Challenges in Forensic Analyses

Same or Different?

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Petroleum Forensics

- Required to determine sources of contamination.
- Want to know what the contamination is, where it came from, and approximate time of release.
- Questions are driven by insurance underwriters and the legal profession.
- If questions are answered appropriately then cleanup can proceed and cost sharing agreed upon.
- Considering the high cost of cleanup, the information has to be legally defensible, *i.e.*, no discrepancies that can be challenged. Otherwise cleanup will be delayed.

How Do We Perform Forensic Investigations?

- Search the literature and find:
  - ASTM methods.
  - Peer reviewed publications.
  - Books.
  - European/International Standards.
- Have hands on experience.

Methods used in the United States

- Following the Deepwater Horizon spill saw a flurry of lab activity Most labs involved in petroleum forensics use ASTM methods, primarily, ASTM-D3328 and ASTM-D5739. **Currently recommended by US-Coast Guard.**

- **These methods are "qualitative" and rely on profile matching of chromatograms obtained using GC/FID and GC/MS**. Weathering can cause problems with data interpretation.

- Labs specializing in petroleum forensics in the U.S. (*e.g.*, Newfields Environmental Forensics Practice, Woods Hole Oceanographic Institution, etc.) use CEN/TR 15522-2: 2012. This method is qualitative, quantitative and performs weathering checks. These labs are a member of OSINET (Oil Spill Identification Network of Experts).

METHODS used in CANADA

- **Environment Canada uses CEN/TR 15522-2: 2012**
- Several Environment Canada labs are members of OSINET.
- Private labs offer a variety of services under the umbrella of "forensics".
- No consistency among private labs. Some use profile matching of chromatograms obtained using GC/FID and GC/MS. Some use GC/MS scanning and identify compounds using NIST library searches. Process used usually based upon least cost to customer.
- Some use petroleum biomarker ratios .
- **Birkholz/Life Science Forensics uses CEN/TR 15522-2:2012.**

METHODS Used in Europe and the International community

- **CEN/TR 15522-2: 2012. This will likely be made an ISO standard soon.**
- Used by 79 members of OSINET (Oil Spill Identification Network of Experts) from around the world.
- Information obtained is used in global prosecutions.
- Method has been updated several times. Round robin studies conducted annually for the last ten years. We learn something new from every study.
- Birkholz is a member of this group and continues to participate in round robin studies. **2015 round robin conducted by Life Science Forensics, Calgary, AB.**

Private Lab Forensic Protocol

- Common procedures advocated include: analyses using GC/FID, and GC/MS (scanning and SIM).
- Data interpretation techniques include: profile matching of chromatograms, interpretation of mass spectra, extracted ion-current profiles and ratios for specific compounds including: alkanes, isoprenoids, aromatic compounds, petroleum biomarkers, and PAHs.
- This is viewed as plausible by laboratories engaged in routine testing.

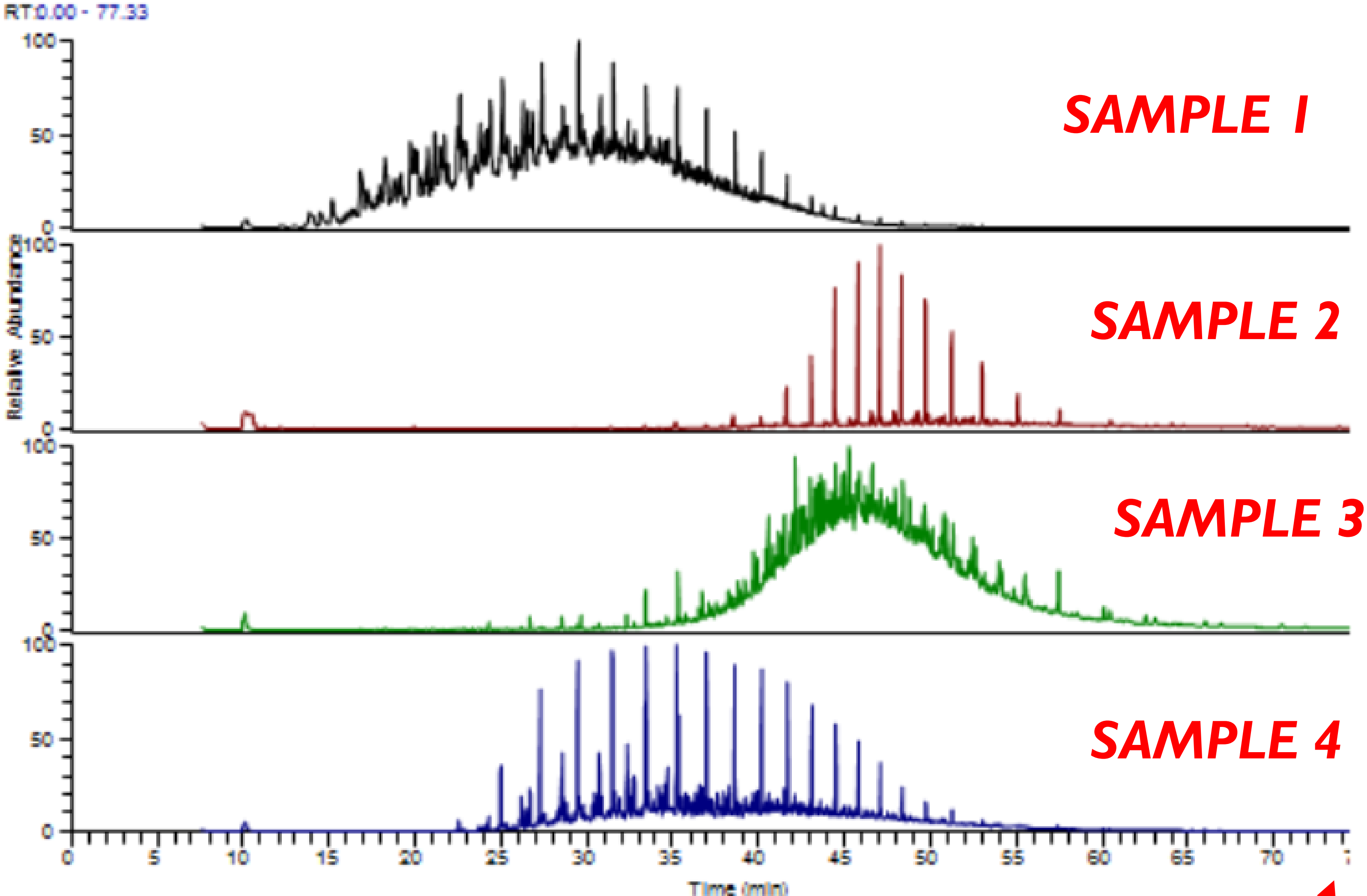
Example of Misuse of Data

- Chromatographic profiles, compound specific ratios, including PAHs were used following the Deepwater horizon spill.
- The objective was to demonstrate that oil found along the Gulf coast (Louisiana, Alabama, Mississippi and Florida) was in fact MC-252.
- A lot of the data caused confusion, and resulted in the belief that oil found anywhere had to be from BP.
- White House organized a conference (JOST) where leading scientists were invited to review findings.
- Much of that data was rejected, especially that relying on PAH ratios and profile matching. Even some biomarker data was rejected (triaromatic steranes).
- Result, a lot of money paid by stakeholders and law firms did not result in outcome expected.

Forensic Protocol

- Can be simple and straightforward especially if very differing products are observed.
- Profile matching can work and is cost-effective.
- Can be challenging if we are dealing with similar products. Depending upon findings can be more costly because of increased need for analyses and data interpretation.

