Use of Chemometrics and Multivariate Statistical Analysis to Determine Toxic Constituents Within Various Crude Oils

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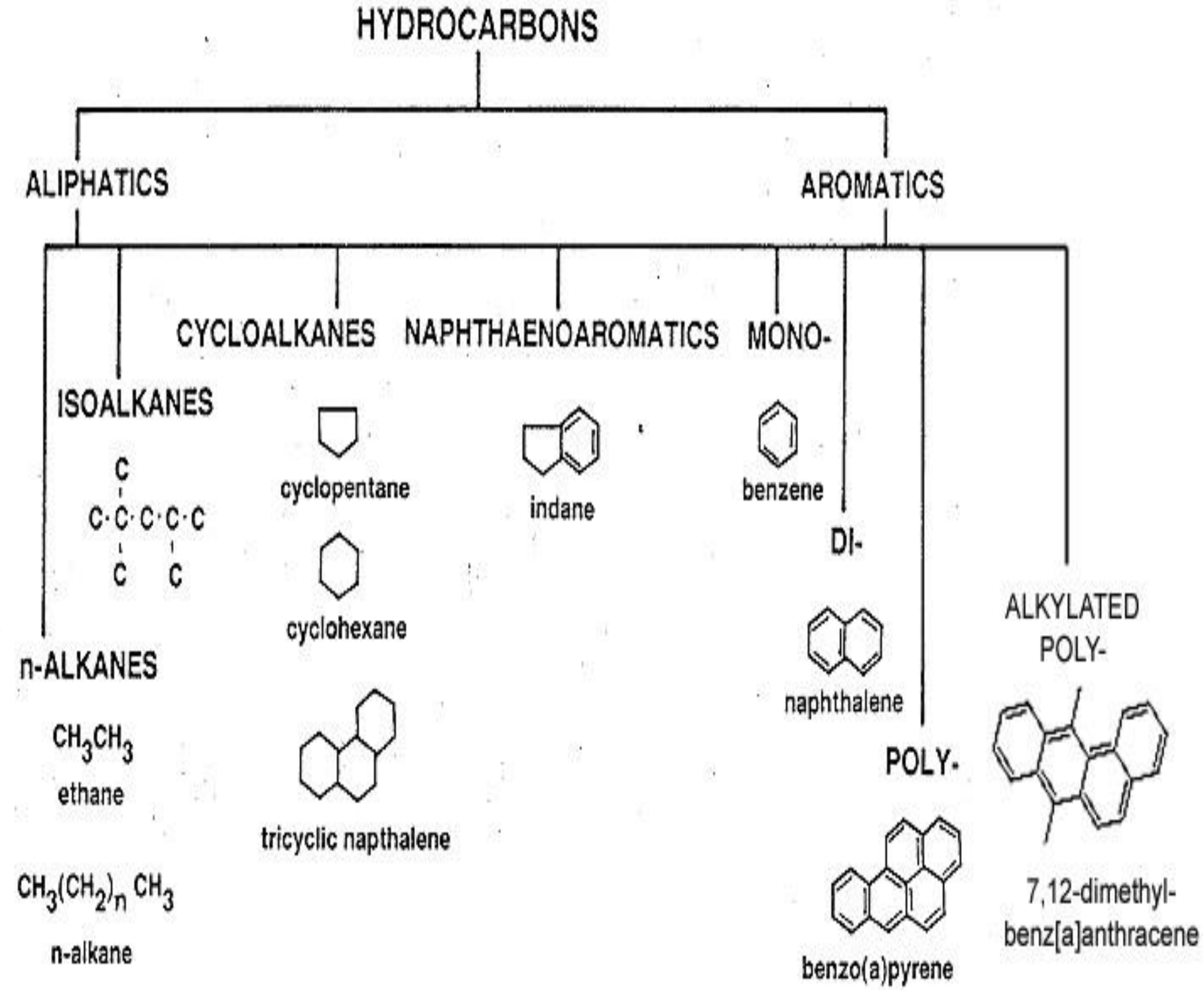
Introduction

- Purpose
  - To develop a procedure for estimating human health risk of crude oil exposure from analysis of its compositional profile.
- Hypothesis
  - The multivariate statistical analysis, Partial Least Squares regression (PLSr), can be successfully used to correlate GC/MS-derived comprehensive profile of various crude oils to a series of measured toxicological endpoints, identification of key toxic constituents, and prediction of in vivo toxicity from untested crude oil samples.
- Materials and Methods
- Biological Effects
- Chemometrics and Multivariate Analyses results
- Conclusion

Information Regarding Characteristics & Classification of Crude Oil

- Paraffins (Aliphatics) – saturated hydrocarbons with straight or branched chains; no ring formations.
- Cycloparaffins (Napthenes) – saturated hydrocarbons containing one or more rings, each of which may have one or more paraffin side-chains.
- Aromatics – hydrocarbons containing one or more aromatic nuclei such as benzene, naphthalene, and phenanthrene ring systems that may be linked up with naphthalene rings or paraffin side-chains



Geraci, St Aubin (1988) Synthesis of Effects of Oil on Marine Mammals.
http://edocs.dlis.state.fl.us/fldocs/oilspill/federal/18892759.pdf

Information Regarding Characteristics & Classification of Crude Oil

- **Density:**
  - Light: <870 Kg/m^3 (API > 31.1°)
  - Medium: 870 – 920 Kg/m^3 (31.1° – 22.3° API)
  - Heavy: 920 – 1000 Kg/m^3 (22.3° - 10° API)
  - Extra Heavy >1000 Kg/m^3 (<10° API)
- **Sulphur, wt%:**
  - When the total sulphur level is > 0.5% the oil is classified as sour.
    - Louisiana Sweet (0.21%); Nigerian (0.12%); N. Dakota Sweet (<0.2%);
    - E. Oriente (1.52%); V. Leona (1.52%); Iraqi (1.95%); V. Merey (2.74%)

- **Light Crudes:**
  - LA Sweet, Nigerian, Iraqi, A010G4, A0083Q, DWH35273, HOOPS Blend
- **Medium Crudes:**
  - E. Oriente, C. Vasconia, V. Leona
- **Heavy Crudes:**
  - V. Merey
- **Sweet Crudes:**
  - LA Sweet, Nigerian, A010G4, A0083Q, DWH35273
- **Sour Crudes:**
  - Iraqi, HOOPS Blend, E. Oriente, C. Vasconia, V. Leona & Merey

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Assessment of Crude Oils' Toxicity

- Study Design:

Acute, High-dose; Female Sprague Dawley (SD) rats (220-260g BW)

Dose: p.o. (10 ml/kg) - 1/day; 2 day

VEH (5%)DMSO in Corn Oil

Benzene* (0.5ml/kg) Toluene (0.8 ml/kg)** Ethylbenzene (0.18ml/kg)***

Crude oil (2.5 ml/kg)

Crude oil (5.0 ml/kg)

1. 48h In-life observations, then euthanize & necropsy
2. Endpoints: -
   - Blood/serum (clin path/hematol; cytokines, vascular biomarkers)
   - Liver (weight; histopath); Spleen (weight; histopath)
   - Bone Marrow (CFU-GM)

'BTEX Distribution by Crude Type' (wt% based off of Light Crude Composition):
0.2 wt% Benzene*, 0.8 wt% Toluene**, 0.18% Ethylbenzene***, 1.18 wt% Xylenes (Figure 4: Page 12; USEPA 2011)

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Materials and Methods

- RELATIVE ORGAN WEIGHTS: Organs were surgically excised from rats, cleaned, and weighed individually. Organ-to-body weight ratios were calculated, and results were compared between treated and non-treated.
- SERUM & BLOOD ANALYSIS:
  - Clinical metabolic parameters for ALP, ALT, AST, Glucose, Albumin, Urea, Total Protein, etc., and
  - Blood parameters such as: Hemocrit, Hemoglobin, RBC and WBC count, CFU-GM, etc..
- STATISTICS: Effects of crude oils at their respective doses on biological and clinical endpoints were determined by ANOVA with post-hoc comparisons of treatment means against vehicle control done with Dunnett's test and a=0.05 (GraphPad Prism, v6). Finally, accuracy for the 'Predicted vs. Observed' endpoints of DWH35273 and HOOPS Blend crude oils were determined using a chi-squared test.

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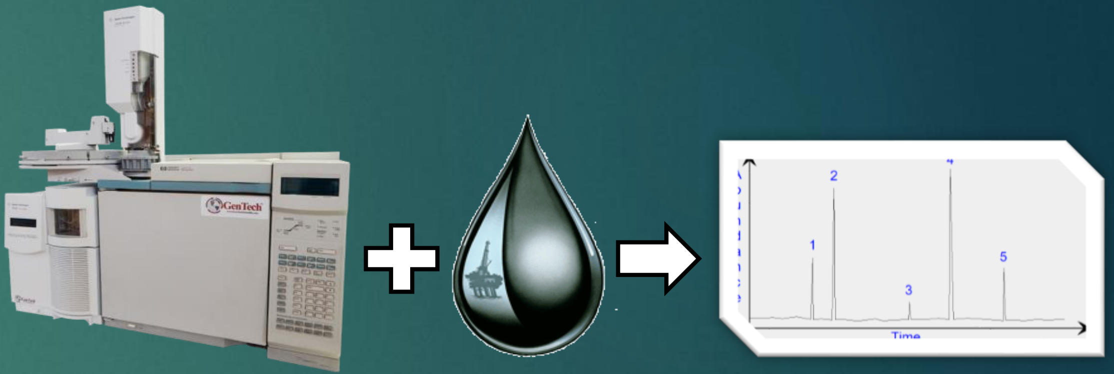
Materials and Methods

- SAMPLE PREPARATION: The carrier internal standard (ISTD) solution was prepared with 0.4734g of 1-bromo-dodecane and 0.5237g of 1-bromo-4-methyl-napthalene. The ISTDs were added to a 500mL volumetric flask and diluted to 500mL with methylene chloride (DCM).
  - 1mL of each crude oil was added into separate 10mL vol. flasks, and diluted to 10mL with the ISTD carrier solvent.
    - 1mL of freshly prepared crude oil sample with ISTD was transferred to a 1.5mL Agilent sample vial for GC/MS analysis
- MULTIVARIATE ANALYSES: PLSr were performed with a total of 11 crude oil chromatographs consisting of 110 selected peaks for the HP-5 column and a total of ten chromatographs consisting of 43 selected peaks for the PAH column.
  - From the results, a plot of Variable Importance in Projection (VIP) was generated for each of the 110 and 43 X-variables, and the top 12 VIPs of constituents ≥ 1.0.
  - These selected VIPs were plotted against the relative spleen weights for each oil by their chromatographic area response for both 2.5mL and 5mL dosages.

