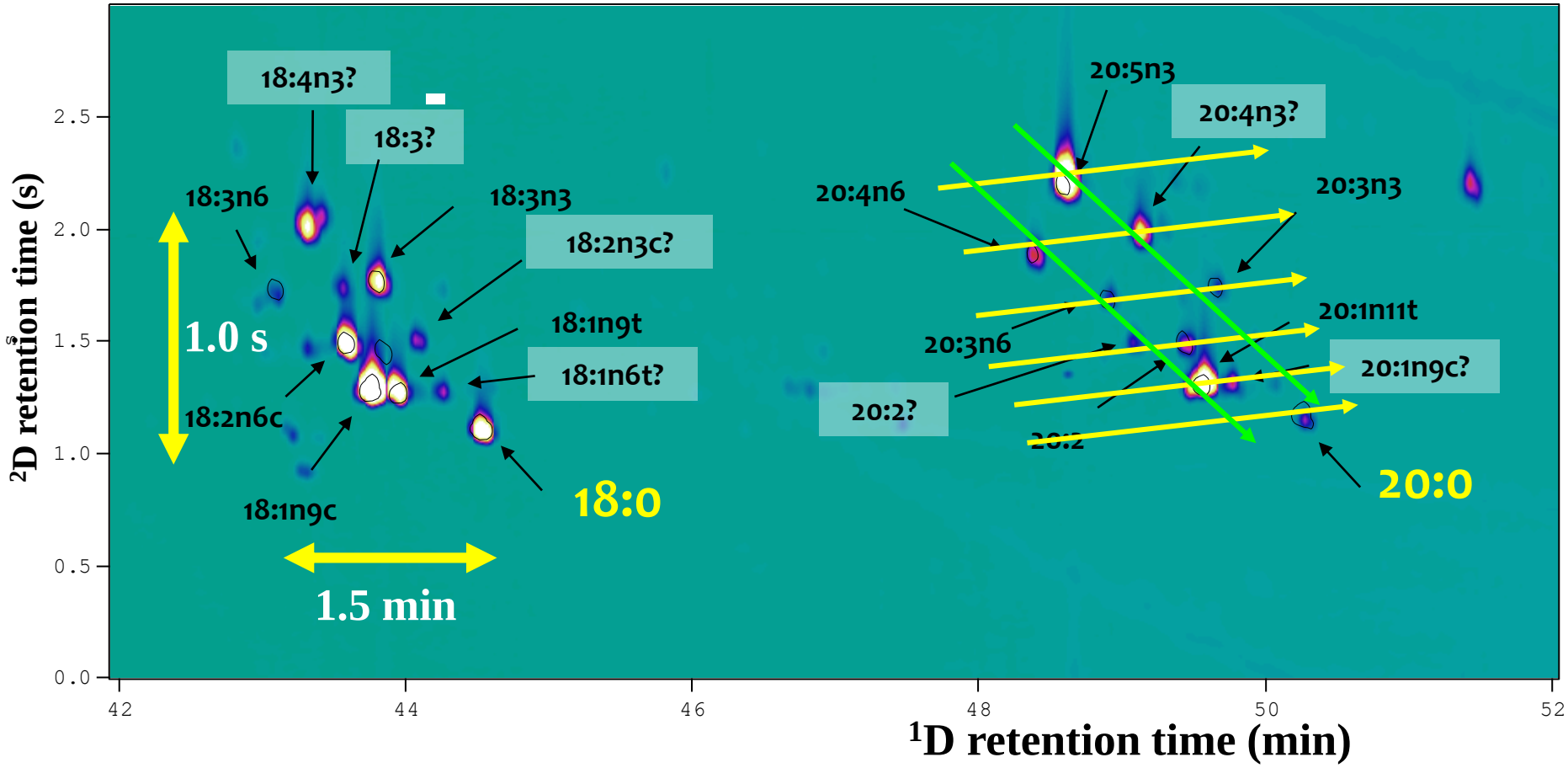


The more polar isomers (i.e. unsaturated) elute earlier on the non-polar ^{1}D column, so before the Cn:0 saturated FAME. No separation of FAME with different bond positions (^1t_R 18:3n6 < 18:3n3) and cis/trans isomers (18:2n6)