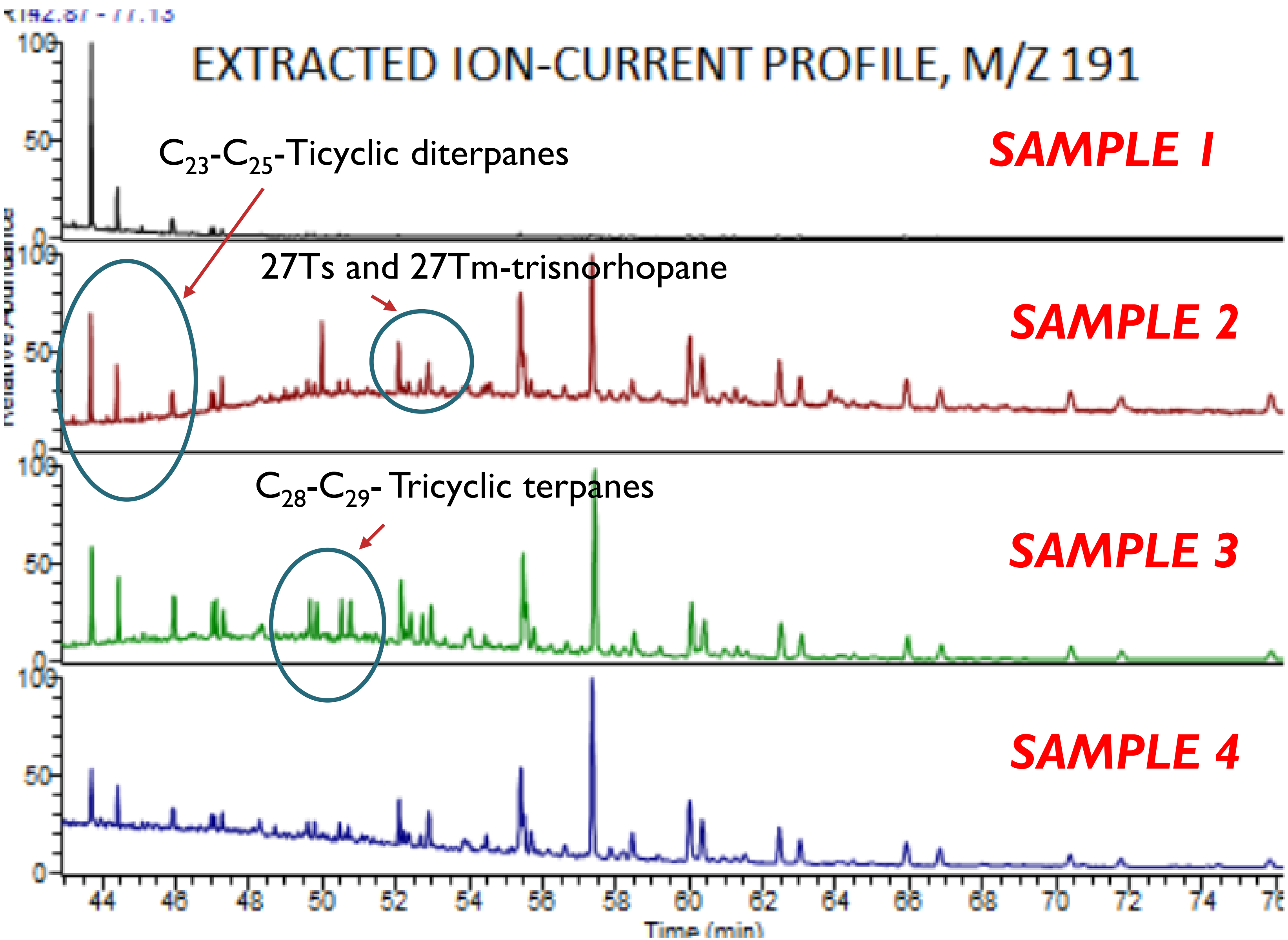
DIFFERING PRODUCTS



TOTAL ION CHROMATOGRAM

Note 80 min run

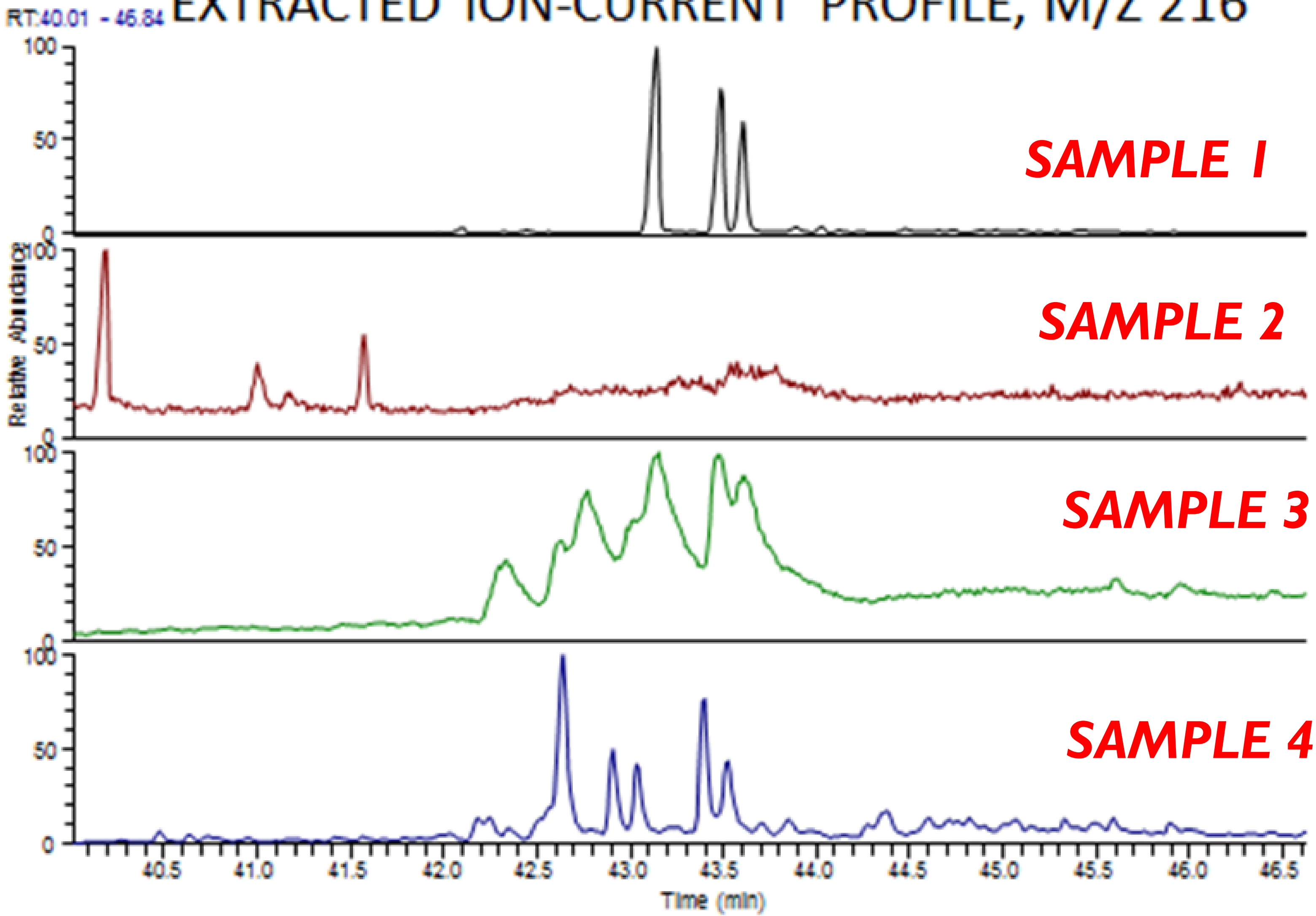
DIFFERING PRODUCTS



Tricyclic terpanes and hopanes

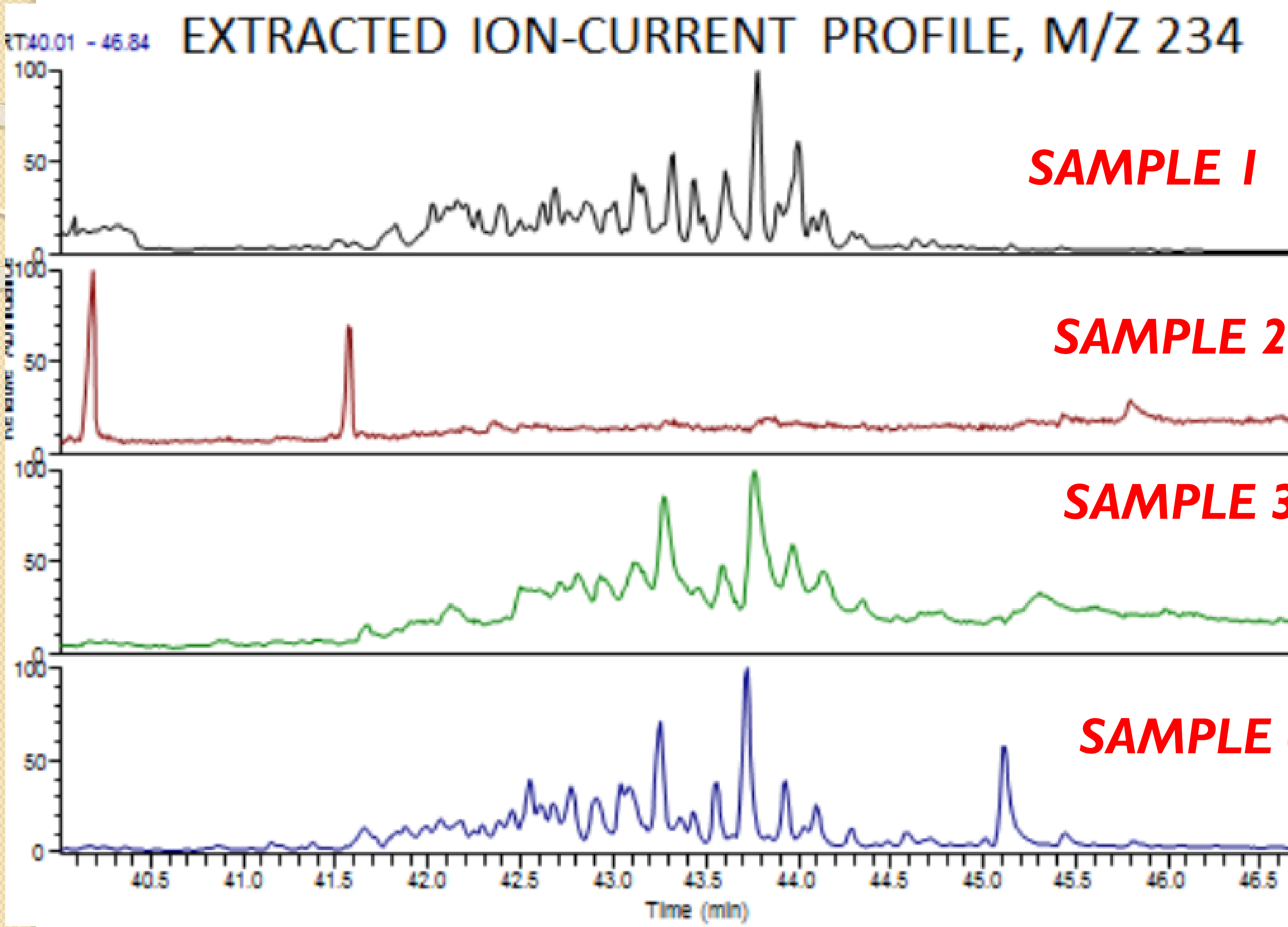
DIFFERING PRODUCTS

EXTRACTED ION-CURRENT PROFILE, M/Z 216



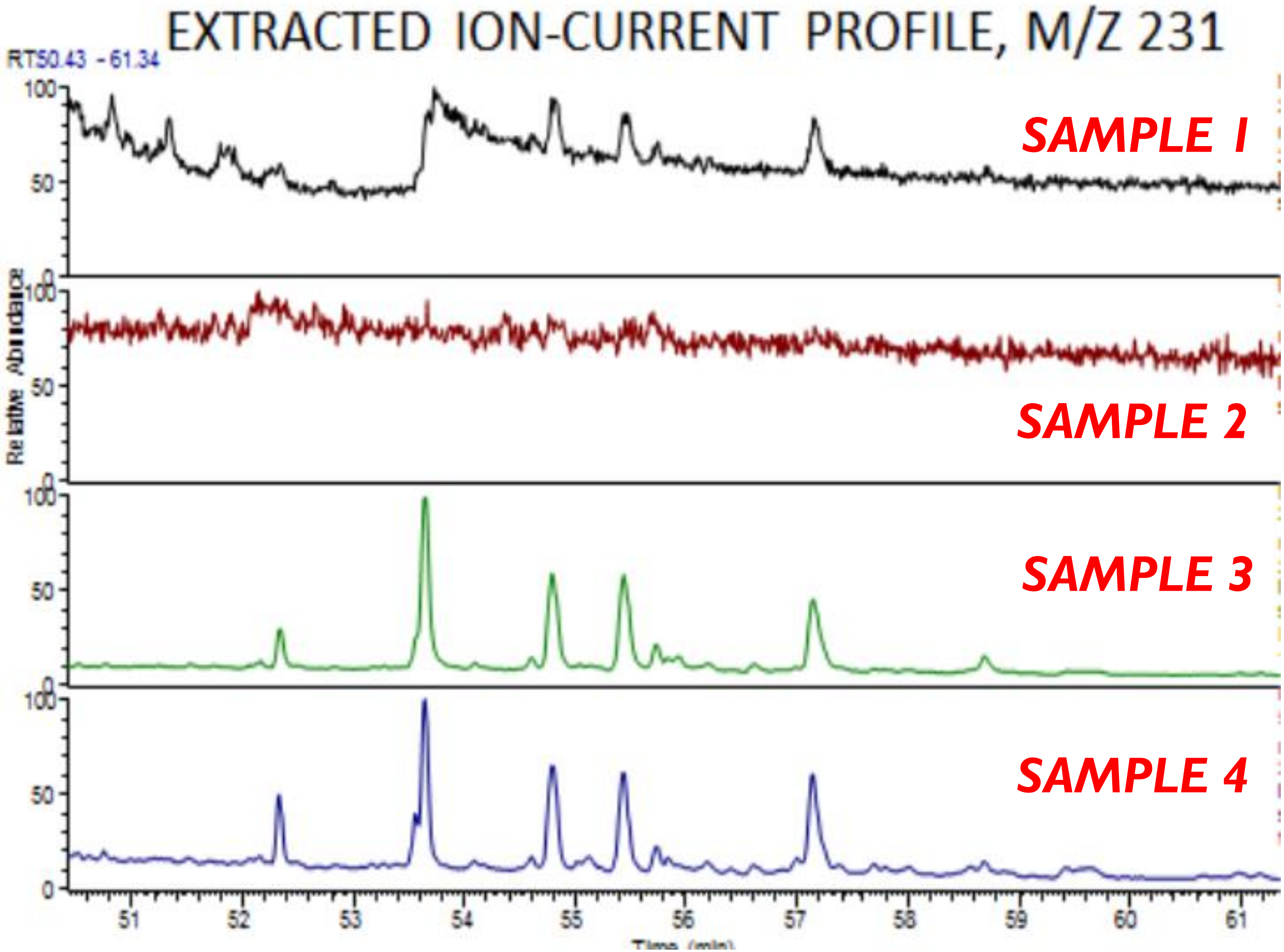
Methyl-fluoranthenes, -pyrenes, -benzofluorenes

DIFFERING PRODUCTS



Retene, tetramethylphenanthrene, benzo[b]naphtha[1,2-d]thiophene

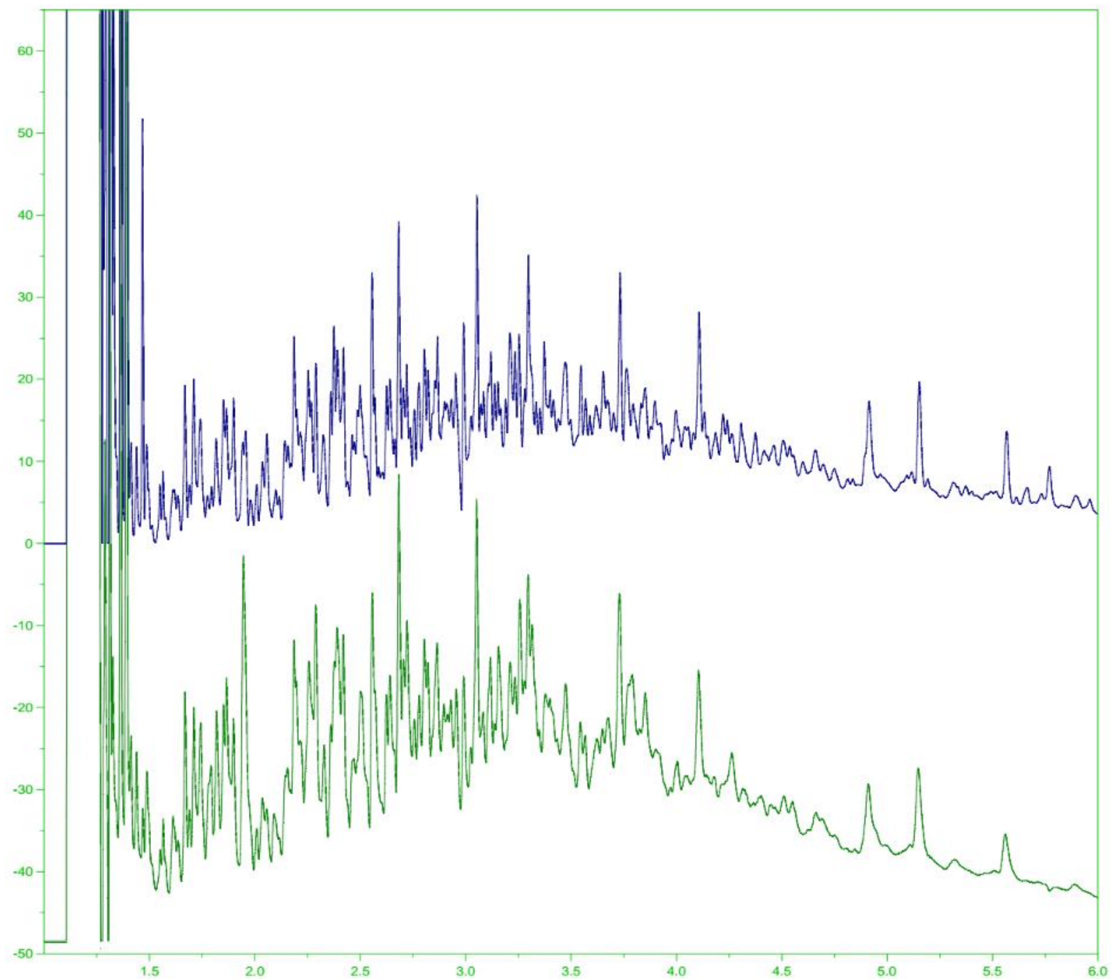
DIFFERING PRODUCTS



Triaromatic steranes

Are these the same product?