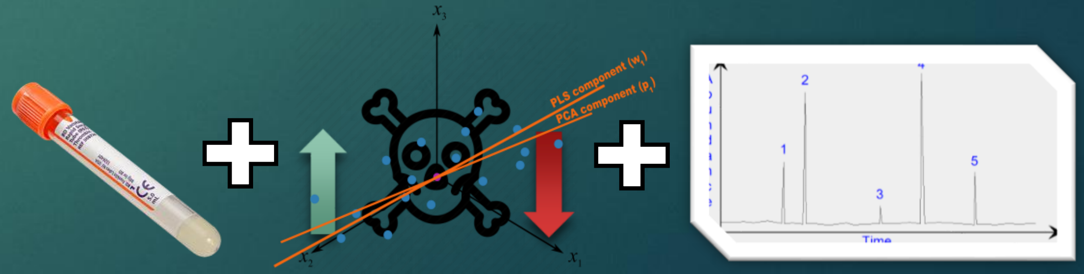
Exploratory Approach Through Multivariate Analysis

Survey selected health effects across different oils

Conduct chemical analysis of high density fingerprints of same oils



Use chemometrics/PLS to relate chemical constituents to health effects



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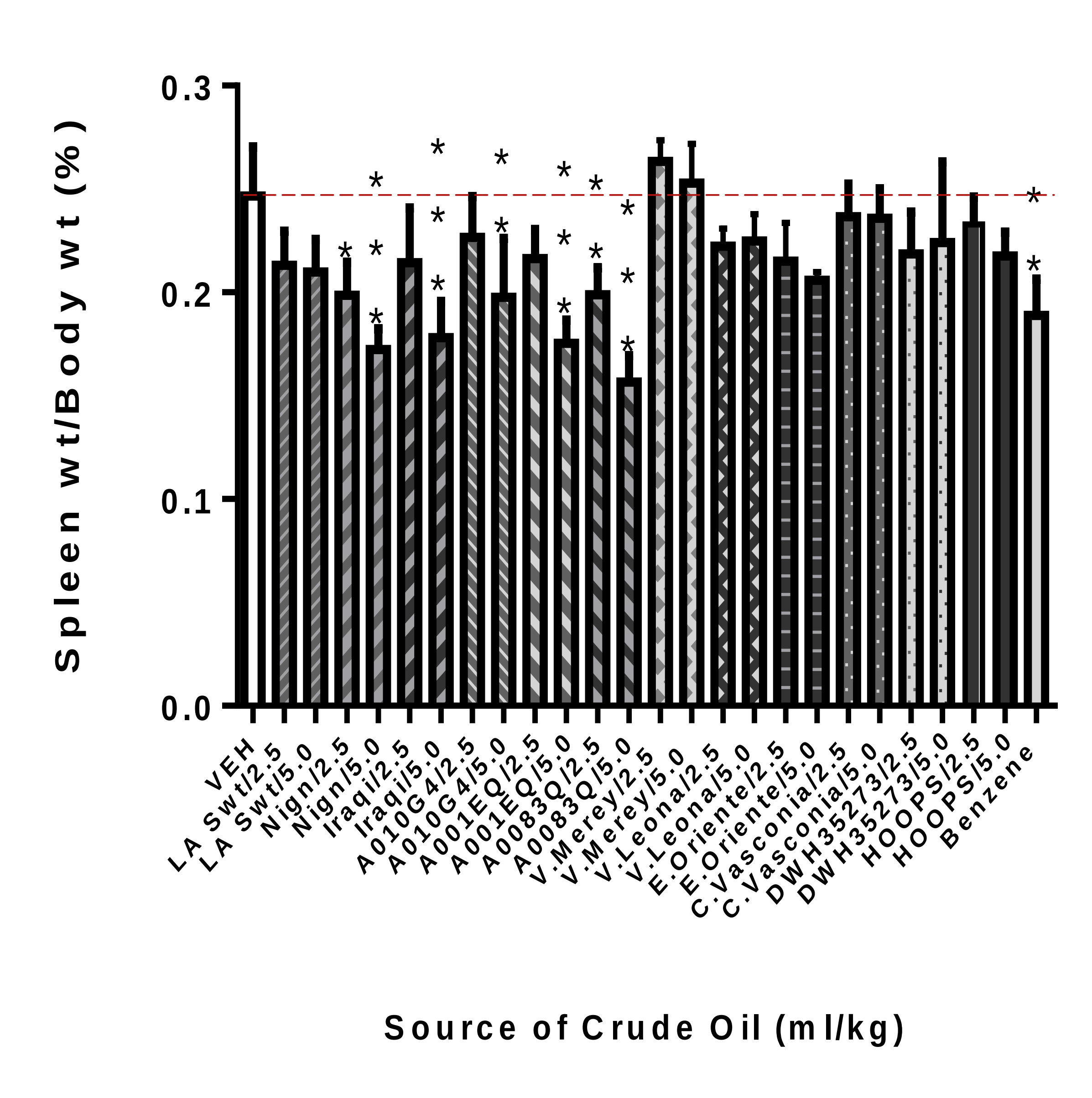
Composite of all Crude Oil Studies with Comparison of Significant Endpoints to Contemporaneous Control

Rel. Spleen Wt (g)	Dose			
Oil Data	Cont. Control	2.5 mL/Kg	5.0 mL/Kg	Significance
LA Sweet	0.2390	0.2132	0.2099	No
Nigerian	-	0.1985	0.1723	P<0.05; p<0.001
Iraqi	-	0.2142	0.1781	No; p<0.001
A010G4	-	0.2267	0.1976	No; p<0.01
A001EQ	-	0.2164	0.1753	No; p<0.001
A0083Q	-	0.1988	0.1567	p<0.05; p<0.001
V. Merey	-	0.2634	0.2528	No
V. Leona	-	0.2224	0.2248	No
E. Oriente	-	0.2150	0.2060	No
C. Vasconia	-	0.2366	0.2358	No
DWH35273	-	0.2245	0.2304	No
HOOPS	-	0.2393	0.2235	No



Use of Chemometrics and Multivariate Statistics to Identify Toxic Constituents, and Predict Toxicity of a Mixture

Agilent Technologies

sas

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Methods and Procedures: HP-5

Crude Oil Samples:
LA Sweet – 1mL/10mL in DCM w/ Int. Std.
Nigerian – 1mL/10mL ...
Iraqi – 1mL/10mL ...
A010G4 – 1mL/10mL ...
A0083Q – 1mL/10mL ...
E. Oriente – 1mL/10mL ...
C. Vasconia – 1mL/10mL ...
V. Leona – 1mL/10mL ...
V. Merey – 1mL/10mL ...
DWH35273 – 1mL/10mL ...
HOOPS Blend – 1mL/10mL ...
10 mL Volumetric Flask; Int. Std.(s):

- GC Column:
  - Agilent HP-5 GC Column, 30 m, 0.25 mm, 0.25 µm
- GC Method Revised:
  - Inlet: Split Ratio: 100:1 – Pressure: 16.086 psi
  - Inlet Temp: 280°C, Aux: 280°C, Detector: 250°C
  - Initial temp: 30°C held for 0.5 min, 30-250°C @ 8°C/min, 250°C held for 12min, Post Run : 2 min, Total run time = 40 mins.
- Sample Injection: 1µL
  - Solvent Delay: 1.95 min
  - [Scan Parameters] Low Mass: 35.0; High Mass 250.0; Threshold: 500
  - Start Time : 8.00 min - Low Mass 50.0 ; High Mass: 300.0 Threshold: 500
  - Start Time : 21.00 min - Low Mass : 50.0; High Mass : 400.0; Threshold : 500
  - MS Source : 230 C (maximum 250 C)
  - MS Quad : 150 C (maximum 200 C)

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Methods and Procedures: PAH

- GC Column:
  - Restek Rxi®-PAH GC Column, 30 m, 0.25 mm, 0.1 µm
- GC Method Revised:
  - Inlet: Split Ratio: 50:1 – Pressure: 23.188 psi
  - Inlet Temp: 280°C, Aux: 280°C, Detector: 250°C
  - Initial temp: 115°C held for 6.0 min, 115-285°C @ 8°C/min, 285-325°C @ 8°C/min held for 3.875min, Post Run : 2 min, Total run time = 32 mins.
- Sample Injection: 1µL
  - Solvent Delay: 1.95 min
  - [Scan Parameters] Low Mass: 50.0; High Mass 250.0; Threshold: 500
  - Start Time : 8.00 min - Low Mass 50.0 ; High Mass: 400.0 Threshold: 500
  - Start Time : 21.00 min - Low Mass : 50.0; High Mass : 500.0; Threshold : 500
  - MS Source : 230 C   (maximum 250 C)
  - MS Quad : 150 C   (maximum 200 C)

Crude Oil Samples:
LA Sweet – 1mL/10mL in DCM w/ Int. Std.
Nigerian – 1mL/10mL ...
Iraqi – 1mL/10mL ...
A010G4 – 1mL/10mL ...
A0083Q – 1mL/10mL ...
E. Oriente – 1mL/10mL ...
C. Vasconia – 1mL/10mL ...
V. Leona – 1mL/10mL ...
V. Merey – 1mL/10mL ...
DWH35273 – 1mL/10mL ...
HOOPS Blend – 1mL/10mL ...
10 mL Volumetric Flask; Int. Std.(s):

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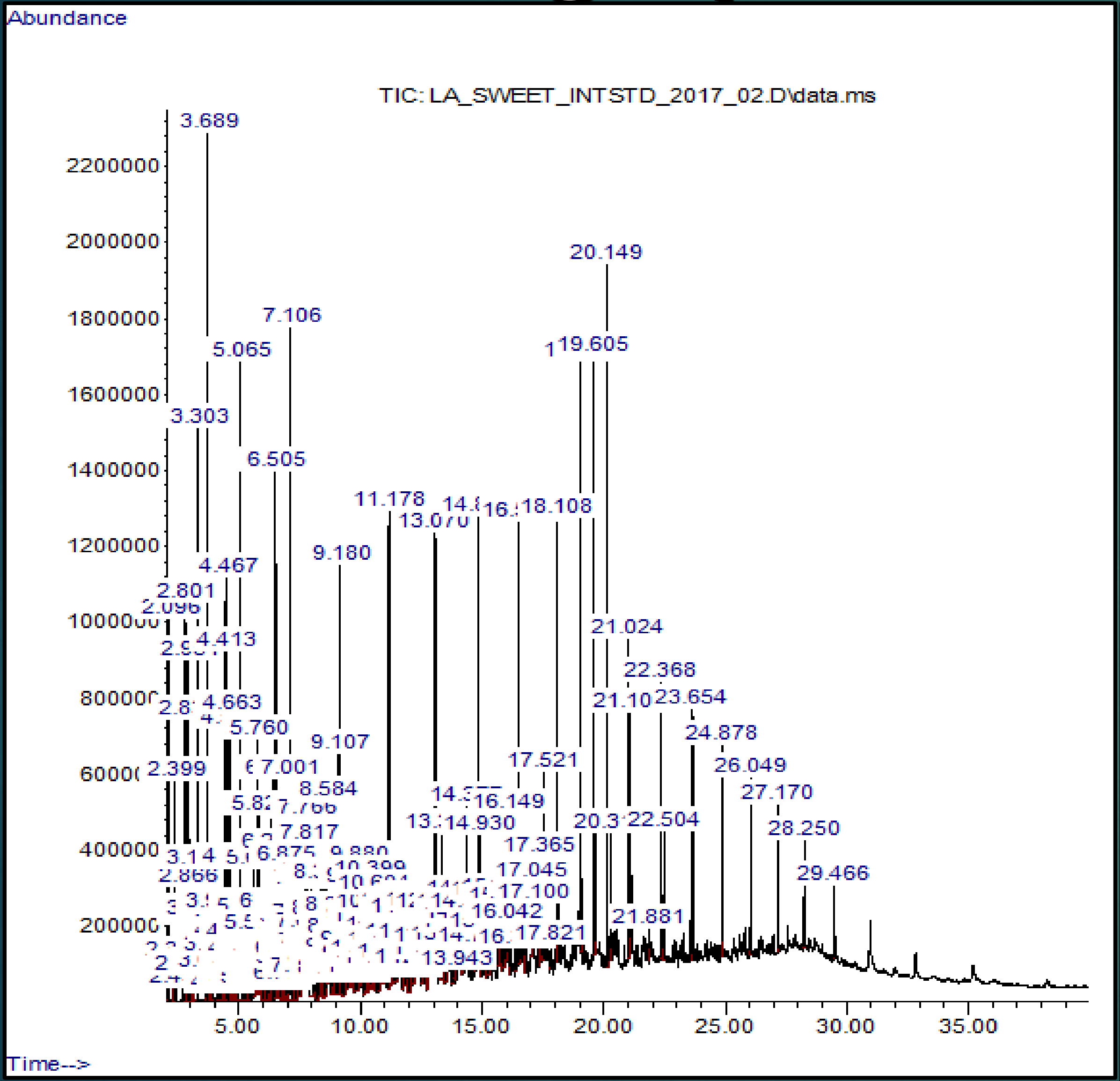
Chromatograph Examples