Cod Liver Oil FAME



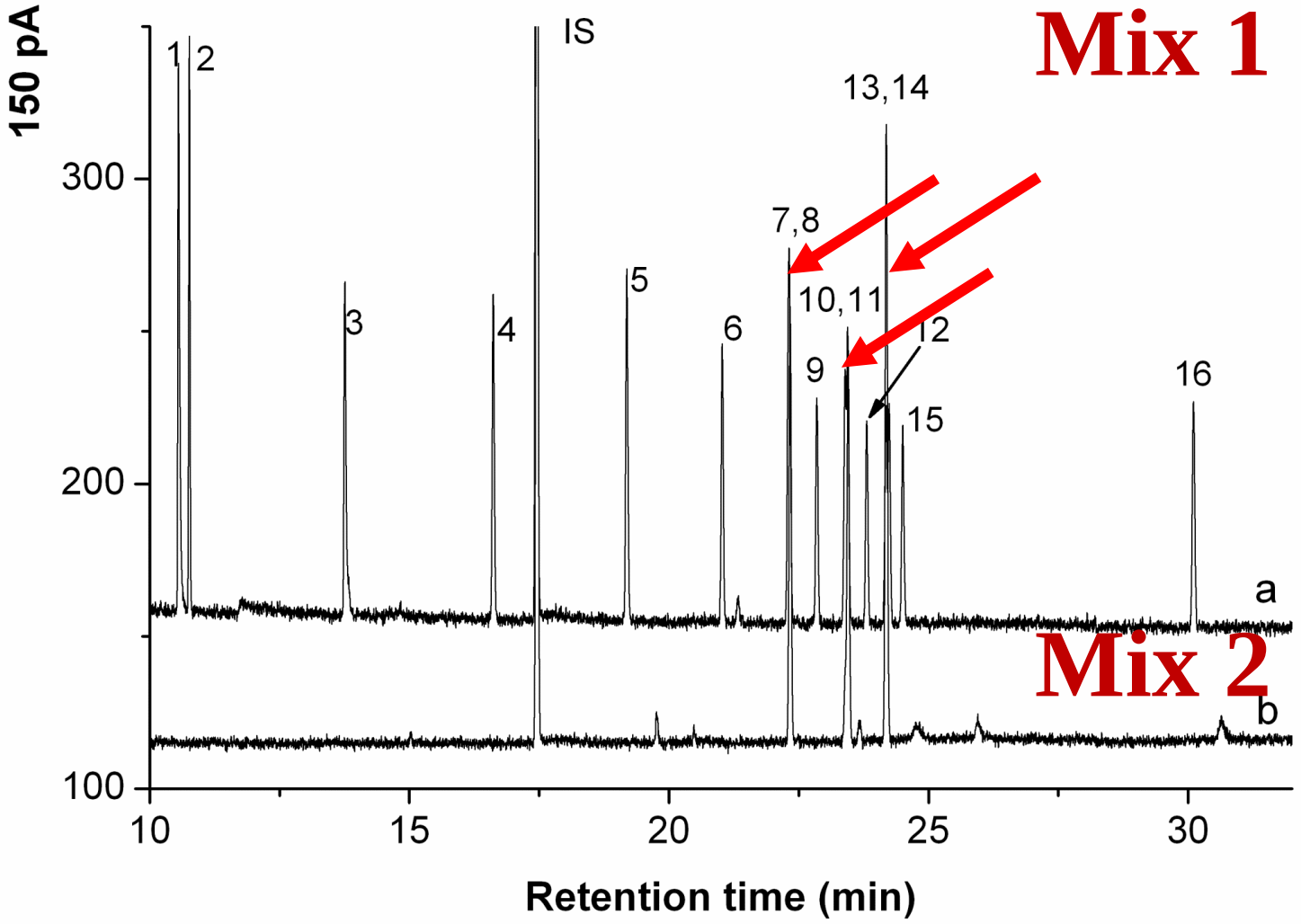
Cod Liver Oil Sample FAME



Many FAME in the CLO sample will overlap in 1D GC. Very many are resolved in GC×GC. And it might be possible to predict 'structure' from the peak pattern in 2D space… CLO is largely polyunsaturated - GOOD