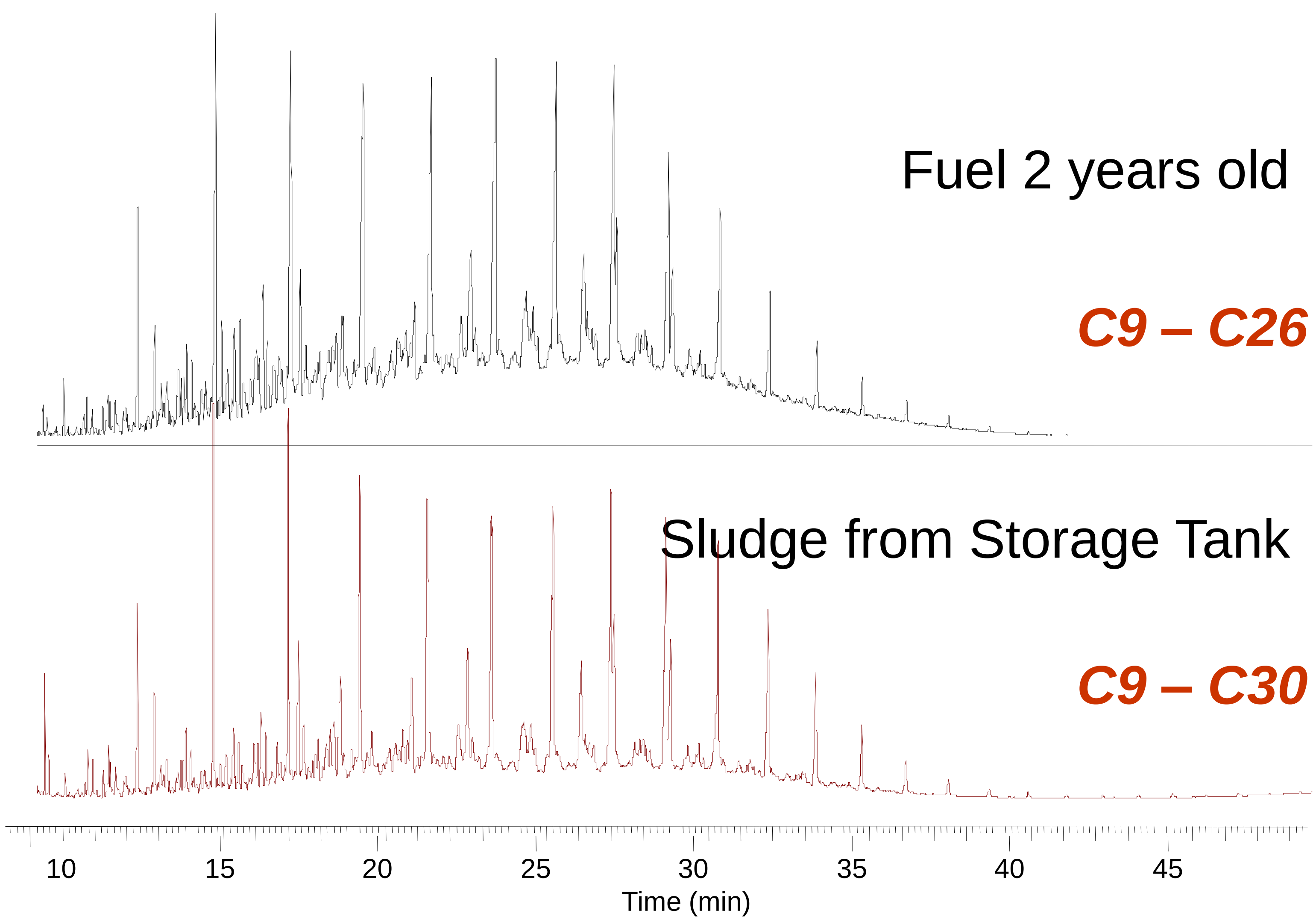
SIMILAR PRODUCTS



6 min run

GC/FID chromatograms

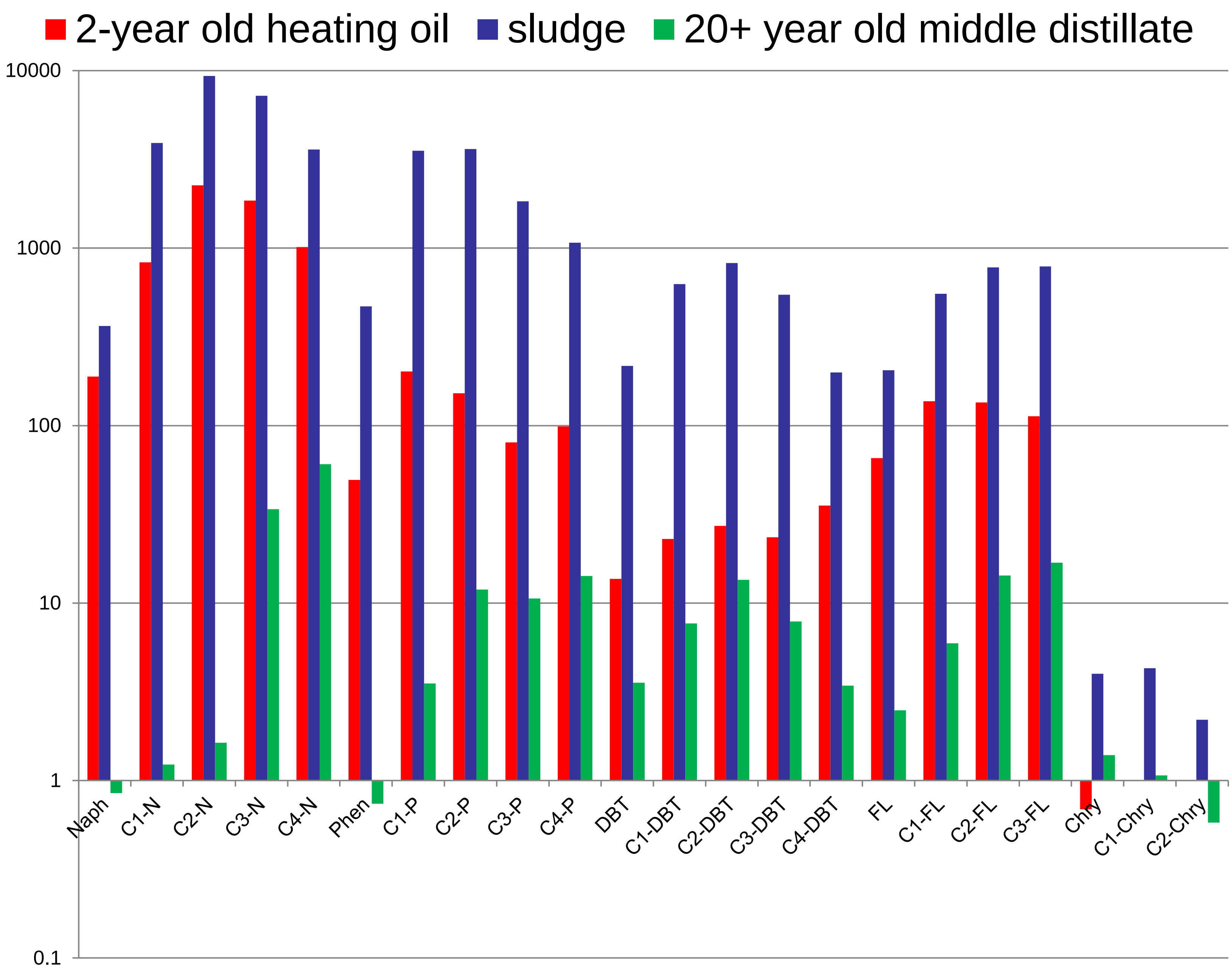
SIMILAR PRODUCTS



ARE THESE DERIVED FROM THE SAME SOURCE?

GC/FID Data

Sample	Pristane/ Phytane	$n\text{-}C_{17}$/pristane	Age (years)
Fresh	1.61 ± 0.19	Ratio based on N.J. 2002-2007 From Oudijk, 2009	
Moderate	1.73 ± 0.21		
Degraded	1.59 ± 0.23		
Very degraded	1.18 ± 0.40		
2-year old fuel	1.42	1.95	3.4 ± 2
Sludge	1.19	1.85	4.3 ± 2
Critical Difference	No Match	Match	

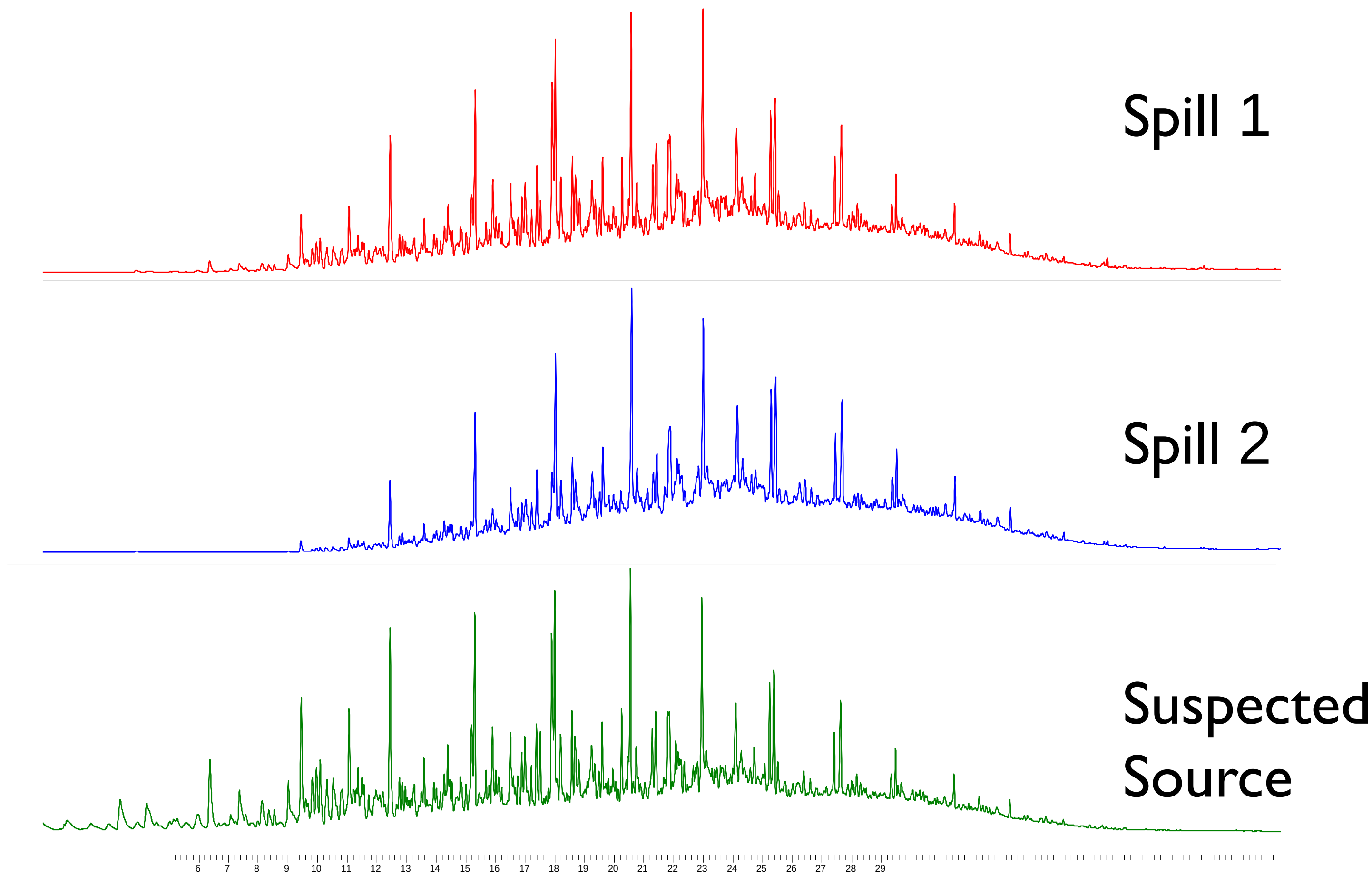


2-year old heating oil
sludge
20+ year old middle distillate
10000
1000
100
10
1
0.1
Naph
C1-N
C2-N
C3-N
C4-N
Phen
C1-P
C2-P
C3-P
C4-P
DBT
C1-DBT
C2-DBT
C3-DBT
C4-DBT
FL
C1-FL
C2-FL
C3-FL
Chry
C1-Chry
C2-Chry

PAH Distribution

PAH Compounds	2-year fuel	sludge
Alkylated naphthalenes/alkylated phenanthrenes	10.5	2.3
Alkylated phenanthrenes/Total PAHs	0.08	0.27
Alkylated Dibenzothiophenes /Total PAHs (%)	1.7	6.1
Percent 3-ring PAHs	5.8	7.4
Percent 4-ring PAHs	0.4	3.3
Percent 5-6 ring PAHs	0.1	0.1

SIMILAR PRODUCTS



Are these spills from the Suspected Source?

Forensic Protocol

- Can be very challenging if products are the same type of product.
- Conclusion by consultant working on this site was wrong – based on data obtained from routine testing lab.
- Concluded this material was from a common source.
- Both spills derived from differing sources and were released at different times.

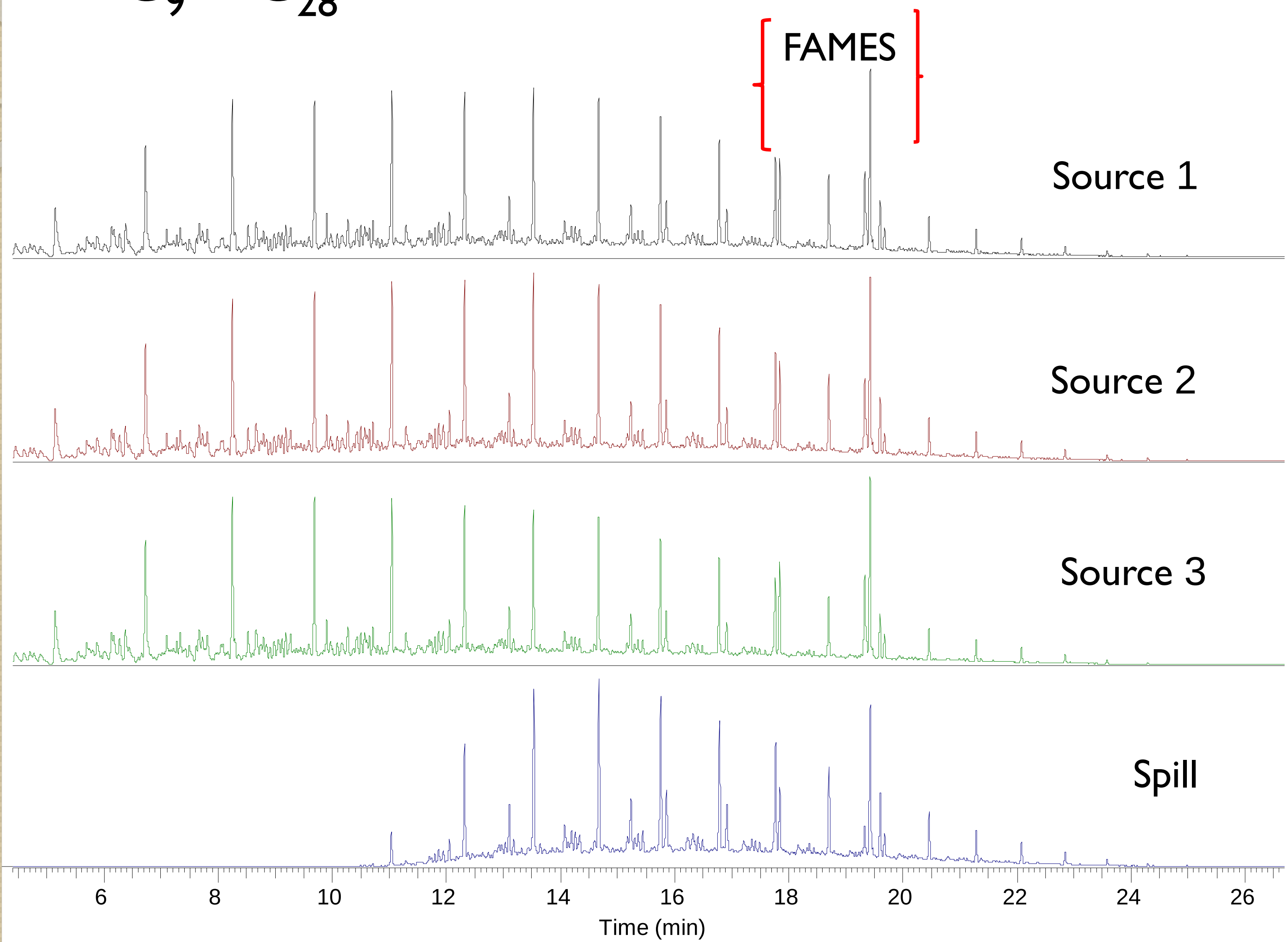
Comment from senior scientist (US), client and colleague – CURRENT STATE OF FORENSICS