Quantitation Report (Not Reviewed)

Data Path : C:\msdchem\1\DATA\Josh_CrudeOil_2016\
Data File : LA_SWEET_INTSTD_2017_02.D
Acq On : 12 Sep 2017 11:54
Operator : Salley
Sample : LA_Swt_IntStd_2017_02
Misc :
ALS Vial : 1 Sample Multiplier: 1

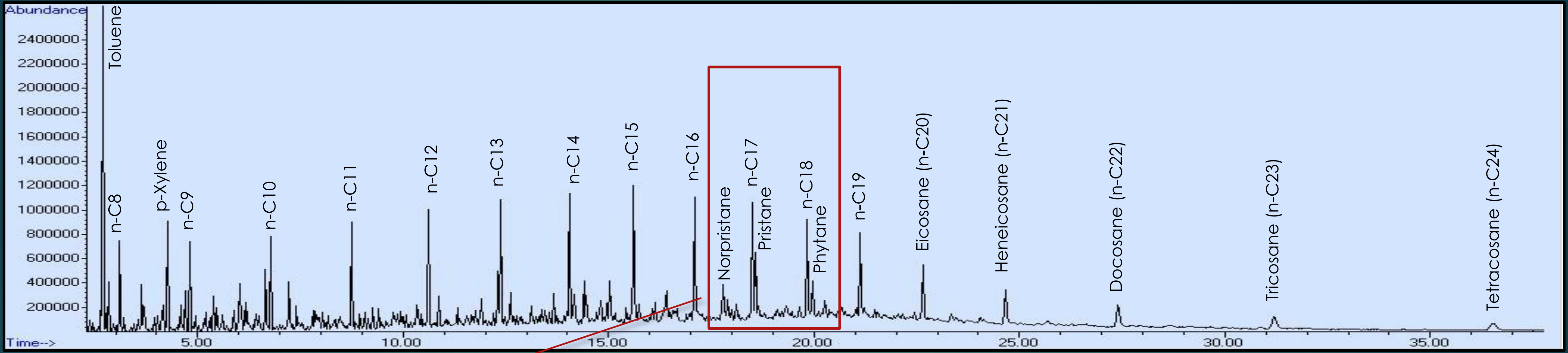
Quant Time: Sep 19 17:35:12 2017
Quant Method : C:\msdchem\1\METHODS\JOSH_METHOD_7.M
Quant Title : LA_Sweet_Int_2017_Quant
QLast Update : Tue Sep 19 17:30:21 2017
Response via : Initial Calibration

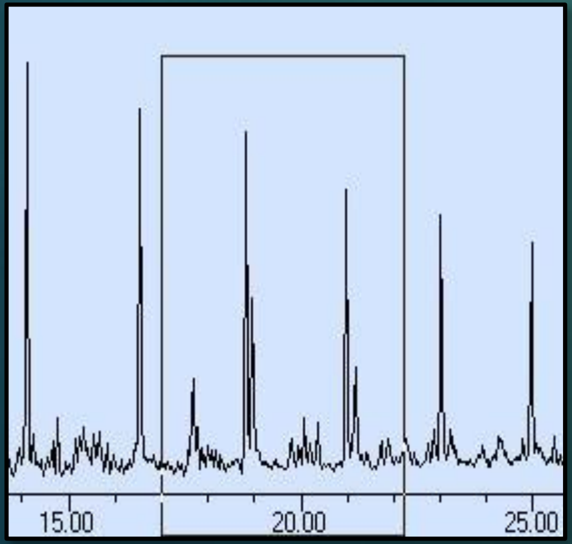
Compound	R.T.	QIon	Response	Conc Units	Dev(Mi
Internal Standards					
1) Naphthalene, 1-bromo-4...	20.149	141	659130	0.95 mg/mL	0
2) Dodecane, 1-bromo-	19.049	135	368431	0.95 mg/mL	0
Target Compounds					Qvalu
3) Hexane	2.096	57	224522	No Calib	
4) Pentane, 2,2-dimethyl-	2.319	57	29317	No Calib	
5) Cyclopentane, methyl-	2.399	56	193834	No Calib	
6) Butane, 2,2,3-trimethyl-	2.455	57	9196	No Calib	
7) Pentane, 3,3-dimethyl-	2.683	43	21579	No Calib	
8) Benzene	2.801	56	193077	No Calib	
9) Hexane, 2-methyl-	2.833	43	209257	No Calib	
10) Pentane, 2,3-dimethyl-	2.866	56	72223	No Calib	
11) Hexane, 3-methyl-	2.951	43	189229	No Calib	
12) Cyclobutane, ethenyl-	3.051	67	63180	No Calib	
13) Cyclopentane, 1,3-dime...	3.080	70	54441	No Calib	
14) Cyclopentane, 1,3-dime...	3.123	56	40471	No Calib #	
15) Cyclopentane, 1,2-dime...	3.161	56	76745	No Calib	
16) Heptane	3.303	43	372826	No Calib	
17) Butane, 2,2,3,3-tetram...	3.633	57	43784	No Calib	
18) Cyclohexane, methyl-	3.689	83	616765	No Calib	
19) Hexane, 2,5-dimethyl-	3.799	57	49408	No Calib	