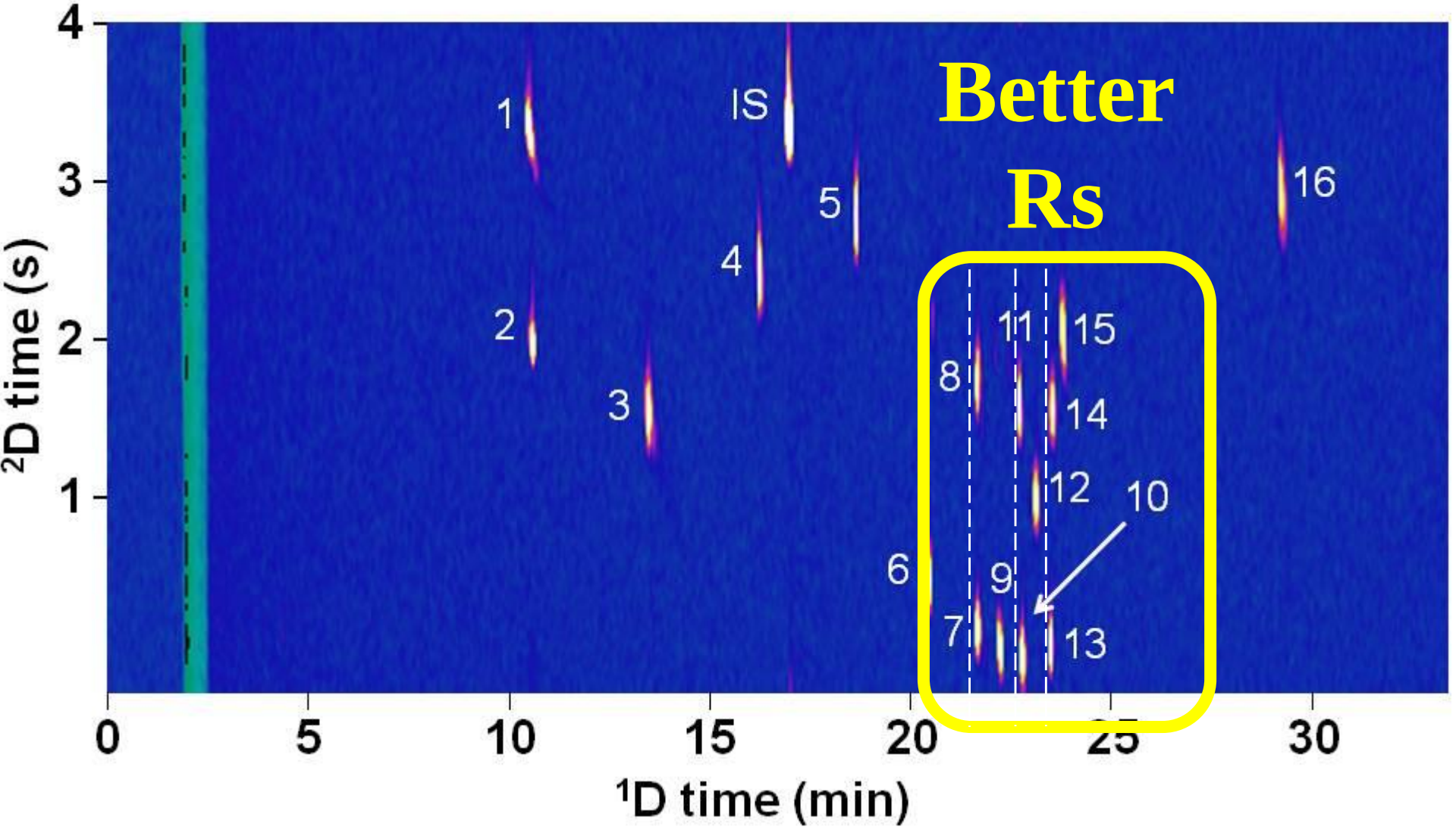
1DGC OP Pesticides in Vegetable.. (See GC Lecture)

OP Standards; even in this SIMPLE MIX (16 compounds), peaks do overlap. See the ARROWS