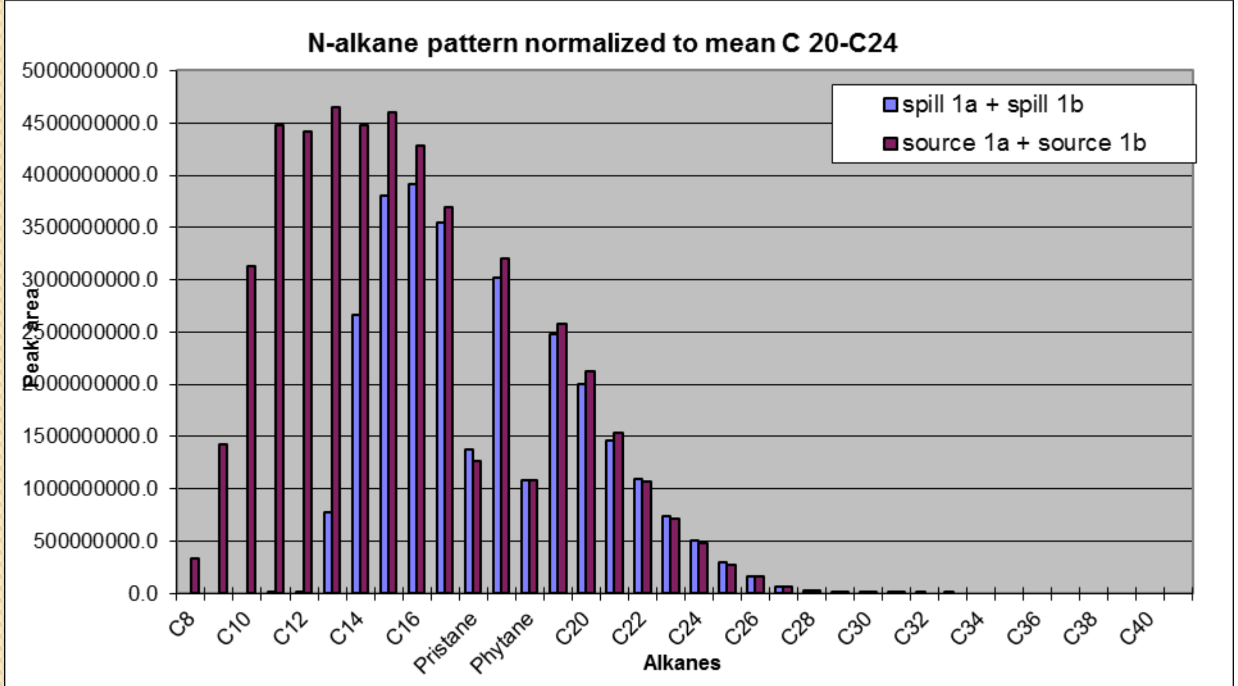
- Some labs have already forgotten how to test for high-quality crude petroleum fingerprints.
- I can only shake my head.
- The reason is you have to continually perform investigations to stay on top of protocols.
- Another reason: pressure to reduce costs means protocols are changed which can be a disaster .

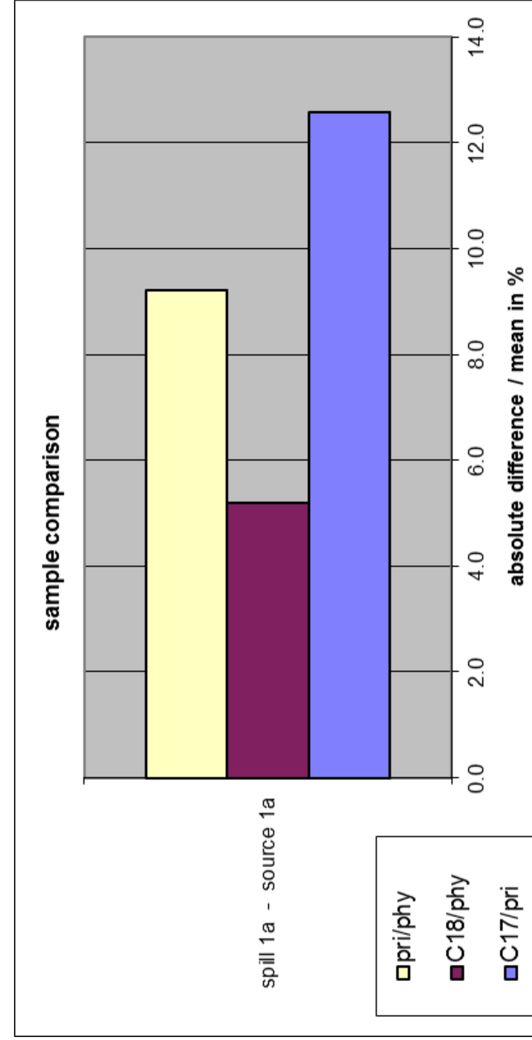
Challenge

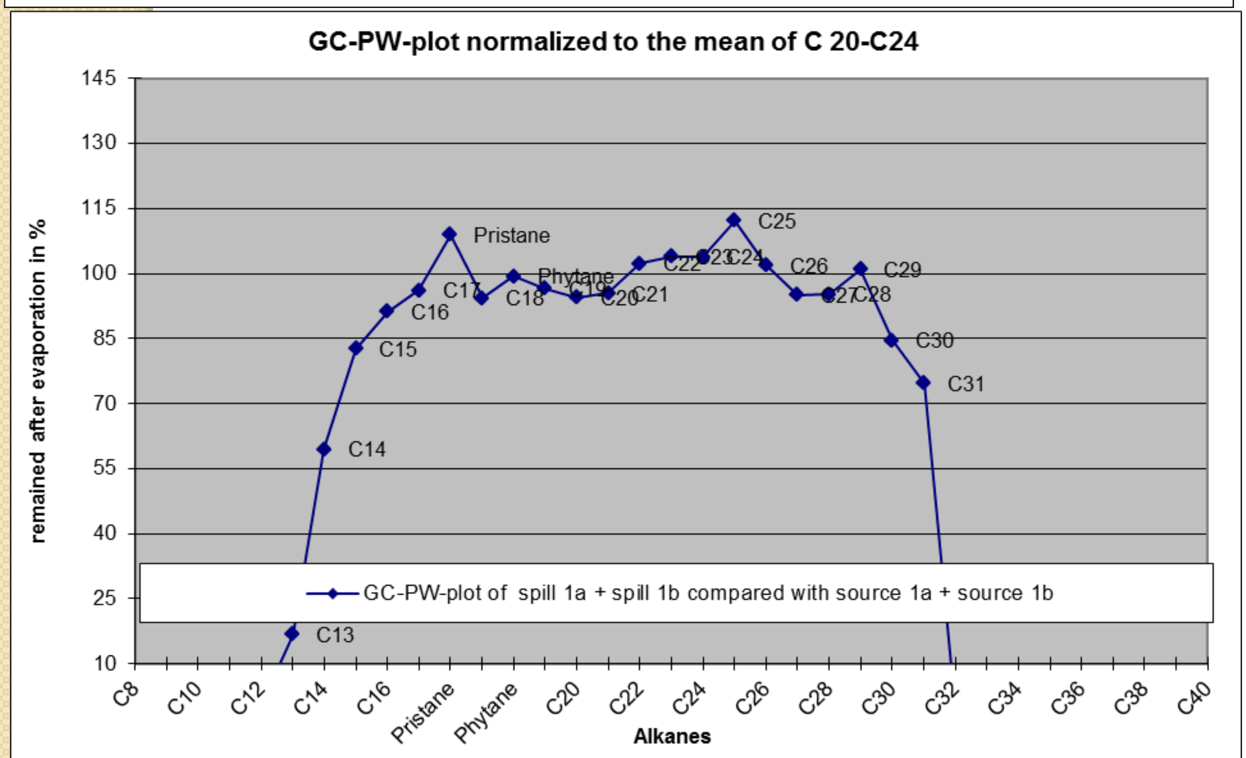
- We were provided three source samples and one spill sample.
- The challenge was to determine which source was responsible for the spill.
- We were also challenged to determine the relationship between source samples.

$C_9 – C_{28}$


FAMES
Source 1
Source 2
Source 3
Spill
6
8
10
12
14
16
18
20
22
24
26
Time (min)