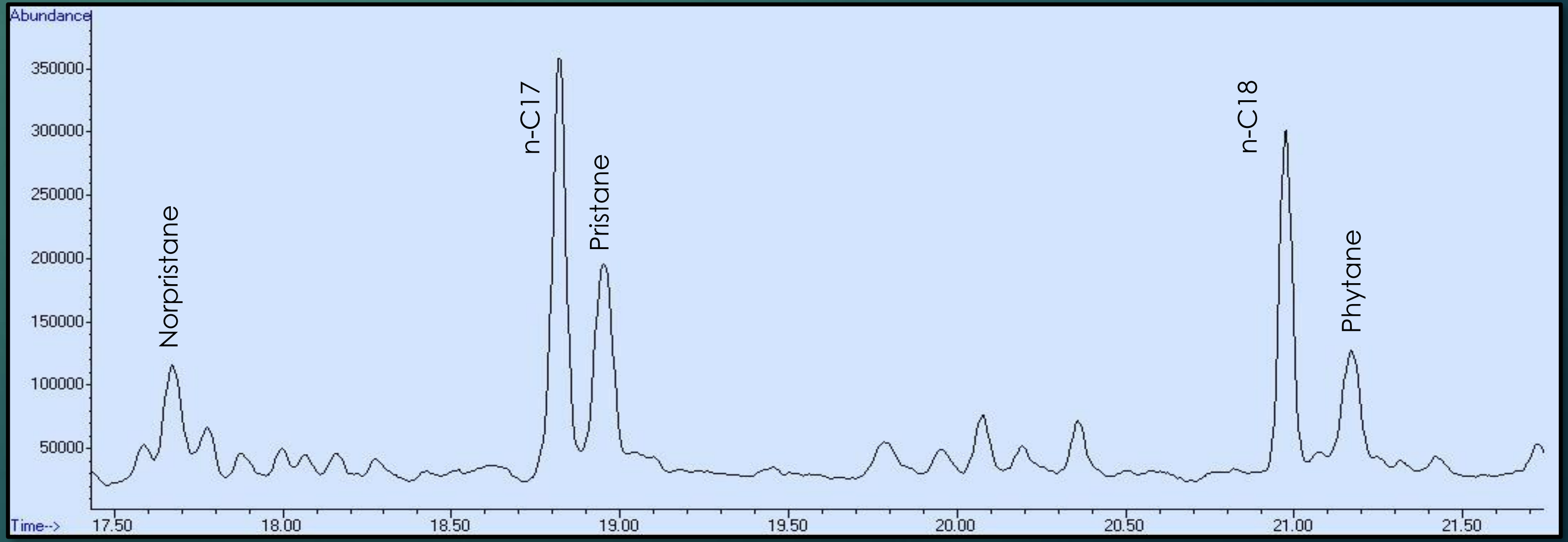
Data Analysis – Pristane

Crude Oil Sample: G4

Integrated Peak Library File



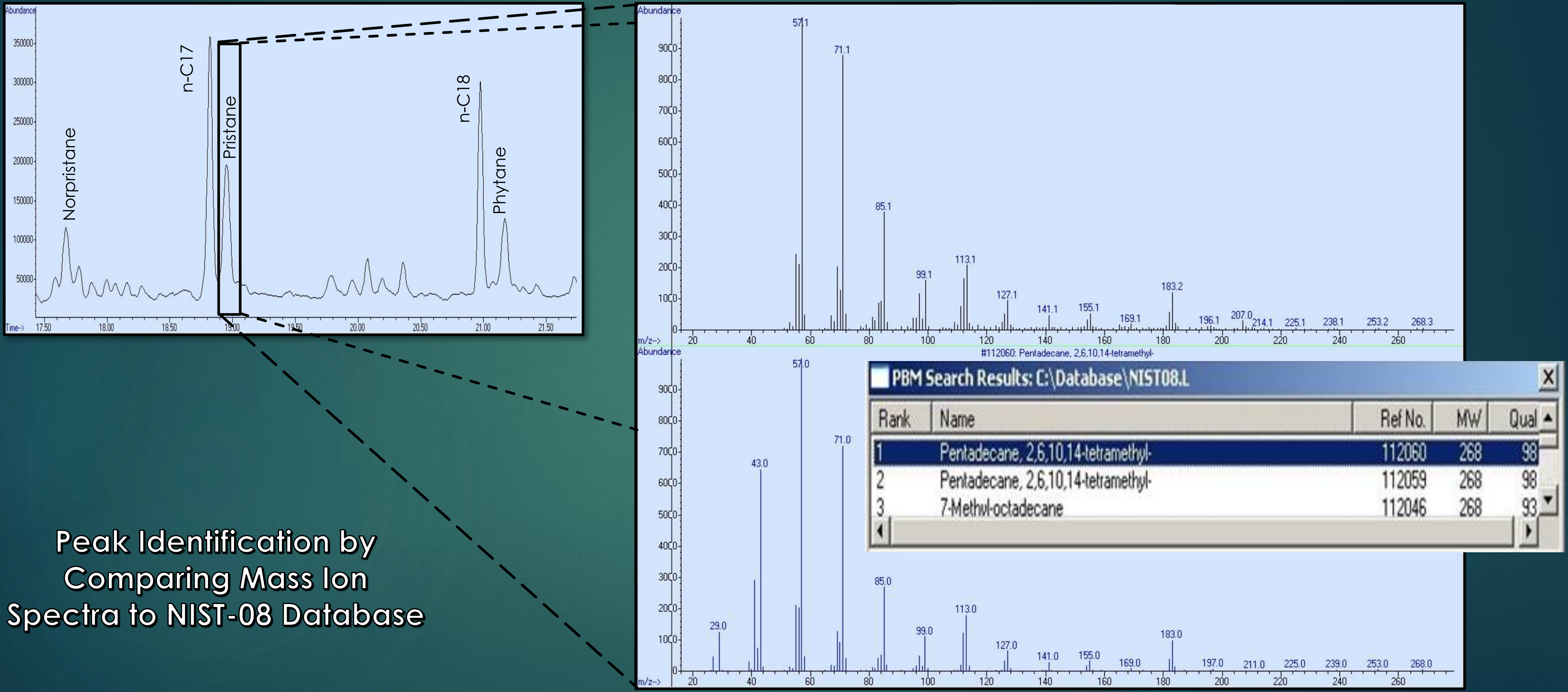




Data Analysis - Pristane

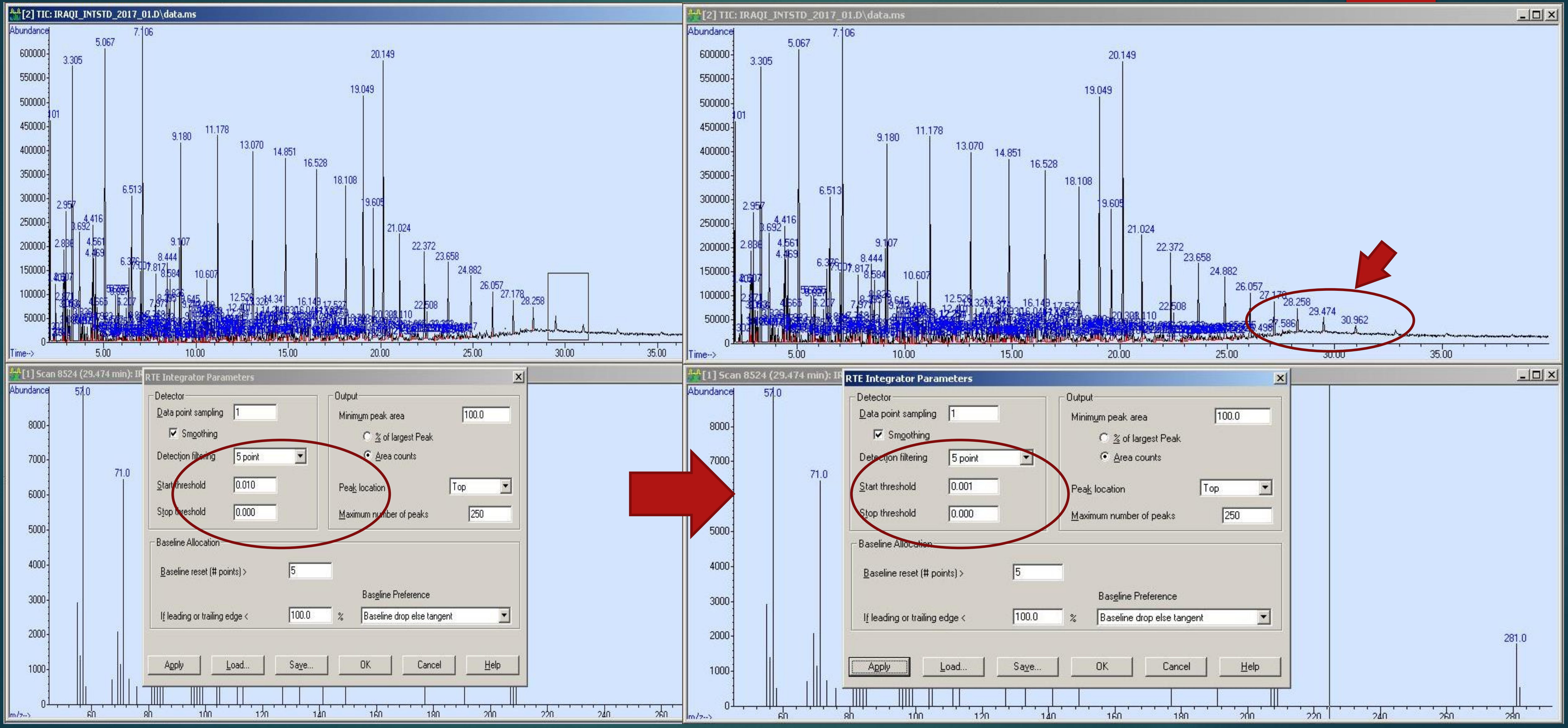
Crude Oil Sample: G4

Integrated Peak Library File

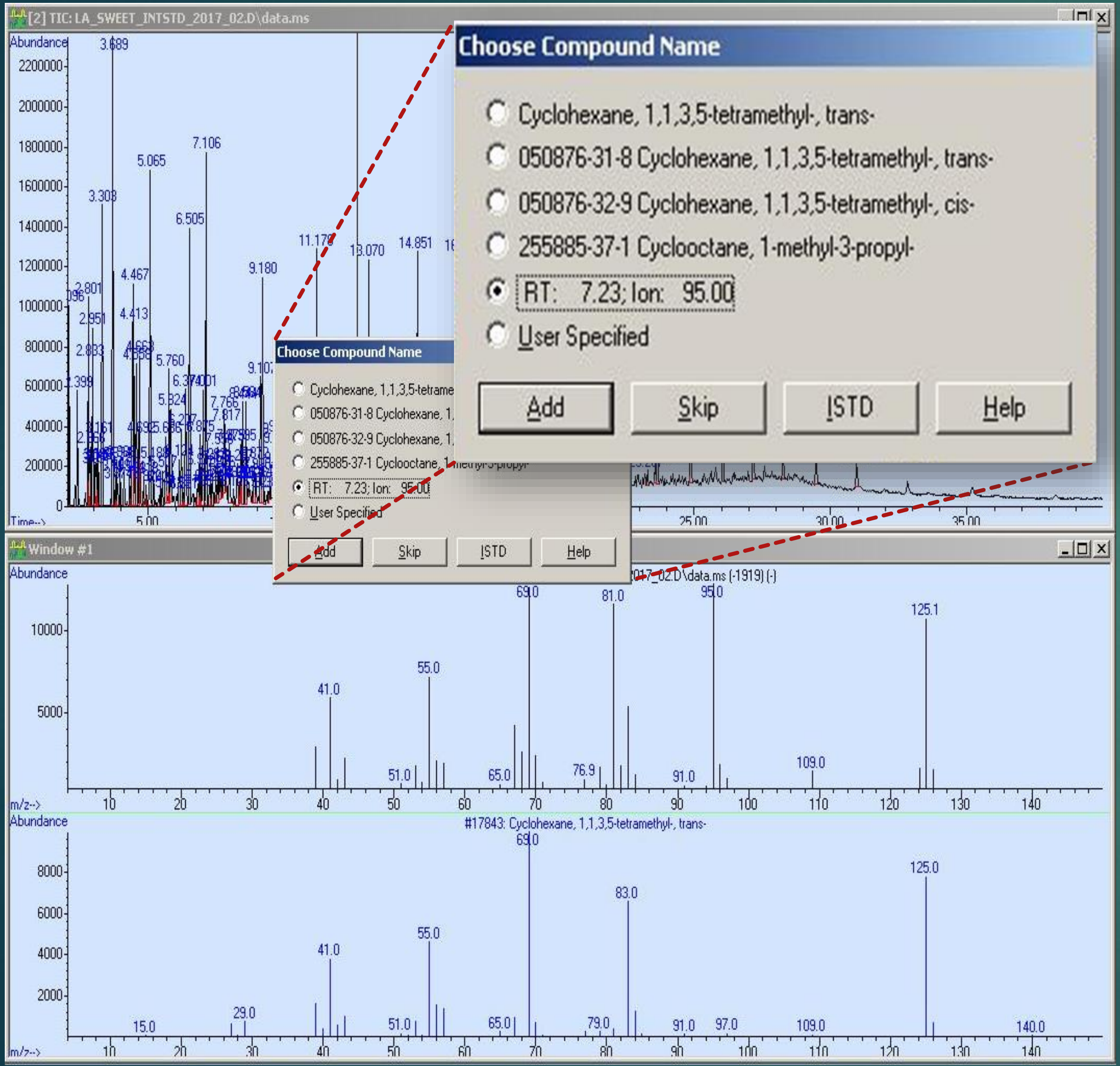


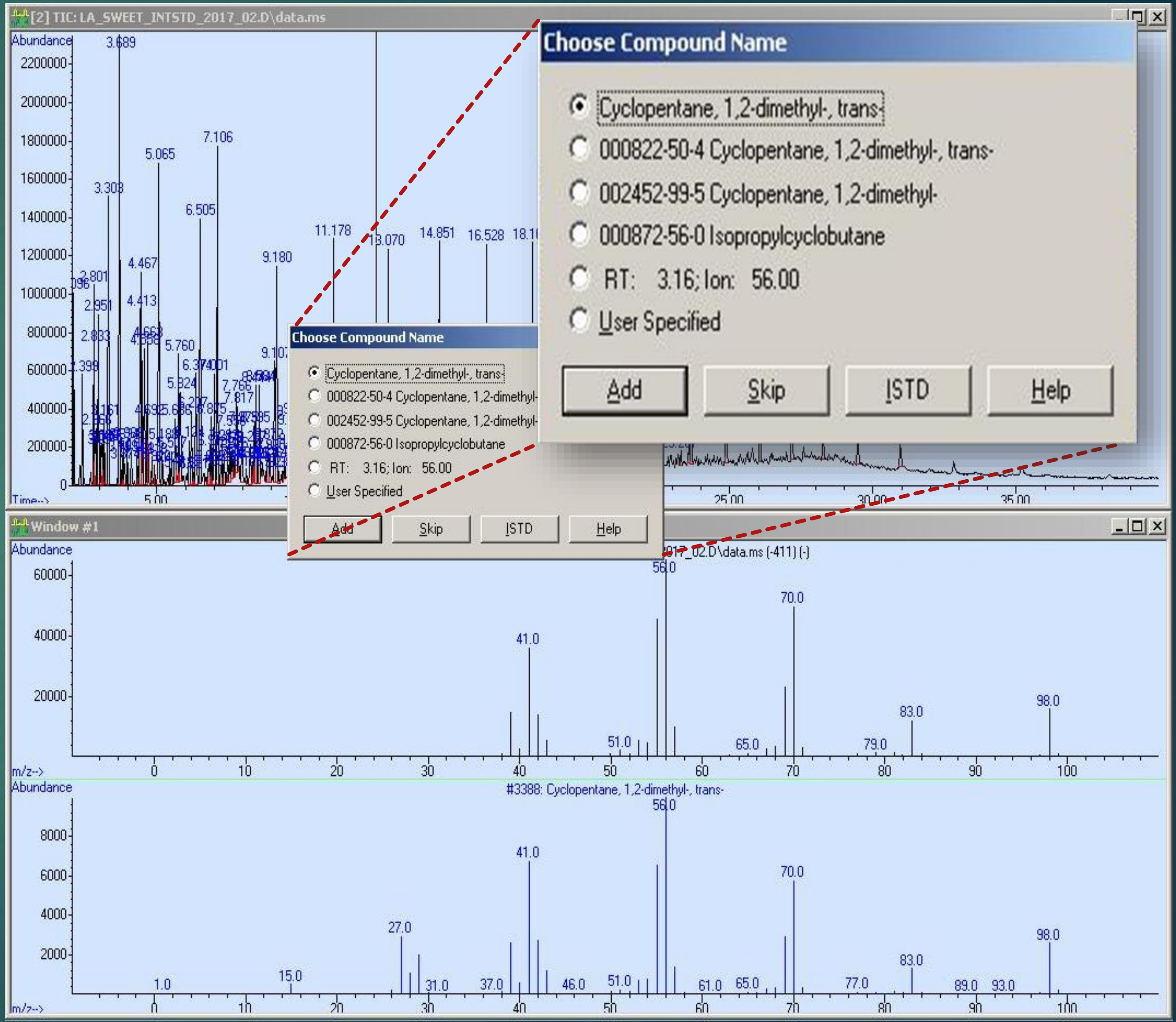
Peak Identification by Comparing Mass Ion Spectra to NIST-08 Database