GC×GC OP Pesticides in Vegetable

Excellent overlay with position of standards



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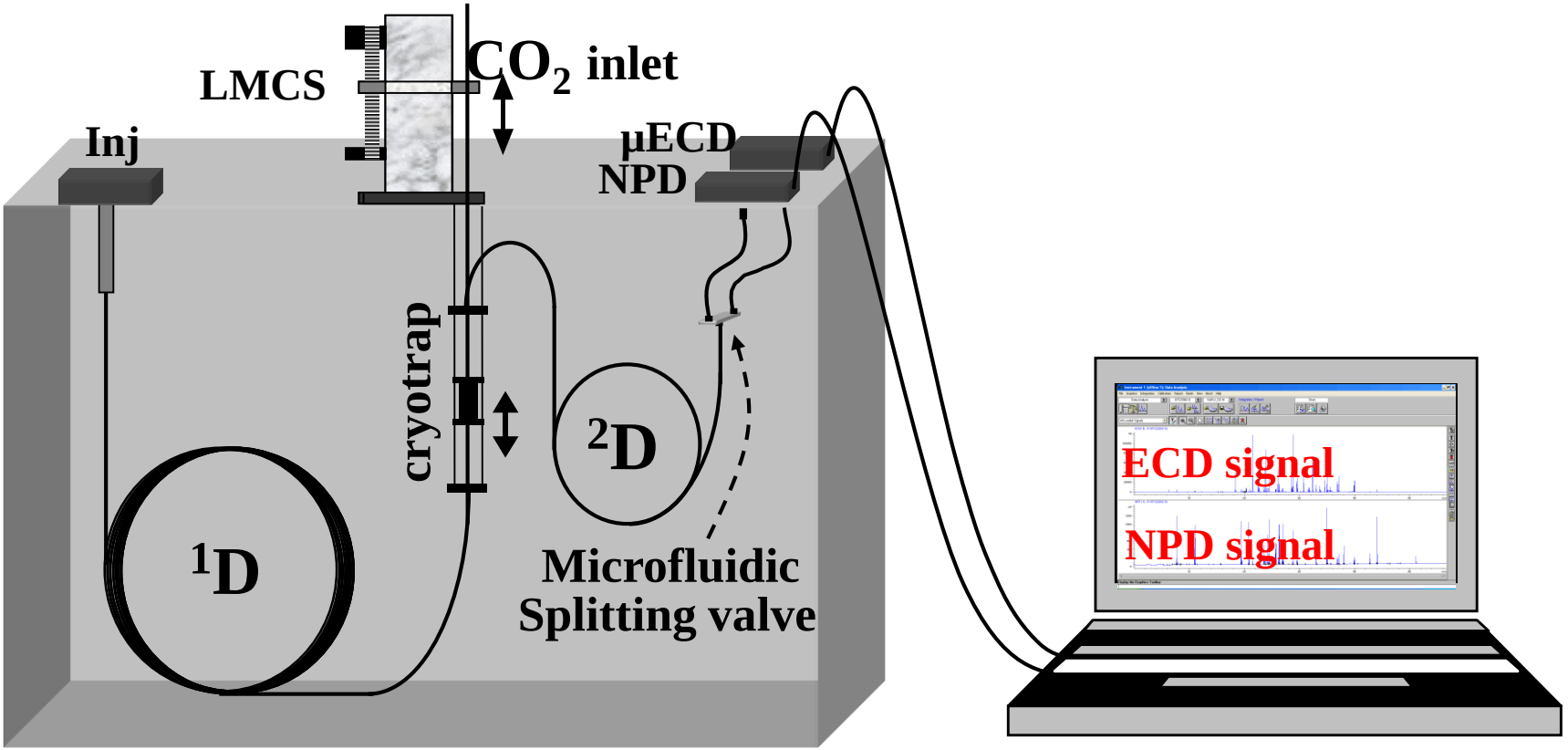
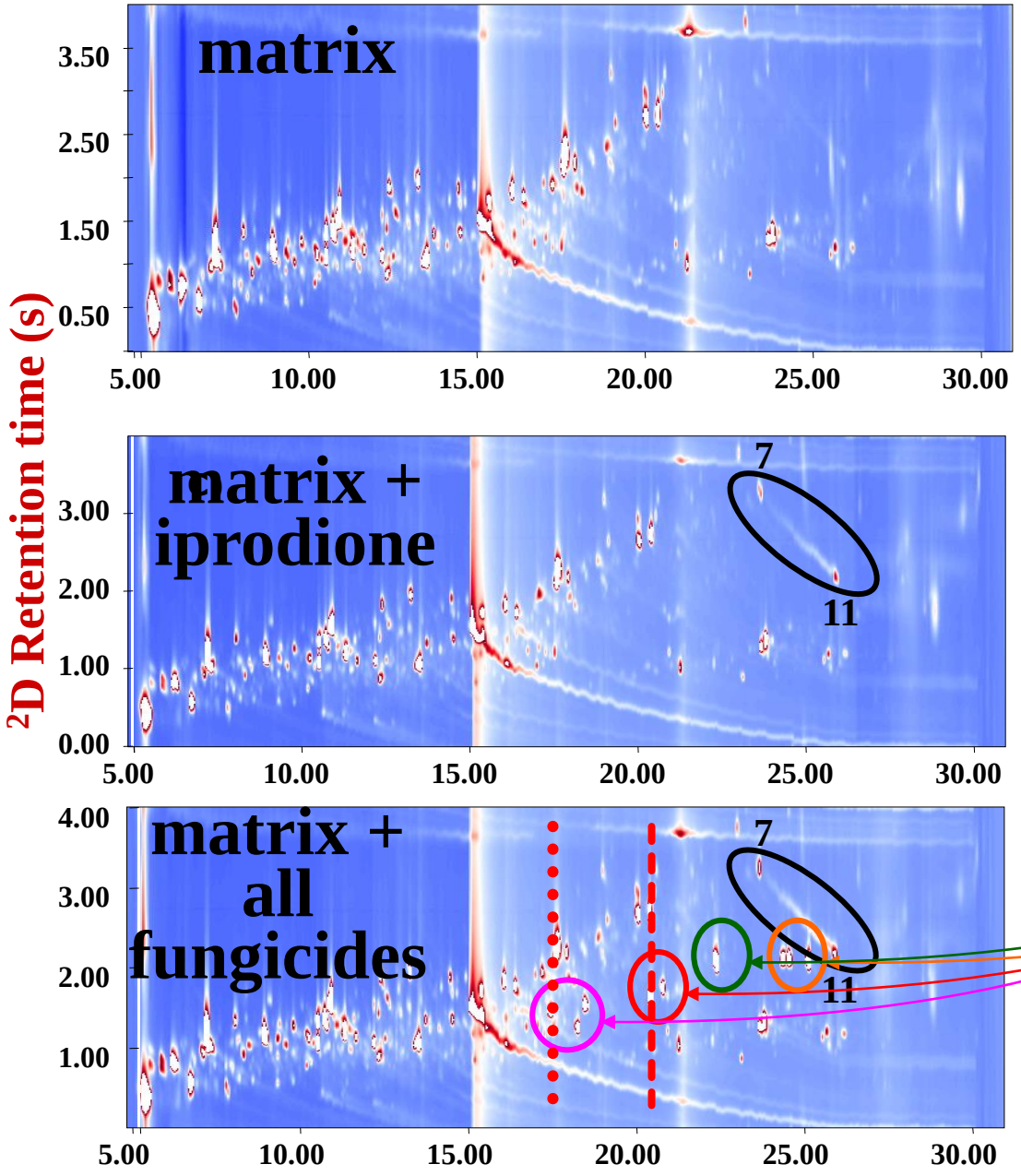
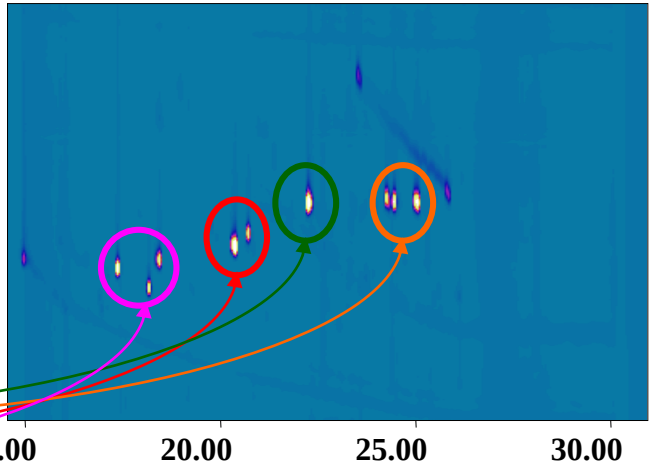
Fungicide
Dual Detection GC×GC system