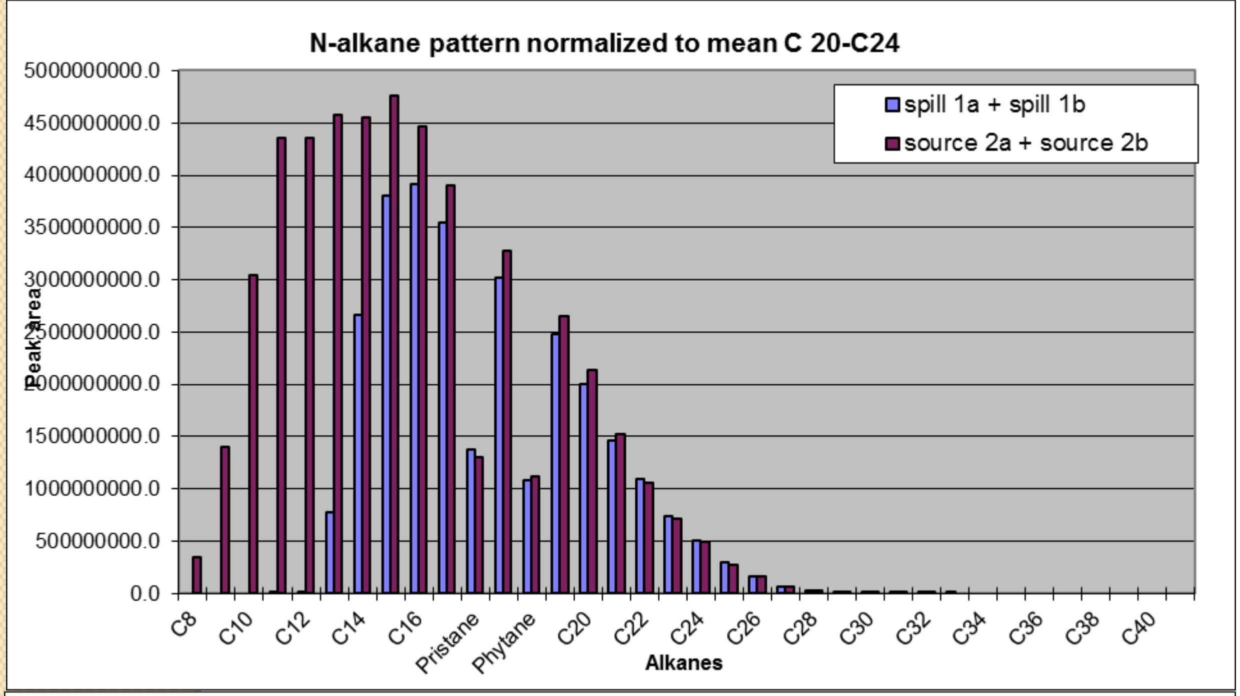


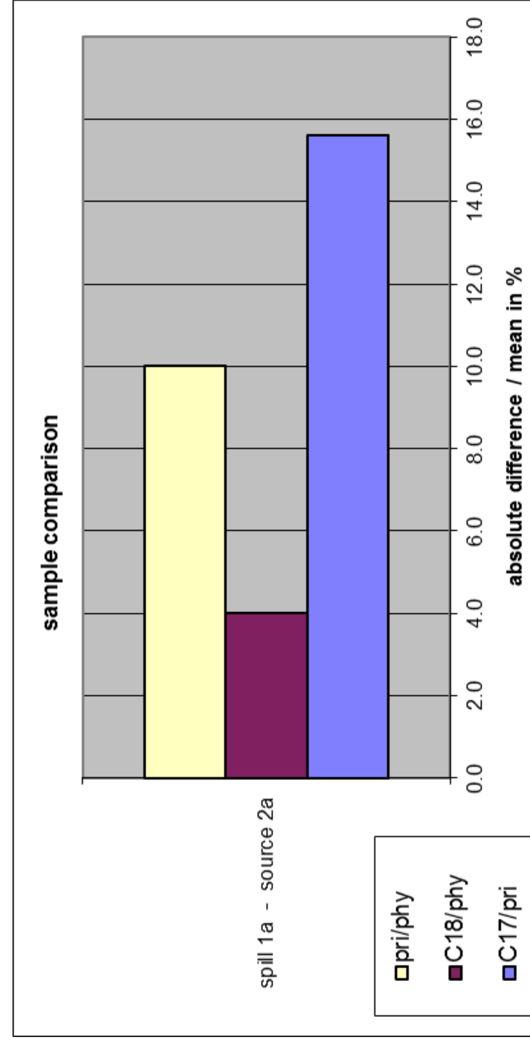
GC/MS – SIM

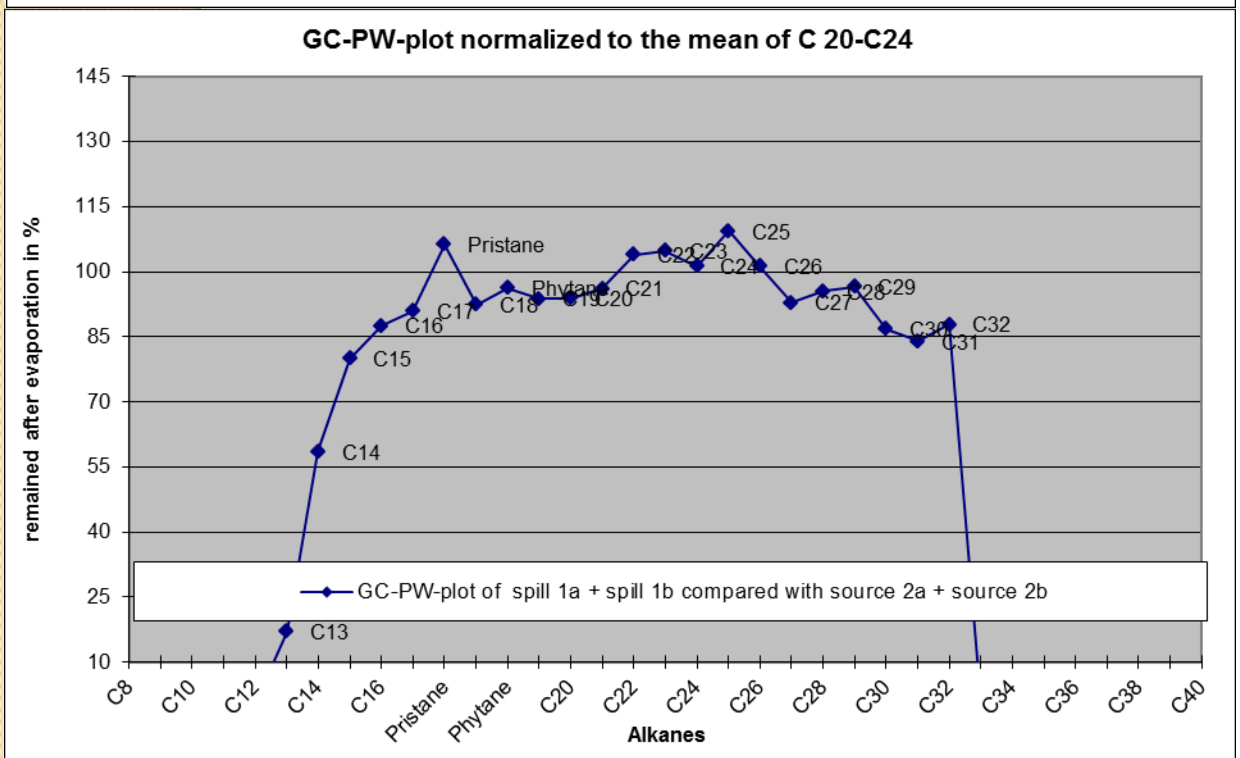
Pris/phy = 0.32%

C_{18}/phy = 10.59%

C_{17}/pris = 10.85%





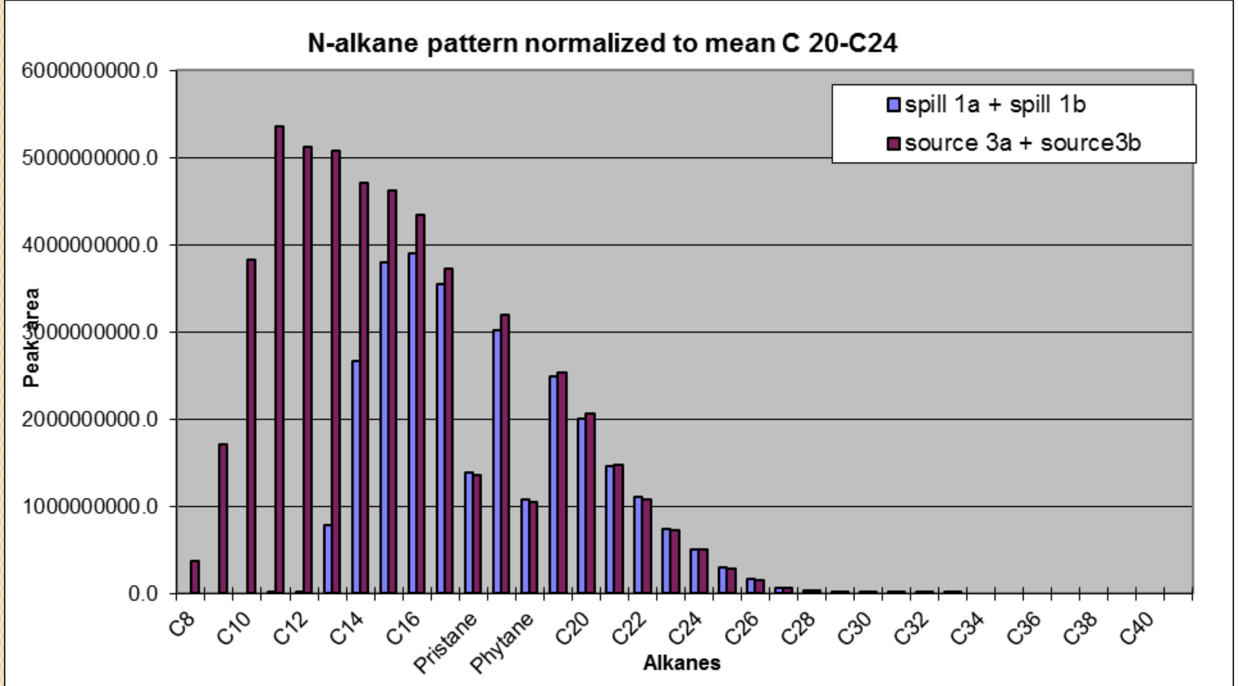


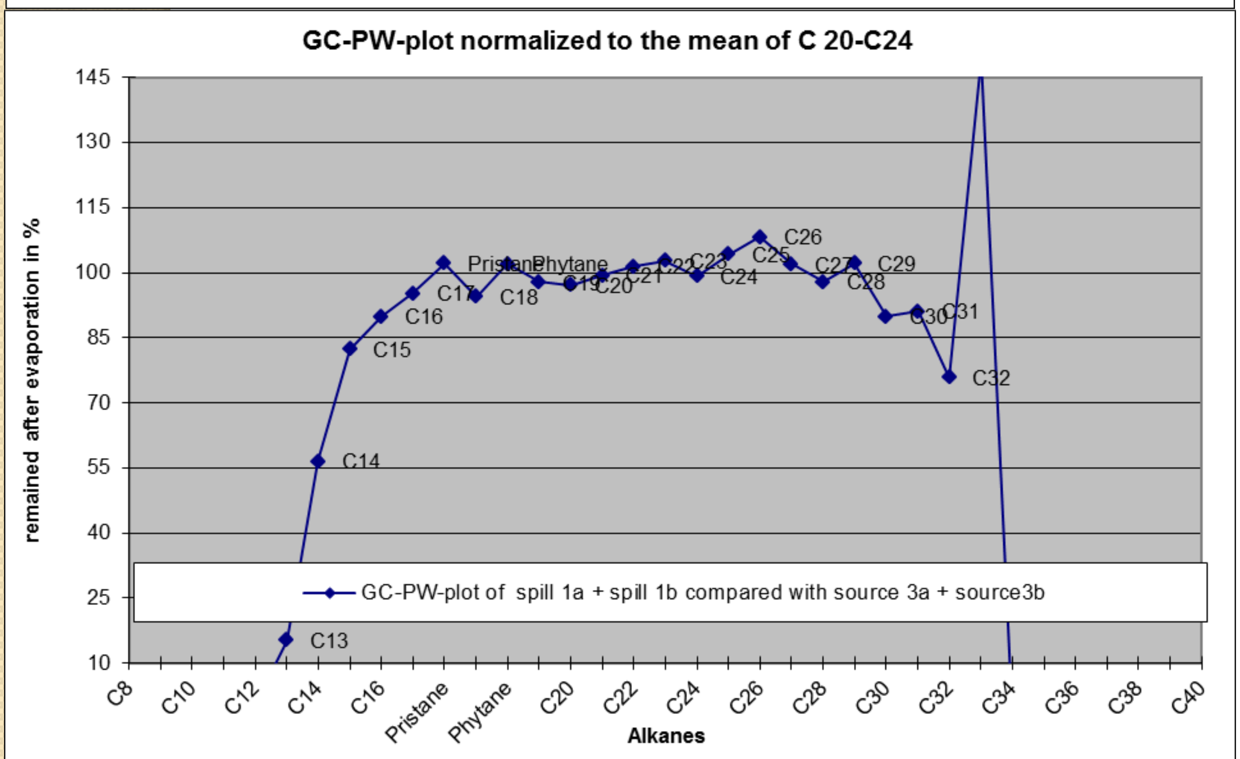
GC/MS – SIM

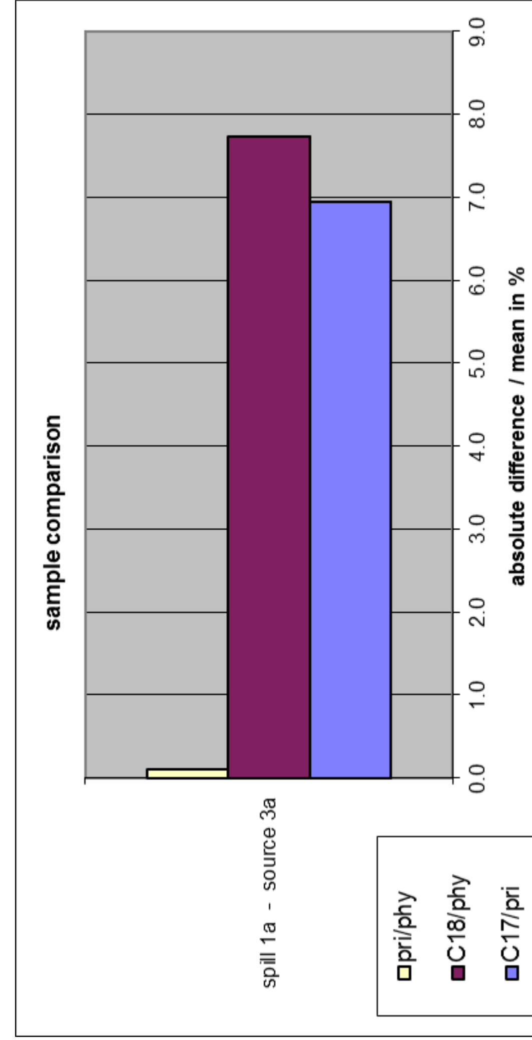
Pris/phy = 5.20%

C_{18}/phy = 7.17%

C_{17}/pris = 11.86%







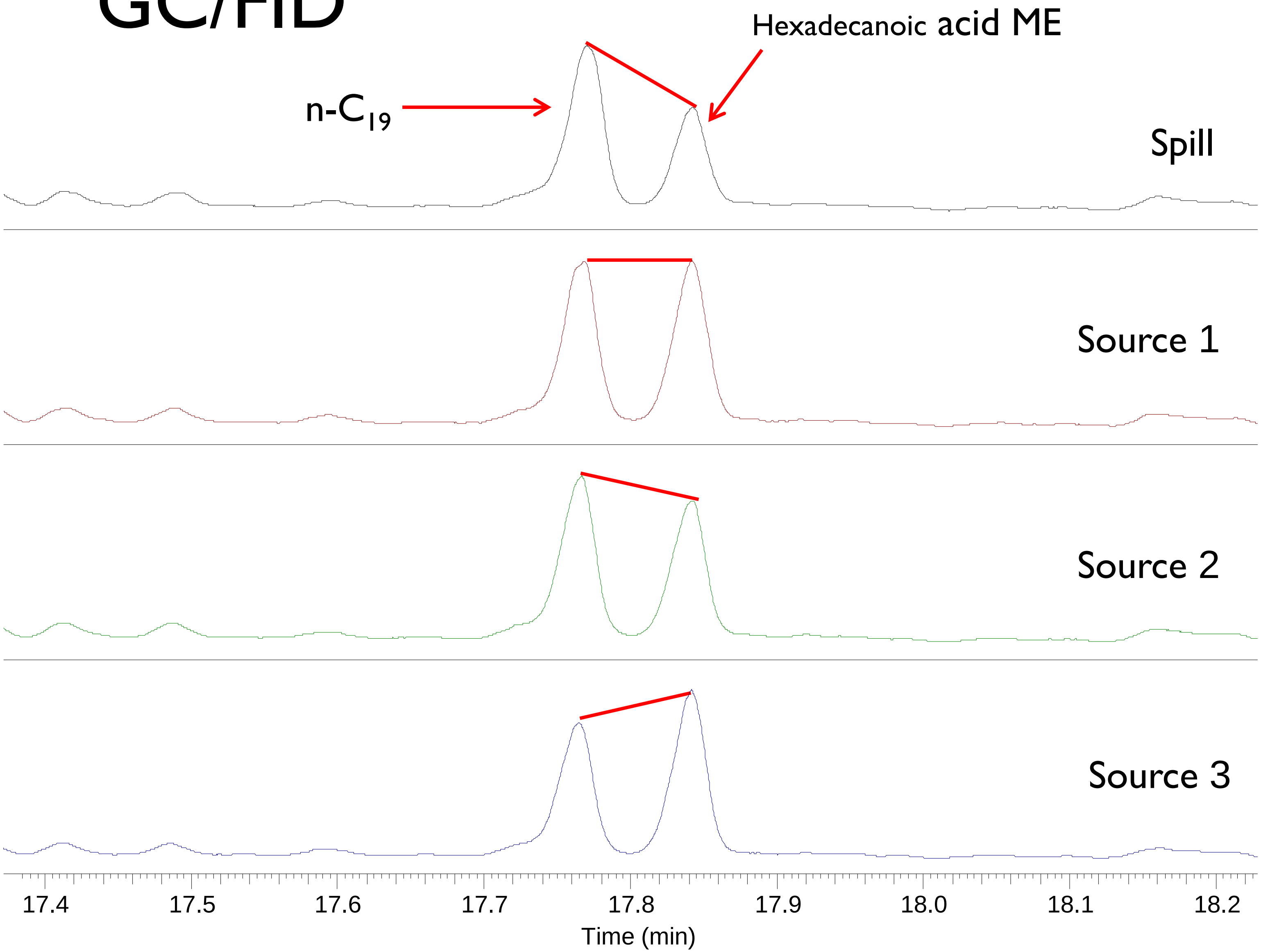
GC/MS – SIM

Pris/phy = 4.11%