Integration Parameters



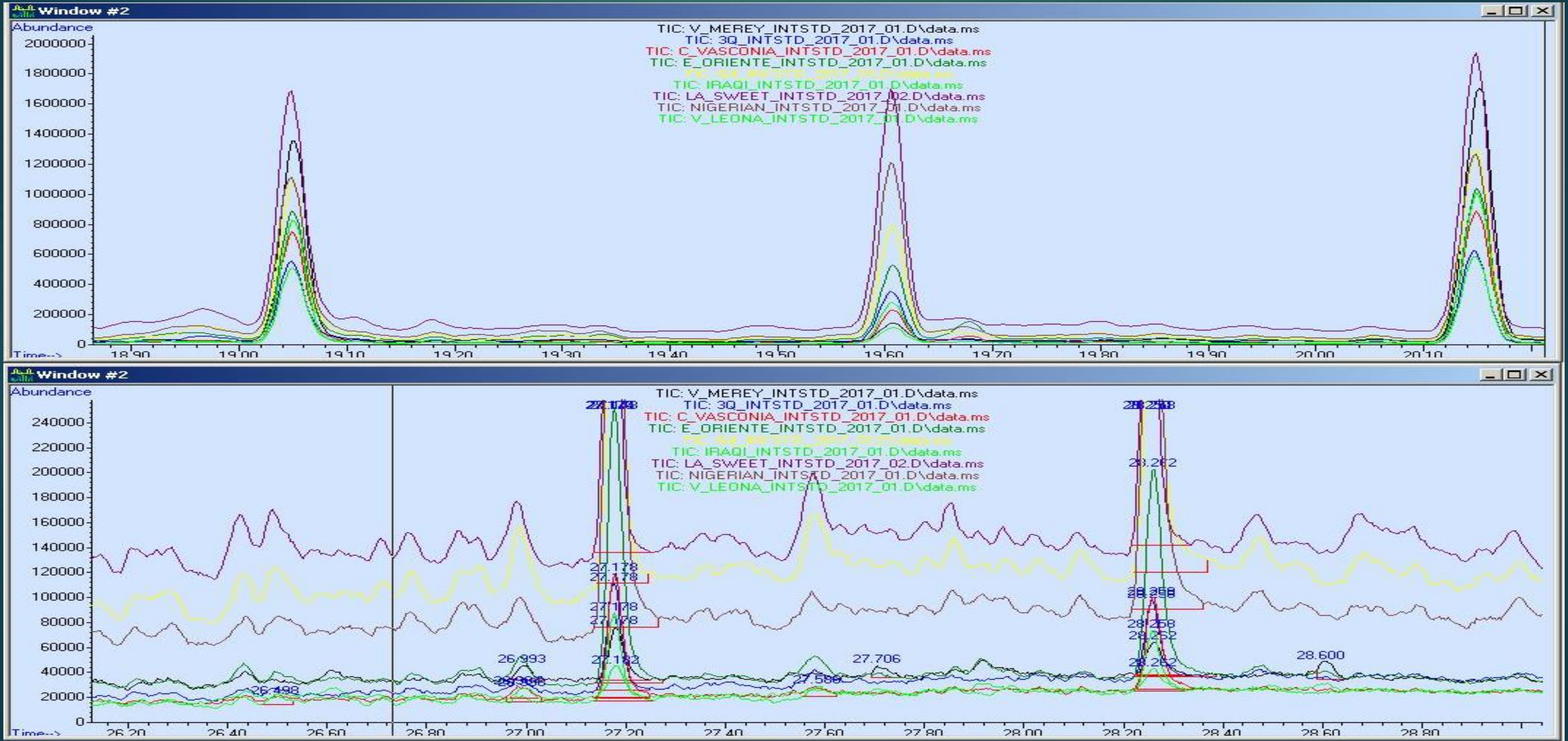
Peak Identification into the Quant. Report



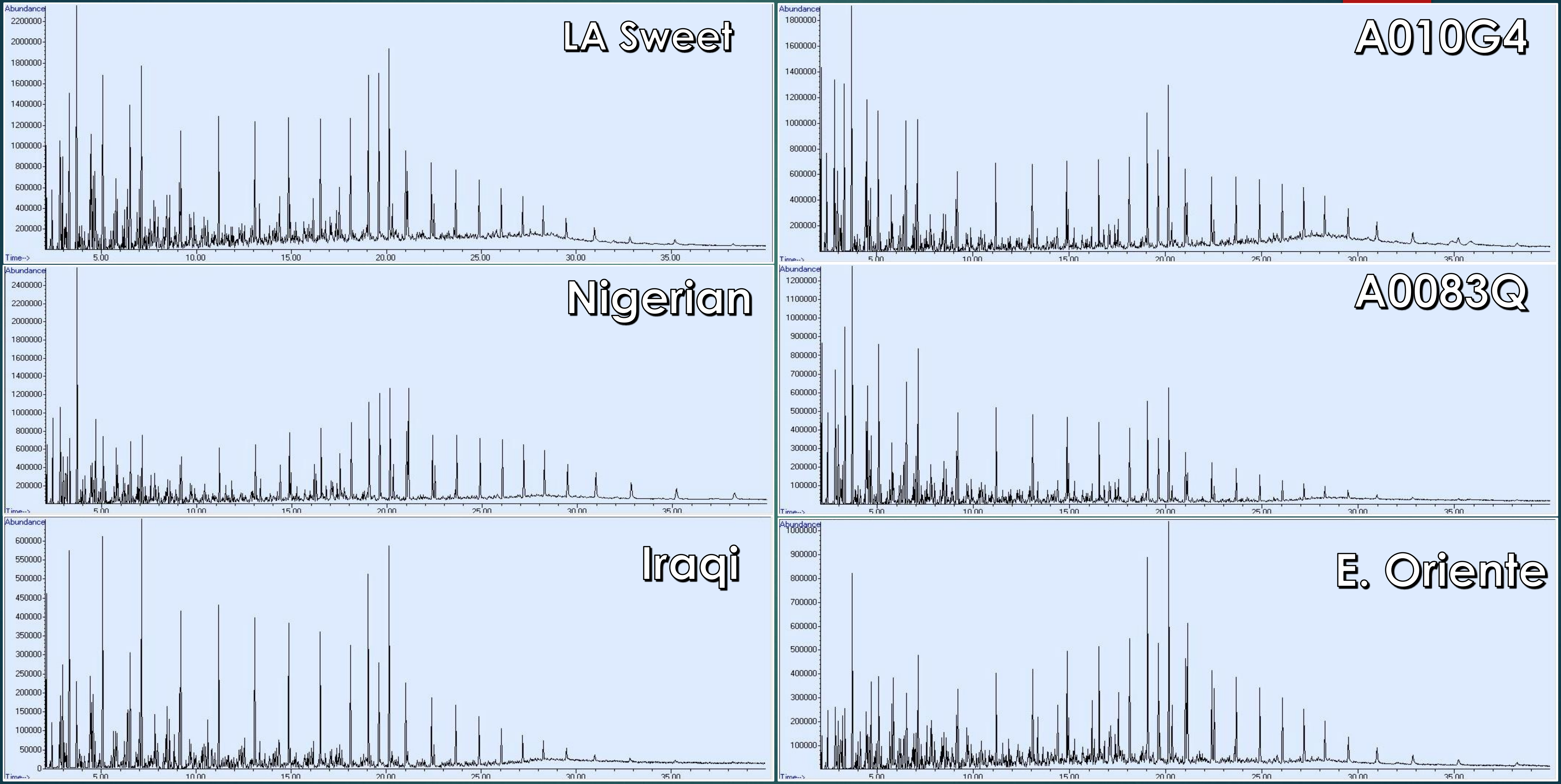


Peak Identification and Sorting

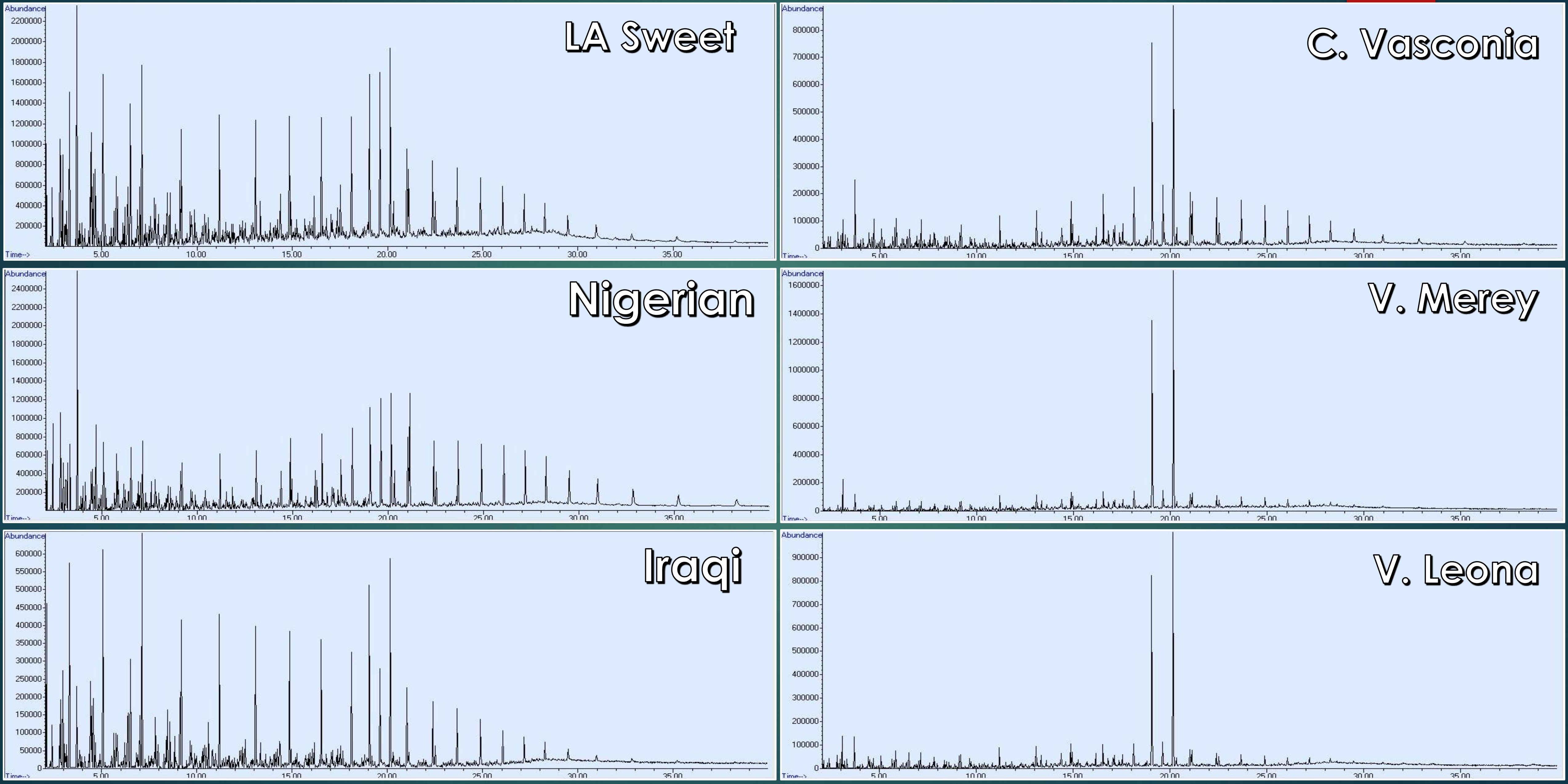
- Response normalized to Int. Std.