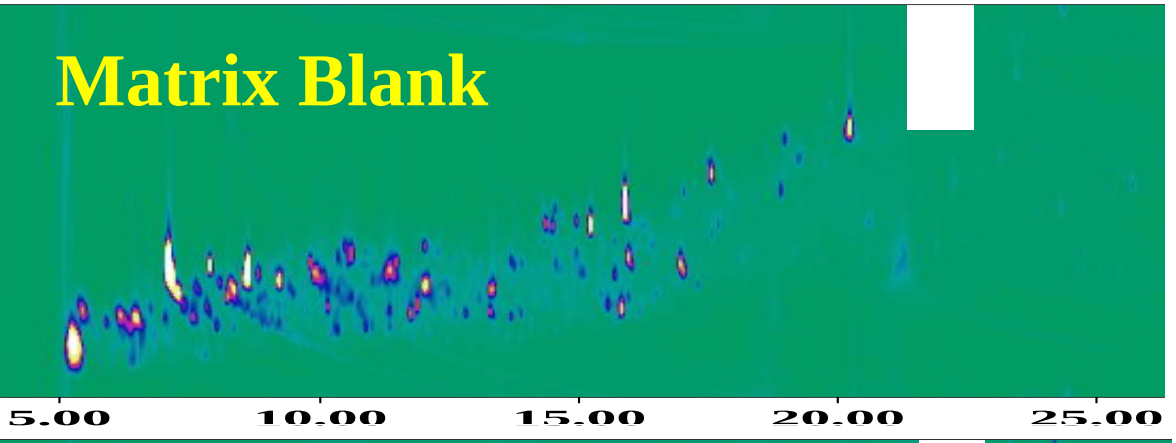


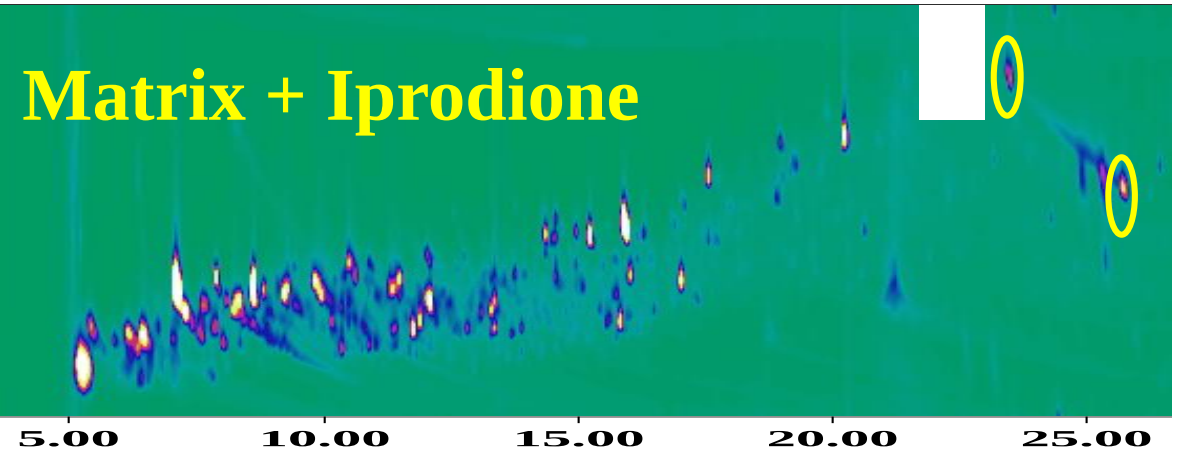
GC×GC-NPD

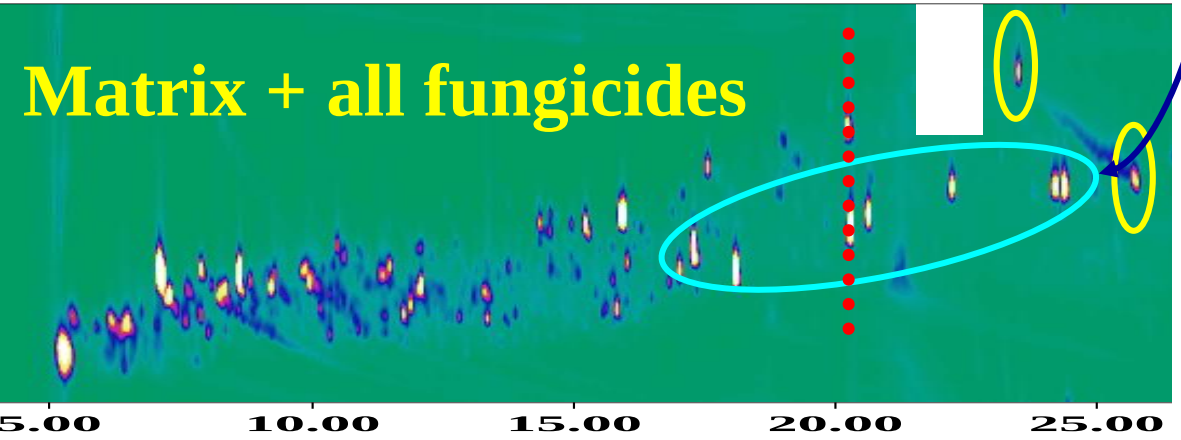


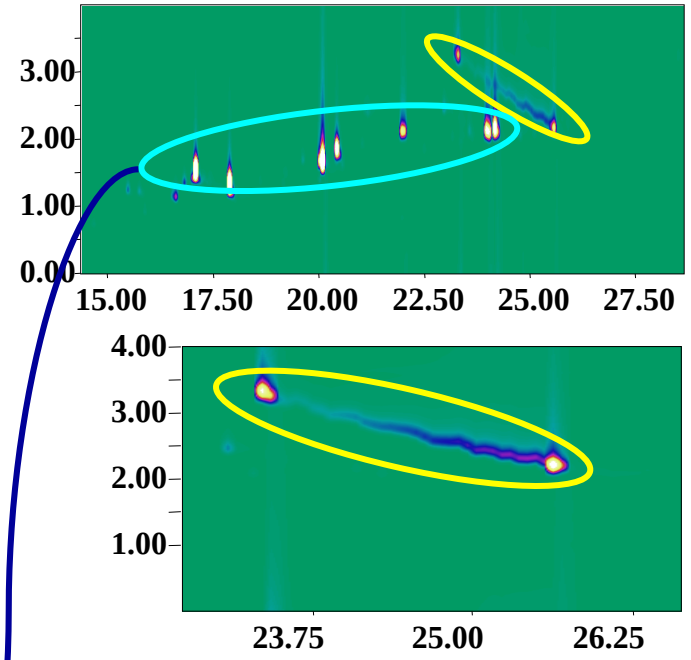
All the fungicides are well separated from the matrix when GC×GC is used.