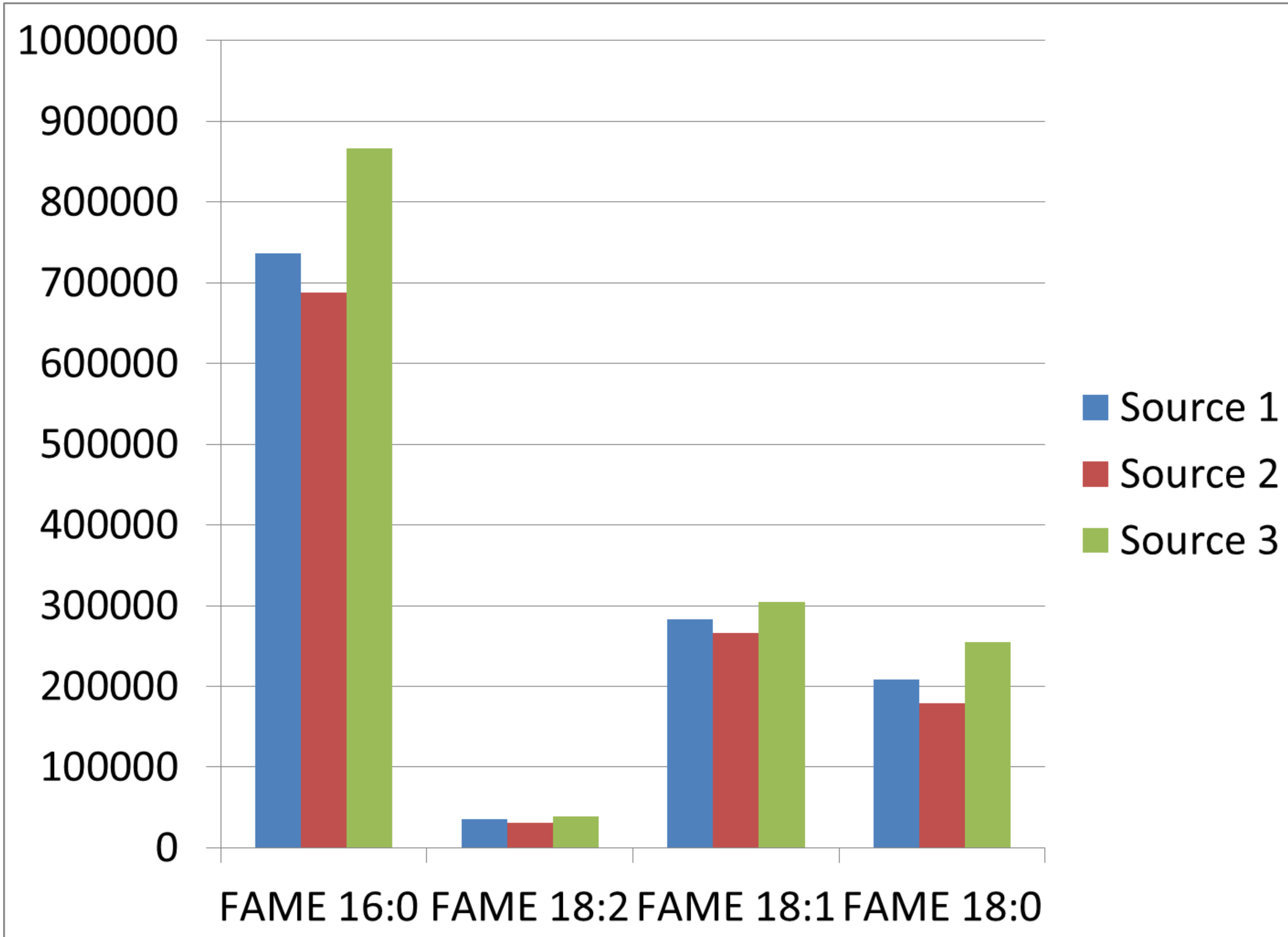
C_{18}/phy = 11.31%

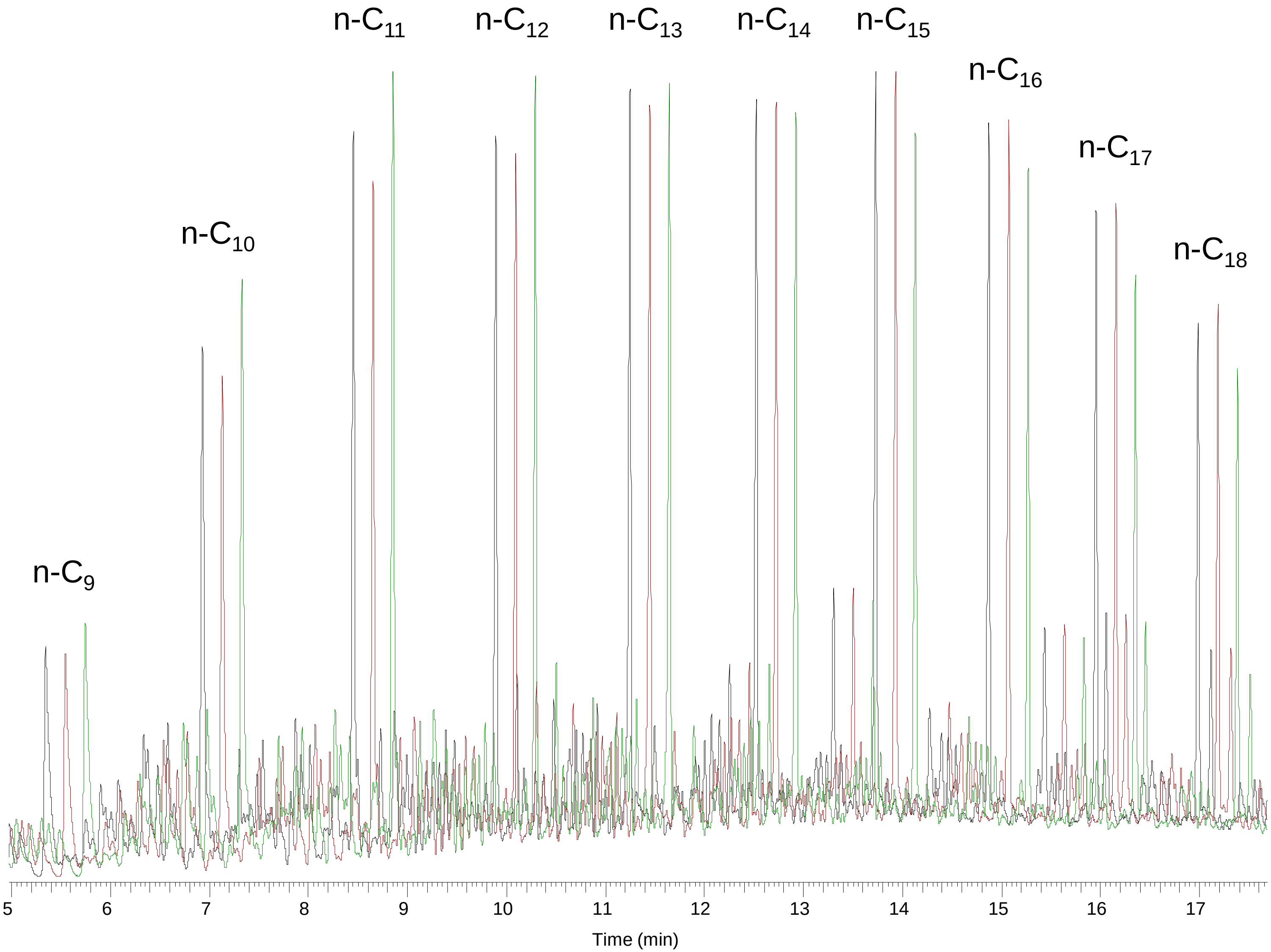
C_{17}/pris = 7.64

GC/FID



Source 1 likely a mixture of source 2 and source 3

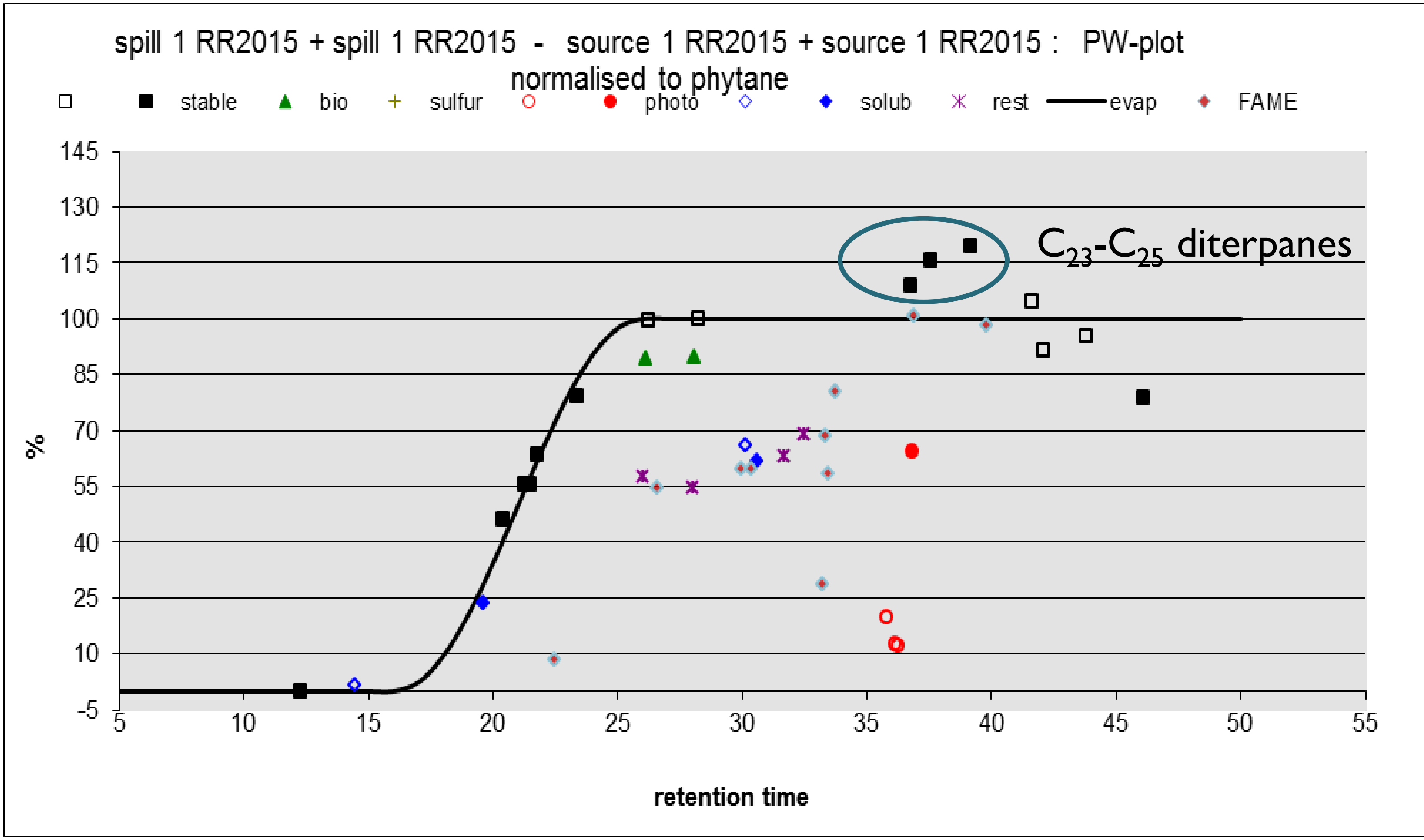

1000000
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
FAME 16:0
FAME 18:2
FAME 18:1
FAME 18:0
Source 1
Source 2
Source 3



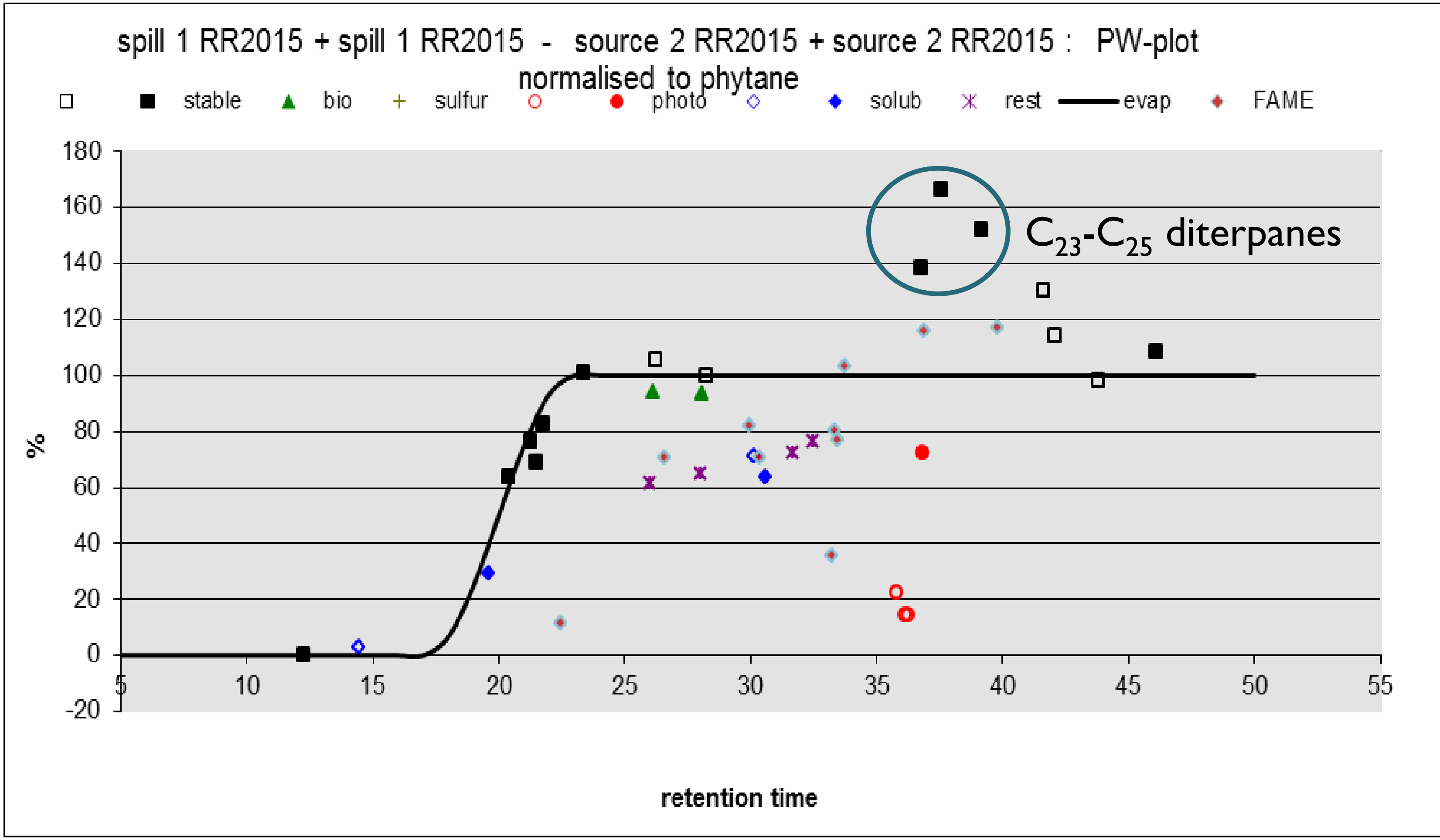
More evidence that source 1 is a mixture of source 2 and source 3