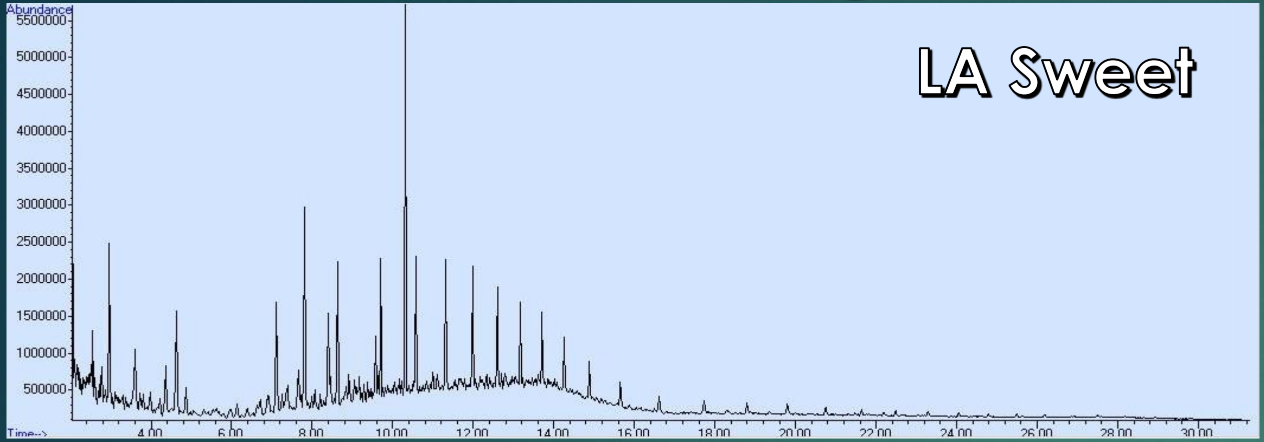
HP-5 Chromatographs

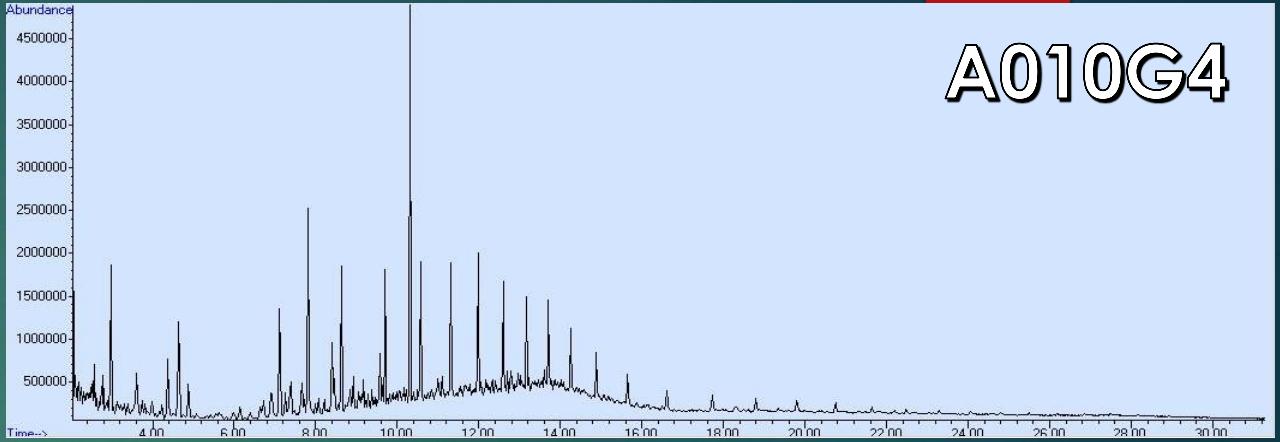


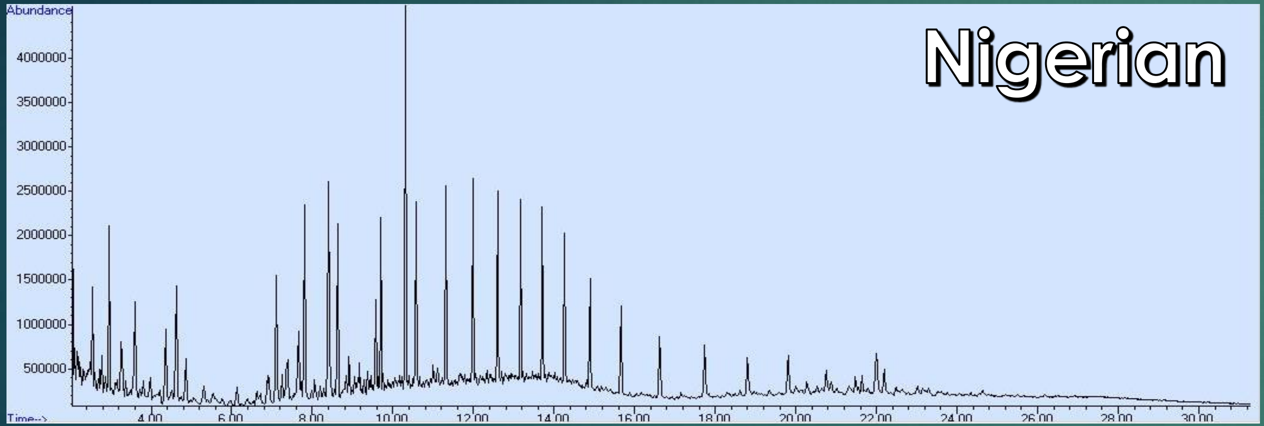
HP-5 Chromatographs

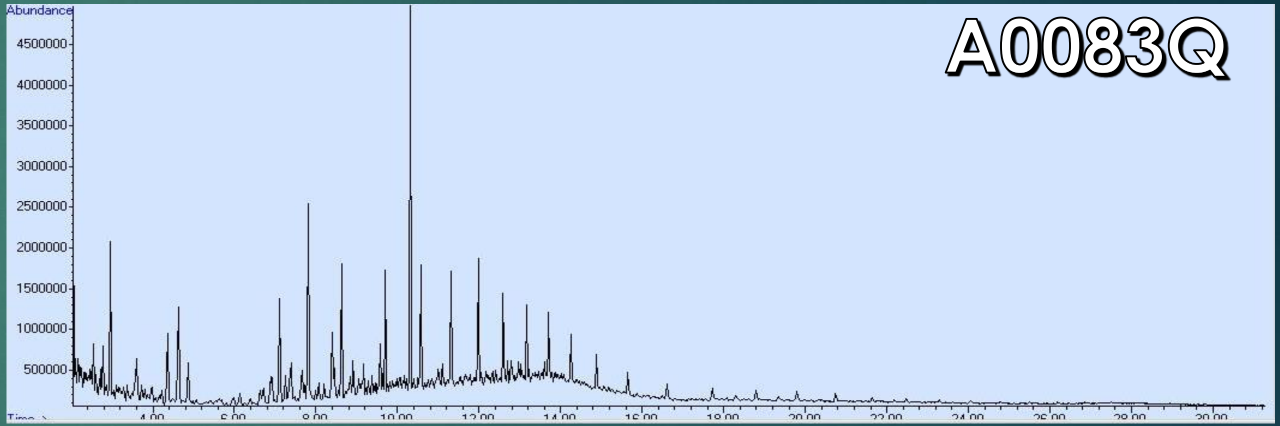


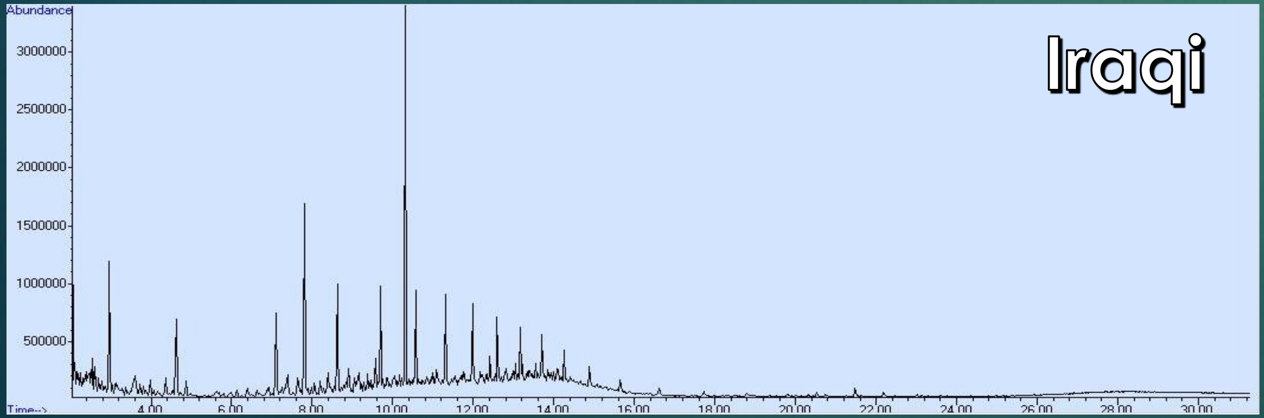
PAH Chromatographs

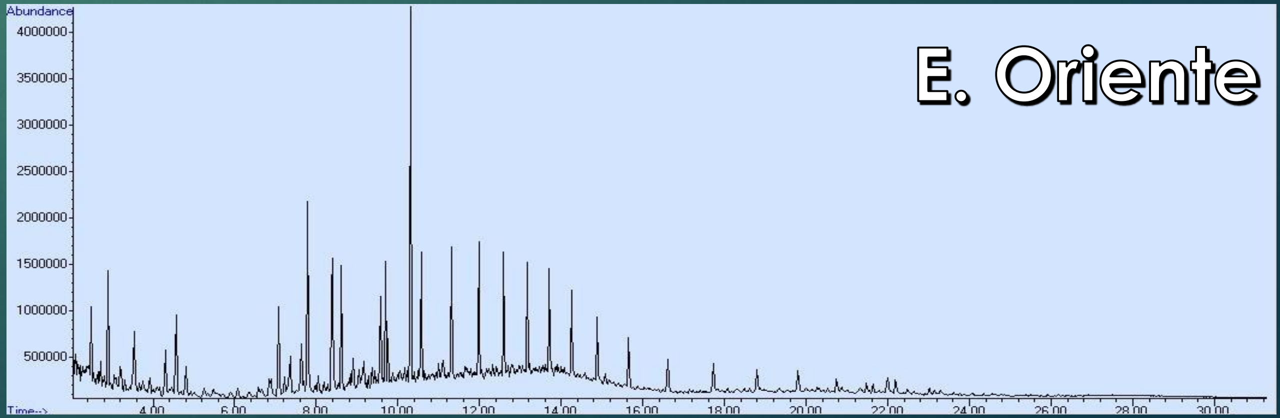




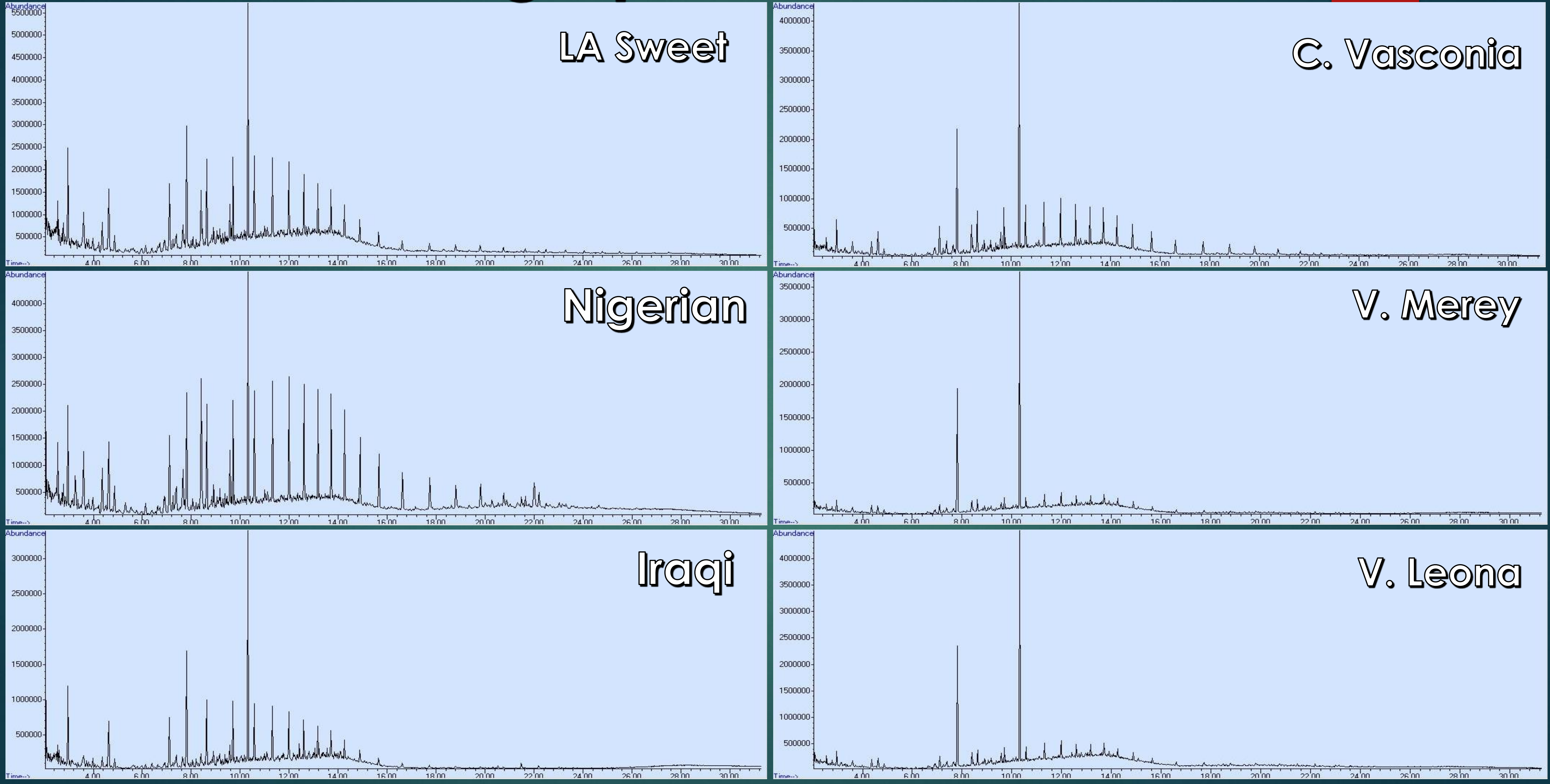








PAH Chromatographs



Applications of PLSr

- The PLS regression is a technique that reduces the predictors to a smaller set of uncorrelated components and performs least squares regression on these components.
- This regression works best when the predictors are highly collinear, or when you have more predictors than observations.
- The emphasis of the PLS regression is to build a predictive model.
  - Therefore, PLS is not usually used to screen out variables that are not useful in explaining the response.

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Normalization of the Data

- PLS is most optimal when operating with fairly symmetrically distributed data, and displays a near constant 'error variance'.
  - Variables that vary more than ten-fold are logarithmically transformed before the analysis.
- The data are also centered and scaled to unit variance before the analysis—scaling all variables to unit variance makes the assumption that all variables are equally important.

PLSr VIPs