In 1D GC, some matrix components exactly overlap a fungicide!











GC×GC-ECD

.. Unspecified overlap of target and matrix = problem for 1D GC

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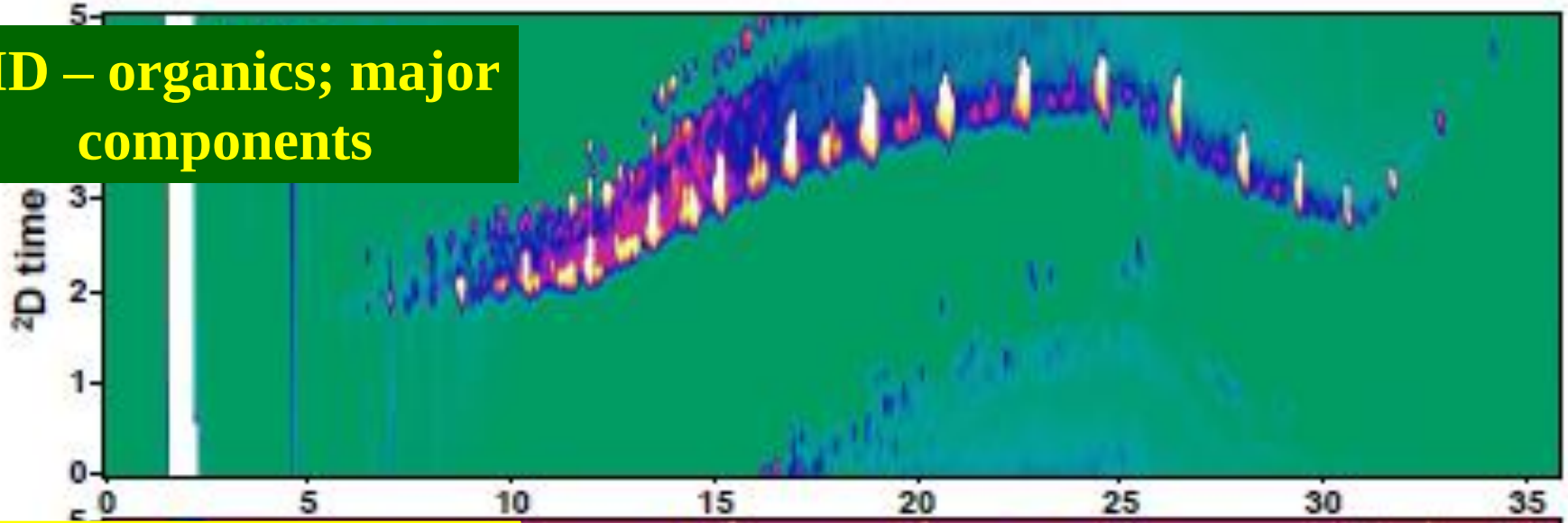
GC×GC-FPD HPO Mode