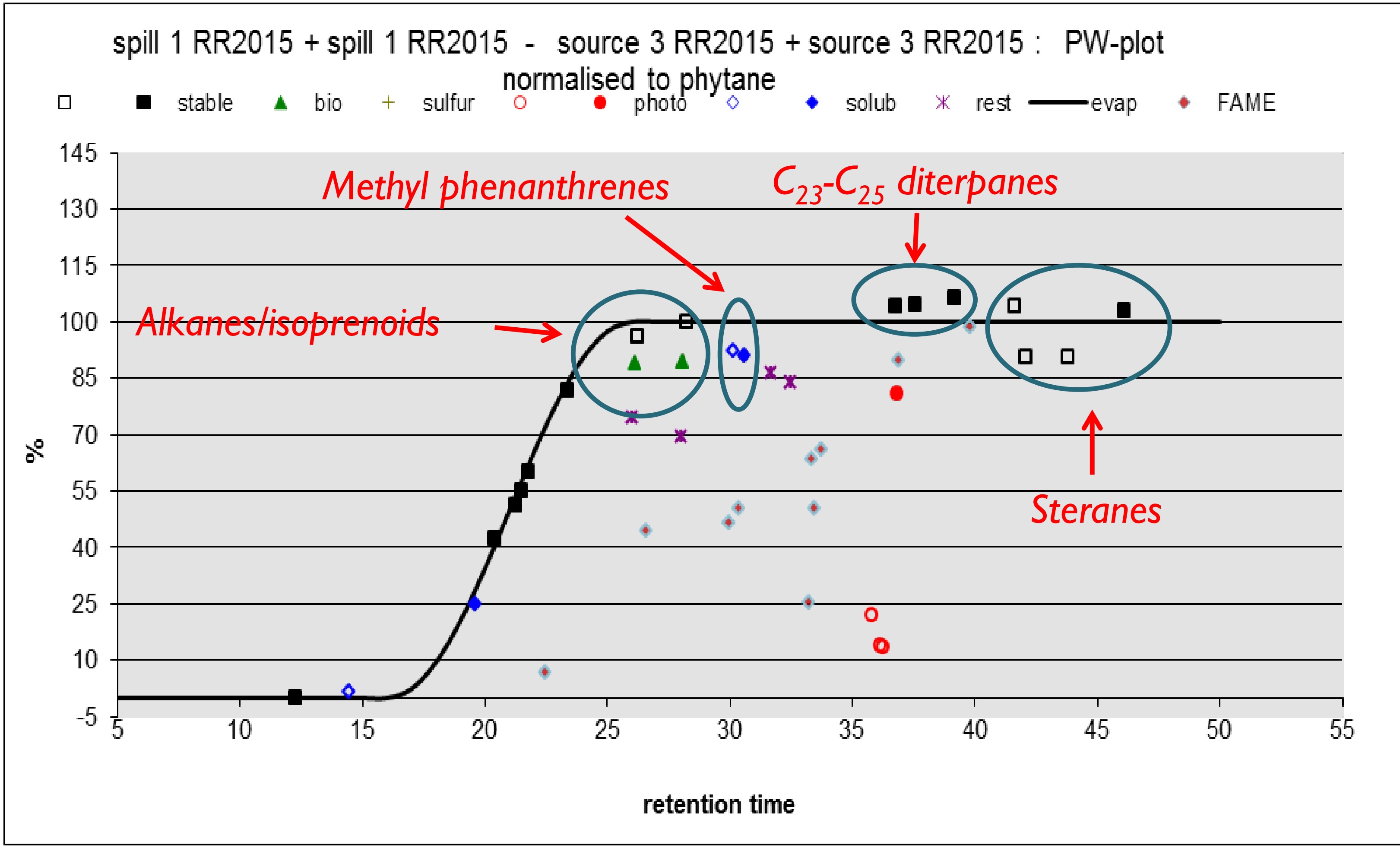
GC/FID Conclusions

- Profiles of spill and all three sources are similar.
- C_{17}/pristane, C_{18}/phytane, and pristane/phytane ratios are all similar, suggesting all three sources responsible for spill. GC/FID PW plots support these diagnostic ratios.
- Based on alkane, and FAME data, it appears Source 1 is a mixture of Source 2 and Source 3.

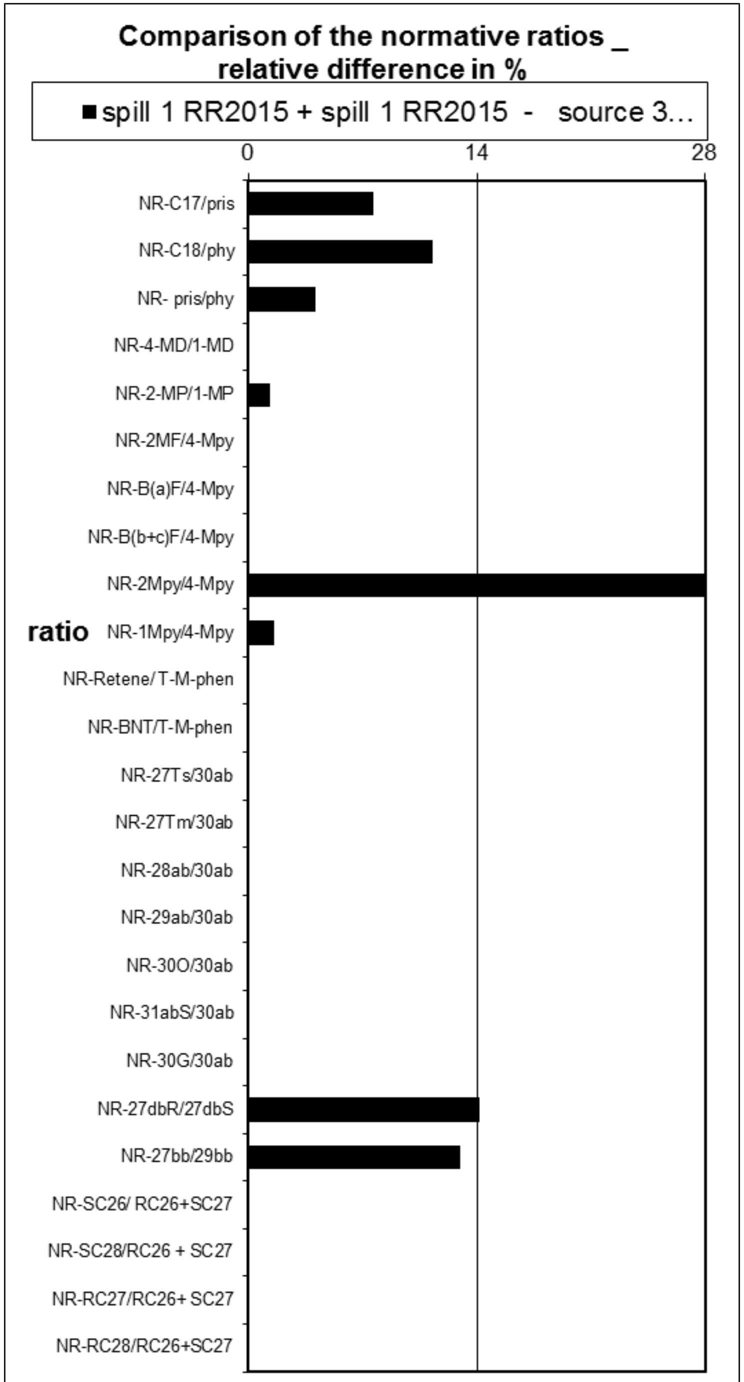


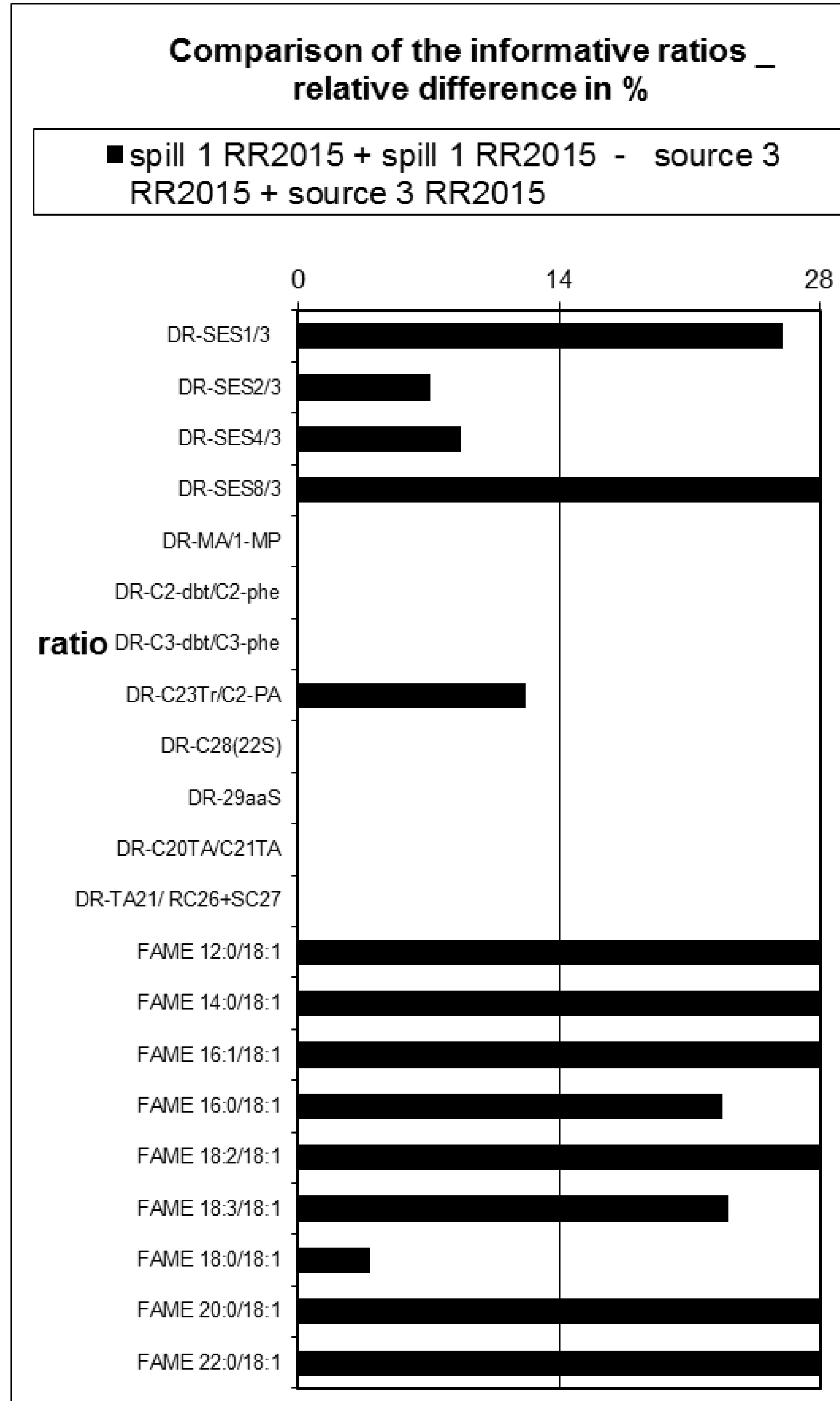
Source 1 is a mixture of source 2 and source 3

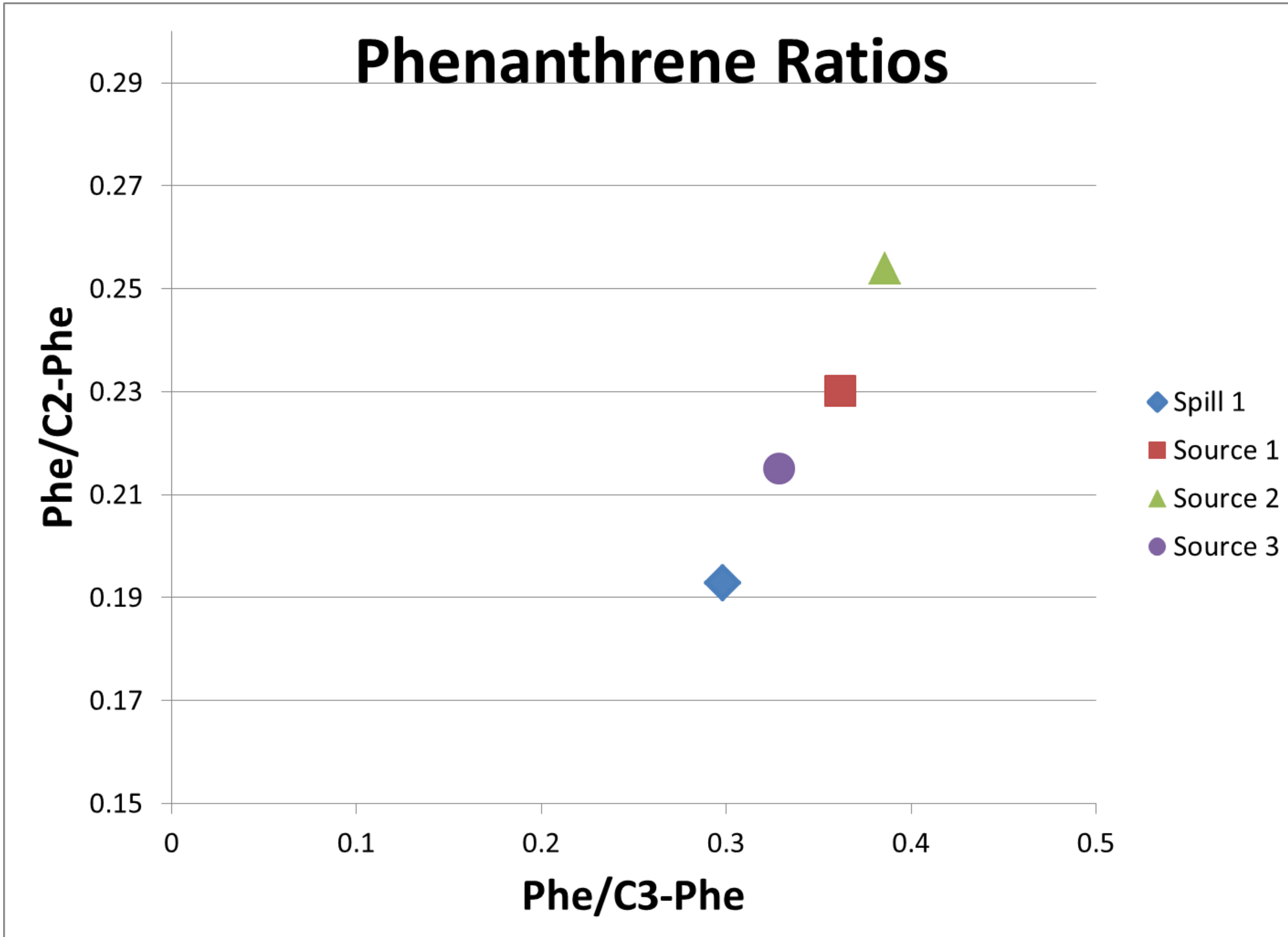

spill 1 RR2015 + spill 1 RR2015 - source 2 RR2015 + source 2 RR2015 : PW-plot
normalised to phytane
stable
bio
sulfur
photo
solub
rest
evap
FAME
C_{23}-C_{25} diterpanes
%
retention time
180
160
140
120
100
80
60
40
20
0
-20
5
10
15
20
25
30
35
40
45
50
55