- When PLS builds the models it essentially performs two *pseudo*-Principal Component Analyses simultaneously for X and Y, and aligns these two models.
  - PCA – maximum variance least squares projection of X
  - PLS – maximum covariance relationship between X and Y
- From the PLSr analysis, we obtained the 'Variable Importance in Projection' (VIP) values which indicate both the loading weights for each component and the variability explained by this component. Essentially, when the VIP for a variable is >1, it is detected as driving the response in the model.

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Principal Component Analysis

1. Principal Component Analysis
   - PCA can be thought of as revealing the internal structure of the data in a way that best explains the variance in the x-variables.
   - Proper treatment of outliers depends on the analysis purpose
   - Should outliers in the PCA analysis be removed from the PLSr?
     - If looking for large-scale tendencies → better to remove outliers.
     - If looking for non-typical data points → best to keep outliers.
   - Iraqi contained unique Thiol-containing constituents (PAH).
     - Maintained similarities in other areas (HP-5).

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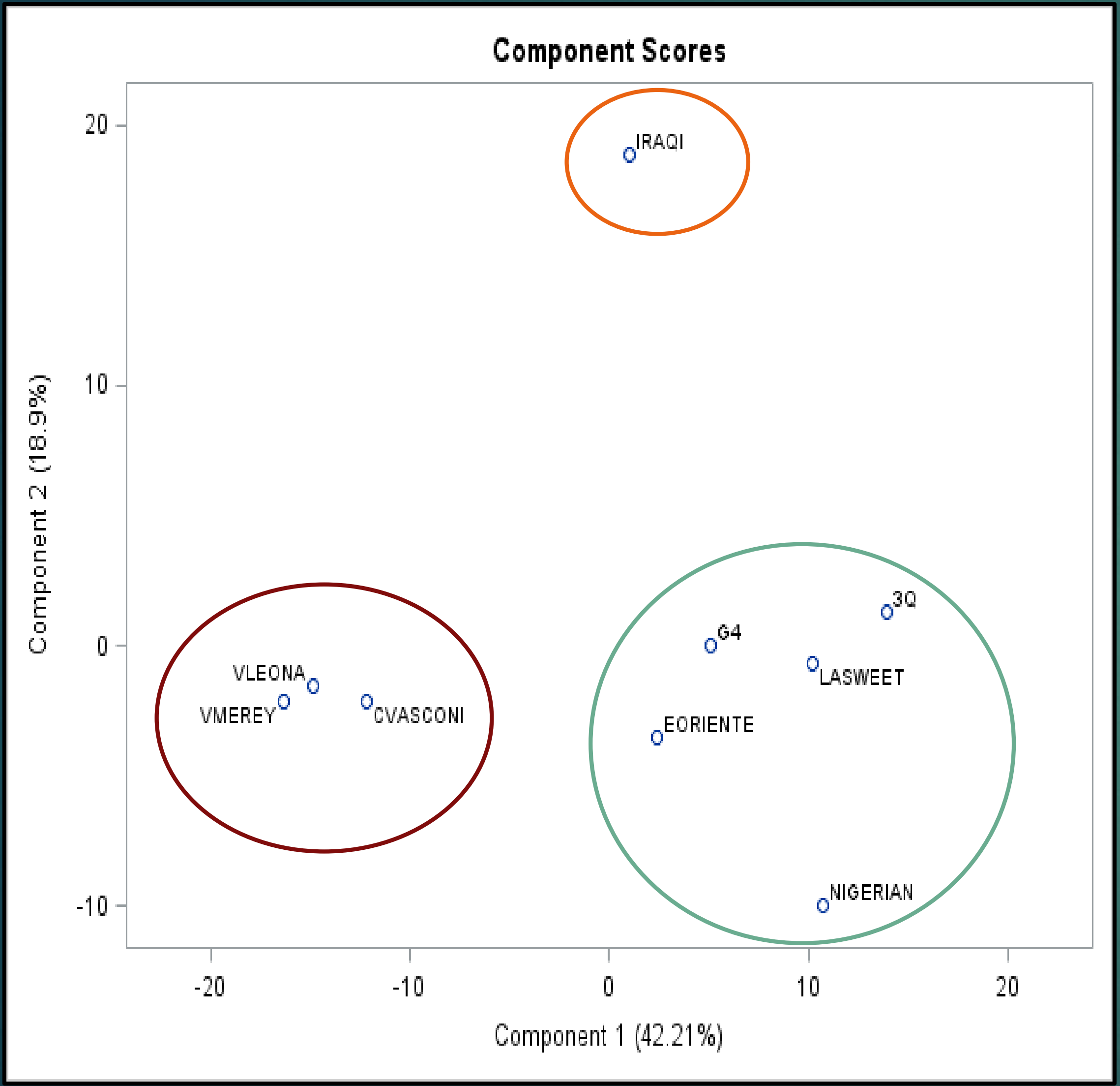
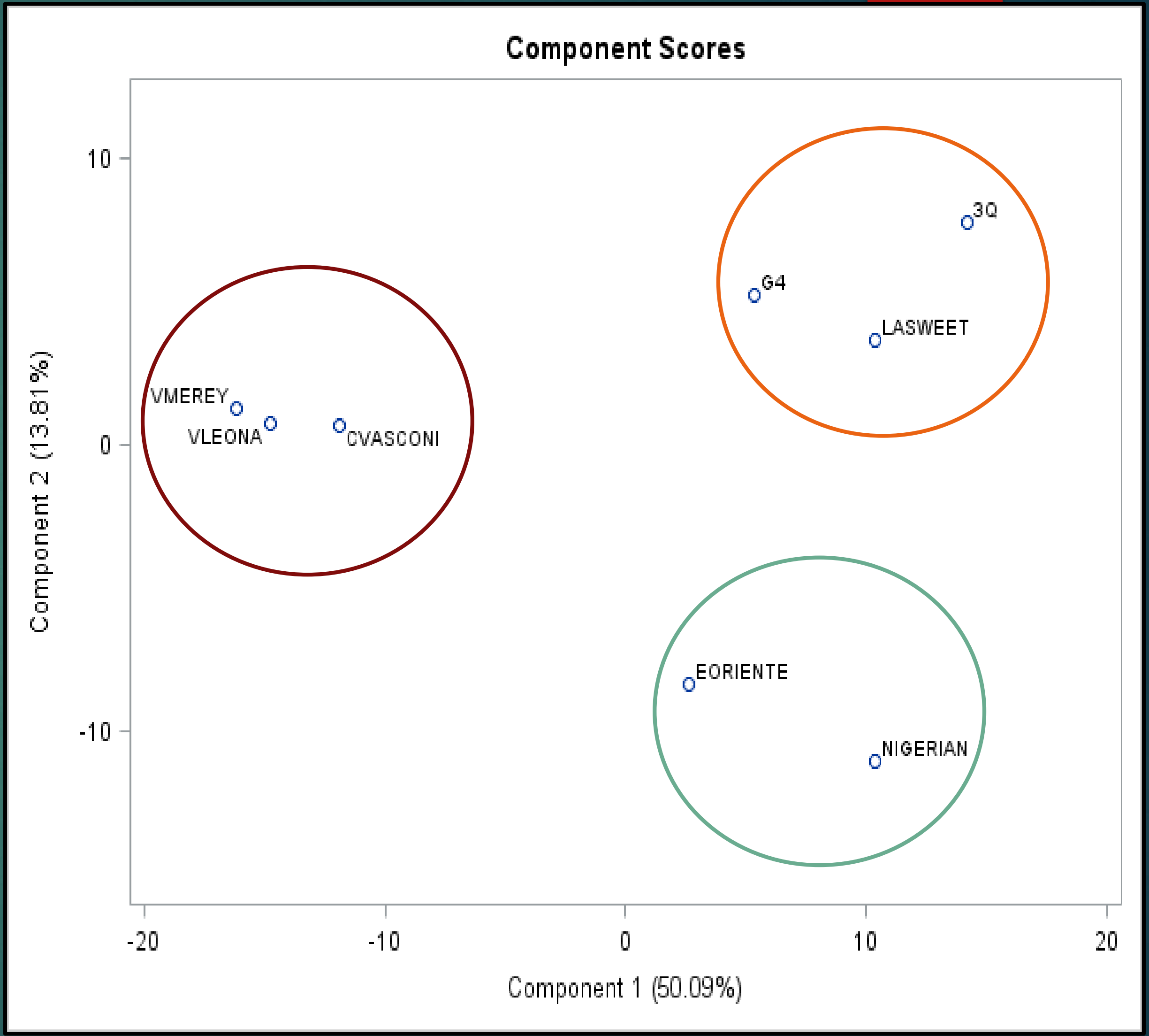
Principal Component Analysis