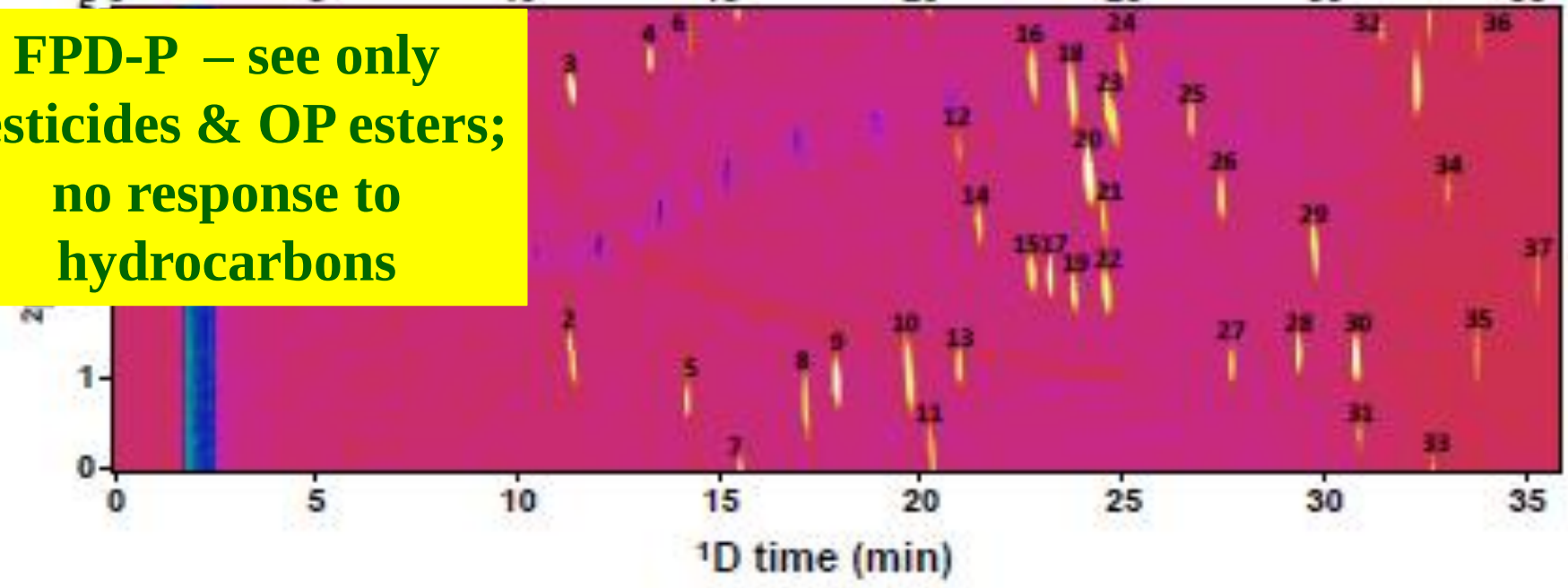
GC×GC performance using FPD-P detection

Model Study: GC analysis of OP Pesticides & Esters in a DIESEL matrix!
(a test for Chemical Weapons & Degradation Products)

FID – organics; major components



FPD-P – see only pesticides & OP esters; no response to hydrocarbons



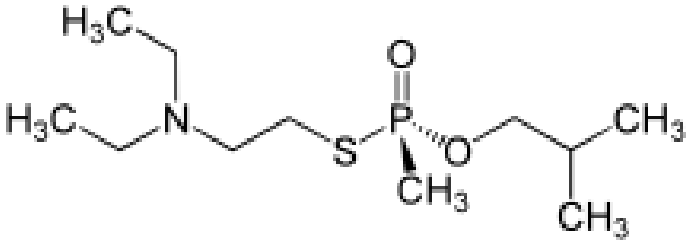
VX (= Russian VR) & Novichok ("Newcomer")

Novichok ("Newcomer"); Lethal, & Unusual; more dangerous & sophisticated than VX or Sarin.

[VX = Kim Jong Nam; Sarin = Tokyo Subway; & Syria]

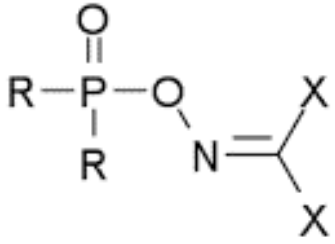
Hard to identify, since component parts are not specifically banned on Chemical Weapons Convention lists; and not readily measured by CWC Tests. Colourless, odourless & tasteless

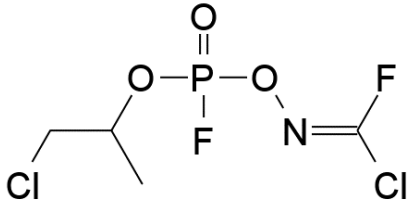
VX = $C_{11}H_{26}NO_2PS$;

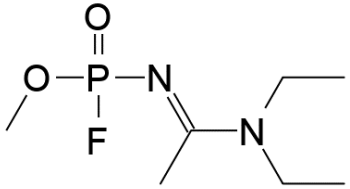


VX v toxic; OP class (thiophosphonate) - nerve agent. Developed for military use as CWA (translation of OP toxicity in pesticide research.

Novichok agents $C_4H_6NO_3PF_2Cl_2$ $C_7H_{16}N_2O_2PF$







Acetaldoxime @ 80°C; 5psi; See GC Lecture