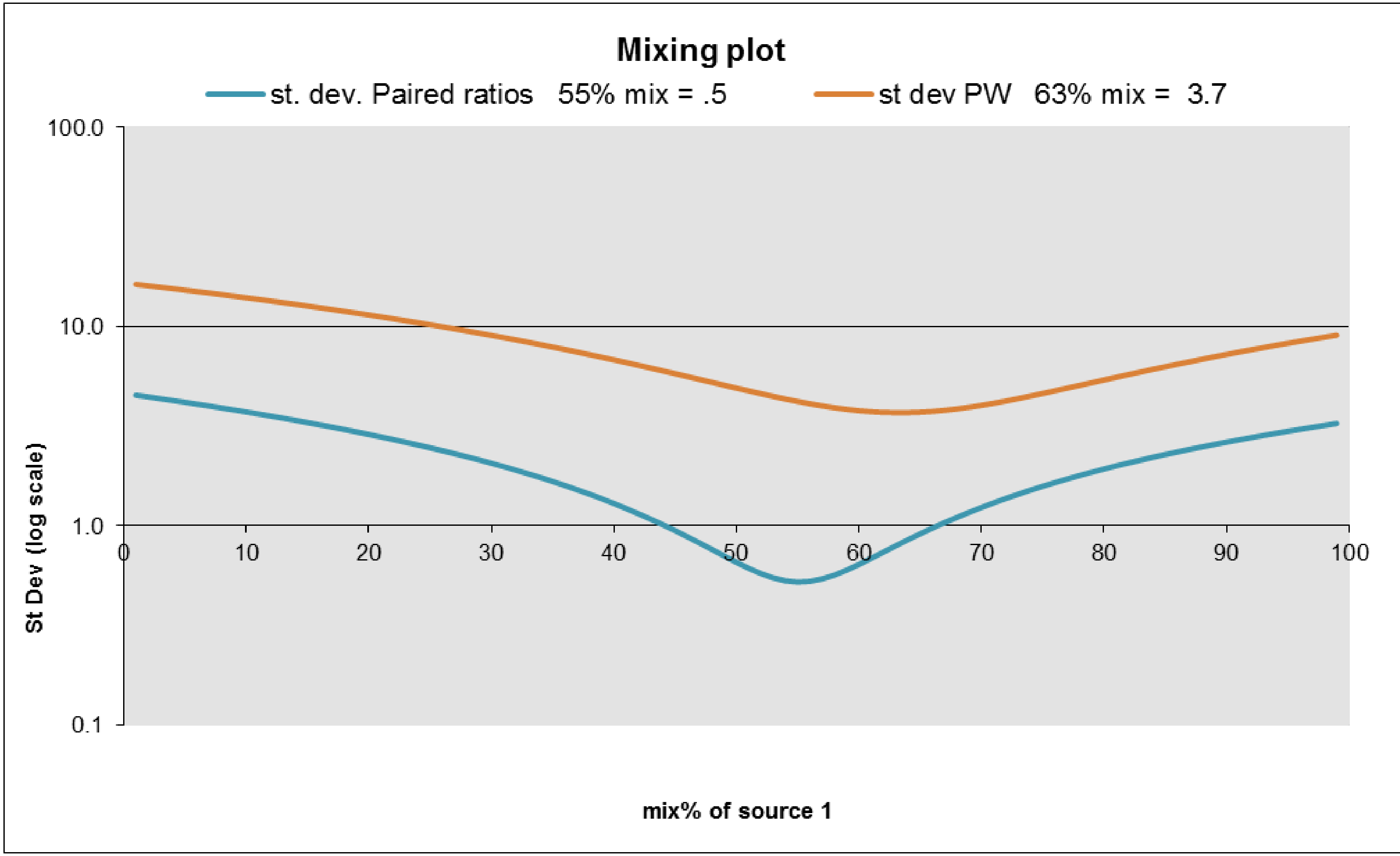
spill 1 RR2015 + spill 1 RR2015 - source 3 RR2015 + source 3 RR2015 : PW-plot
normalised to phytane
stable
bio
sulfur
photo
solub
rest
evap
FAME
Methyl phenanthrenes
C_{23}-C_{25} diterpanes
Alkanes/isoprenoids
Steranes
145
130
115
100
85
70
55
40
25
10
-5
%
5
10
15
20
25
30
35
40
45
50
55
retention time


Comparison of the normative ratios _ relative difference in %
spill 1 RR2015 + spill 1 RR2015 - source 3...
0
14
28
ratio
NR-C17/pris
NR-C18/phy
NR- pris/phy
NR-4-MD/1-MD
NR-2-MP/1-MP
NR-2MF/4-Mpy
NR-B(a)F/4-Mpy
NR-B(b+c)F/4-Mpy
NR-2Mpy/4-Mpy
NR-1Mpy/4-Mpy
NR-Retene/ T-M-phen
NR-BNT/T-M-phen
NR-27Ts/30ab
NR-27Tm/30ab
NR-28ab/30ab
NR-29ab/30ab
NR-30O/30ab
NR-31abS/30ab
NR-30G/30ab
NR-27dbR/27dbS
NR-27bb/29bb
NR-SC26/ RC26+SC27
NR-SC28/RC26 + SC27
NR-RC27/RC26+ SC27
NR-RC28/RC26+SC27

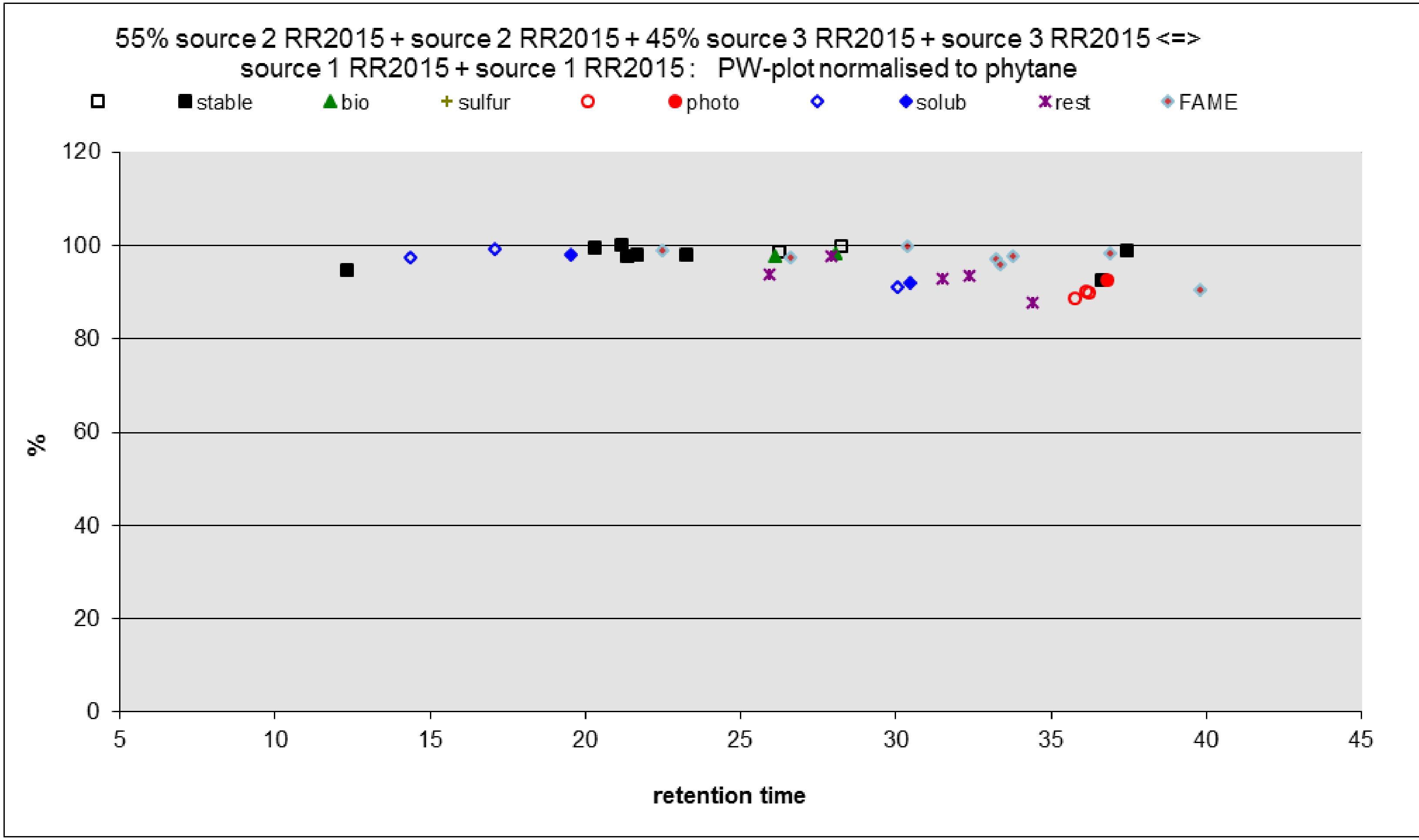

Comparison of the informative ratios _ relative difference in %
spill 1 RR2015 + spill 1 RR2015 - source 3 RR2015 + source 3 RR2015
0
14
28
ratio
DR-SES1/3
DR-SES2/3
DR-SES4/3
DR-SES8/3
DR-MA/1-MP
DR-C2-dbt/C2-phe
DR-C3-dbt/C3-phe
DR-C23Tr/C2-PA
DR-C28(22S)
DR-29aaS
DR-C20TA/C21TA
DR-TA21/ RC26+SC27
FAME 12:0/18:1
FAME 14:0/18:1
FAME 16:1/18:1
FAME 16:0/18:1
FAME 18:2/18:1
FAME 18:3/18:1
FAME 18:0/18:1
FAME 20:0/18:1
FAME 22:0/18:1



Phenanthrene Ratios
0.29
0.27
0.25
0.23
0.21
0.19
0.17
0.15
Phe/C2-Phe
0
0.1
0.2
0.3
0.4
0.5
Phe/C3-Phe
Spill 1
Source 1
Source 2
Source 3



Source 1 = 55% Source 2 and 45% Source 3


55% source 2 RR2015 + source 2 RR2015 + 45% source 3 RR2015 + source 3 RR2015 <=>
source 1 RR2015 + source 1 RR2015 : PW-plot normalised to phytane
stable
bio
sulfur
photo
solub
rest
FAME
%
120
100
80
60
40
20
0
5
10
15
20
25
30
35
40
45
retention time

GC/MS Conclusions

- Due to weathering very few ratios can be used to compare sources to spill.
- n-C_{17}/pristane, n-C_{18}/phytane, and pristane/phytane confirmed as per GC/FID.
- 1-methylphenanthrene/2-methylphenanthrene important.
- C_{23}, C_{24} and C_{25} terpanes critical to assessment.

GC/MS Conclusions

- Source 1 is a mixture consisting of 55% source 2 and 45% source 3.
- Source 3 is the best match for the Spill material based on terpane data.
- Phenanthrene data supports this decision.

Conclusions

- Some forensic studies can be extremely challenging.
- In this study we learned that injection concentrations for GC/FID and GC/MS were critical to coming up with the correct conclusions.
- Too high concentration for FID resulted in C_{17}/pristane ratio to be wrong for spill 1 and source 2.
- Too low a concentration for GC/MS resulted in sterane data being incorrect (comparison of 1.75 and 7.0 mg/mL data).

Conclusions

- All data was collected following duplicate analyses and precision was excellent (<5%). Even so, concentrations have a huge impact.
- Also critical to check for mass discrimination when using GC/FID.
- Critical to check peak resolution and mass discrimination using GC/MS.
- Maintaining Control charts for forensic files are important aspect of this kind of work.
- Forensic analyses are not routine

Conclusions

- Although equipment (GC/FID and GC/MS) are common in routine testing laboratories, columns and operating conditions are critical to correct conclusions.
- Furthermore, experience of the investigator is crucial.
- In some instance data other that GC/FID and GC/MS are required to come to a proper conclusion and experience of the investigator is paramount.

Overall Conclusions

- We learned that high resolution GC/FID data can provide misleading information.
- Despite the fact that samples were analyzed in duplicate, and normalized (C_{20} – C_{24}) the information provided was not correct.
- The use of alkane and isoprenoid ratios provided misleading information. In fact there were observed differences between ratios obtained using GC/FID and GC/MS.
- This clearly shows why OSINET assigns Level 1 confidence to GC/FID data.
- This presentation shows that it is essential to participate in international round robin studies aimed at petroleum forensics.

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