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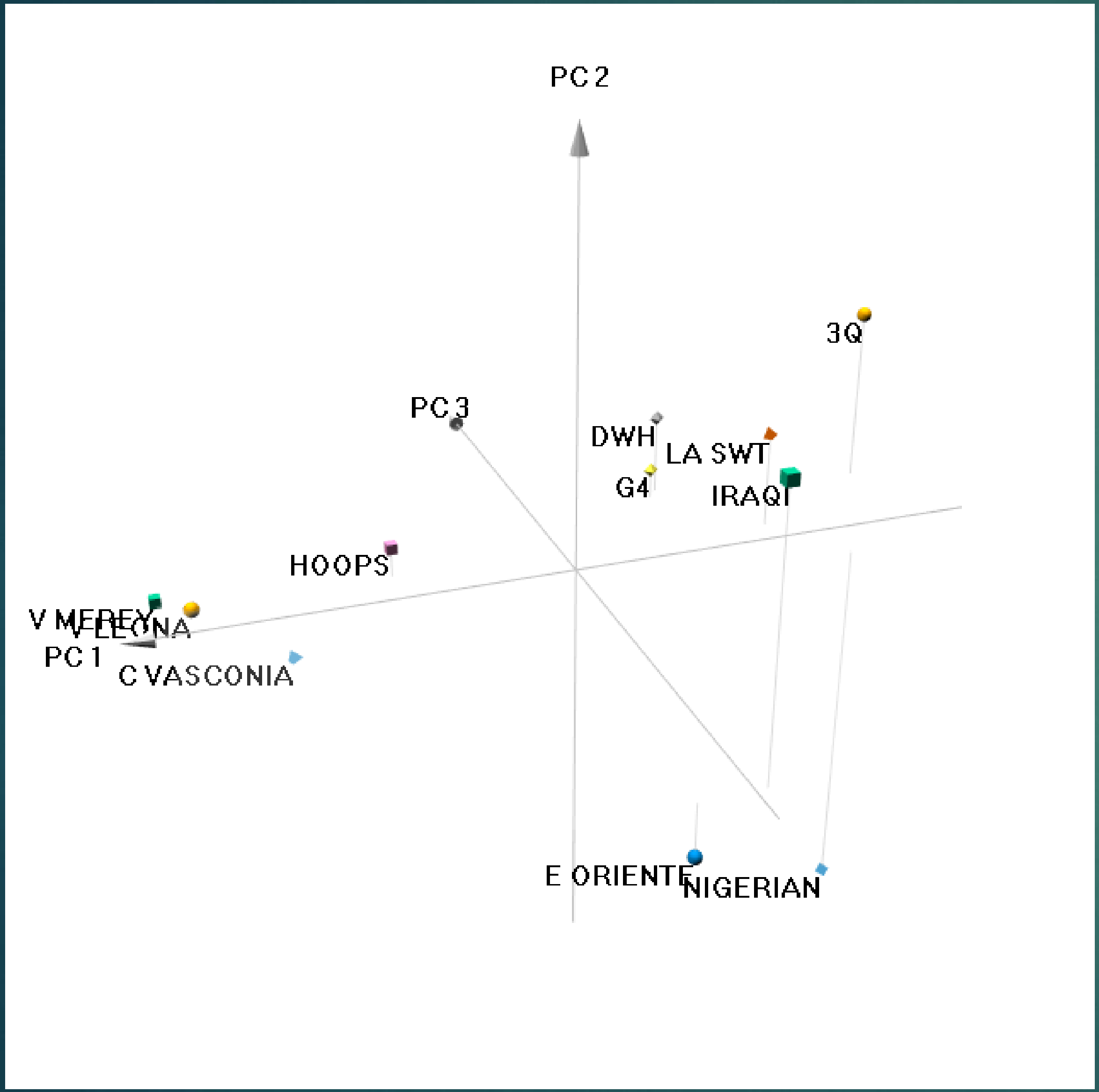
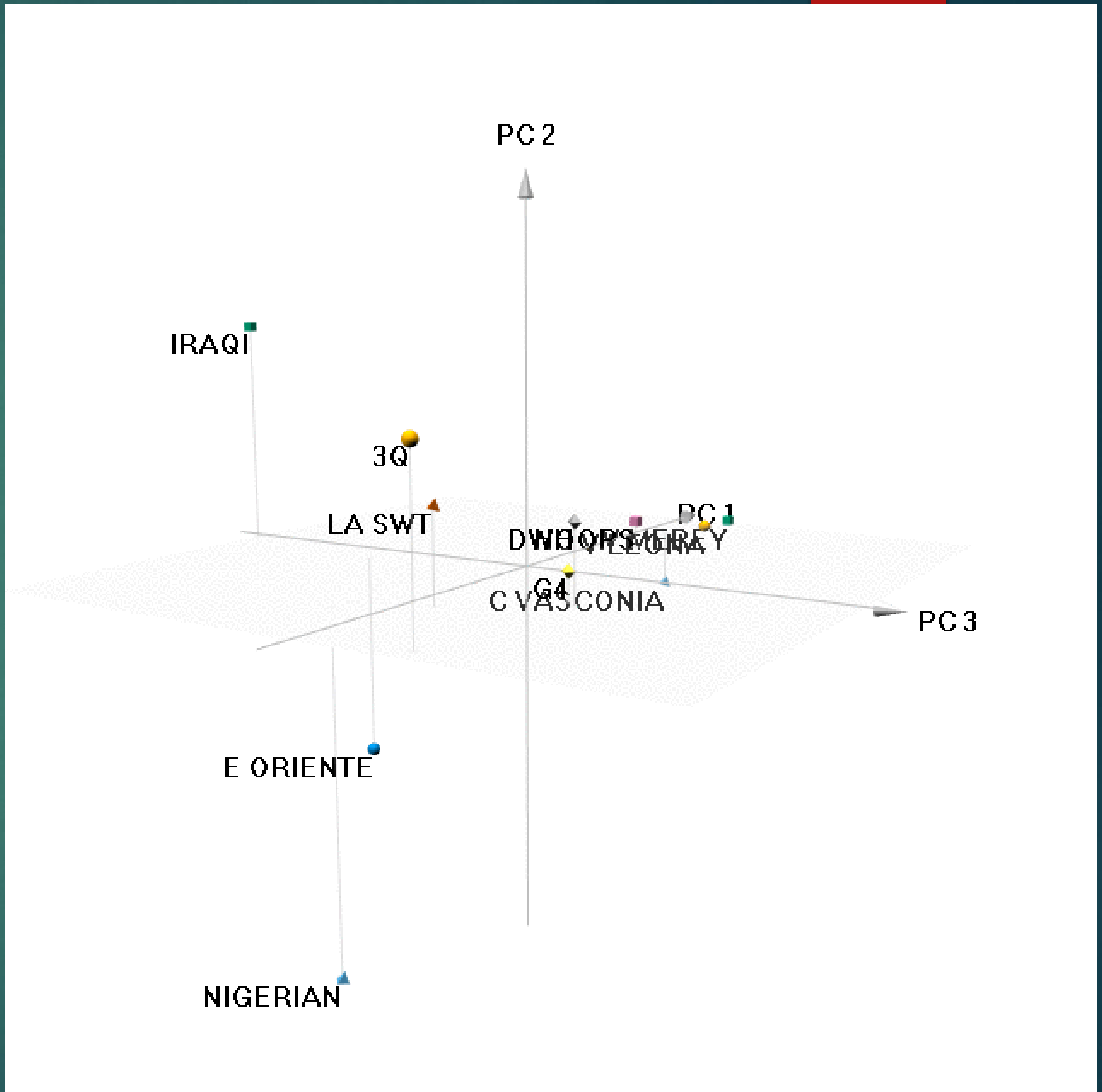
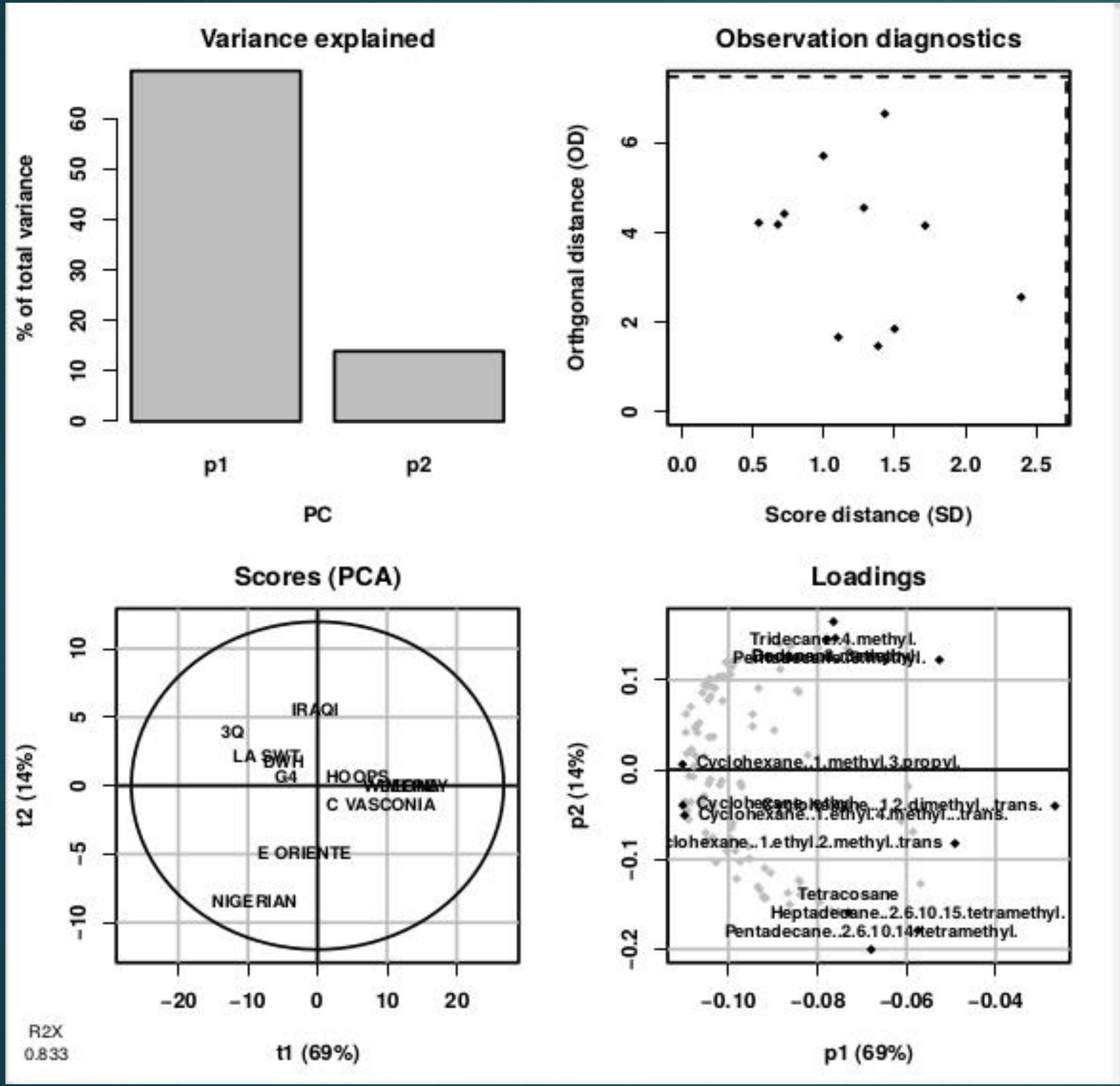
3D PCA of HP-5 Data (No Variables)

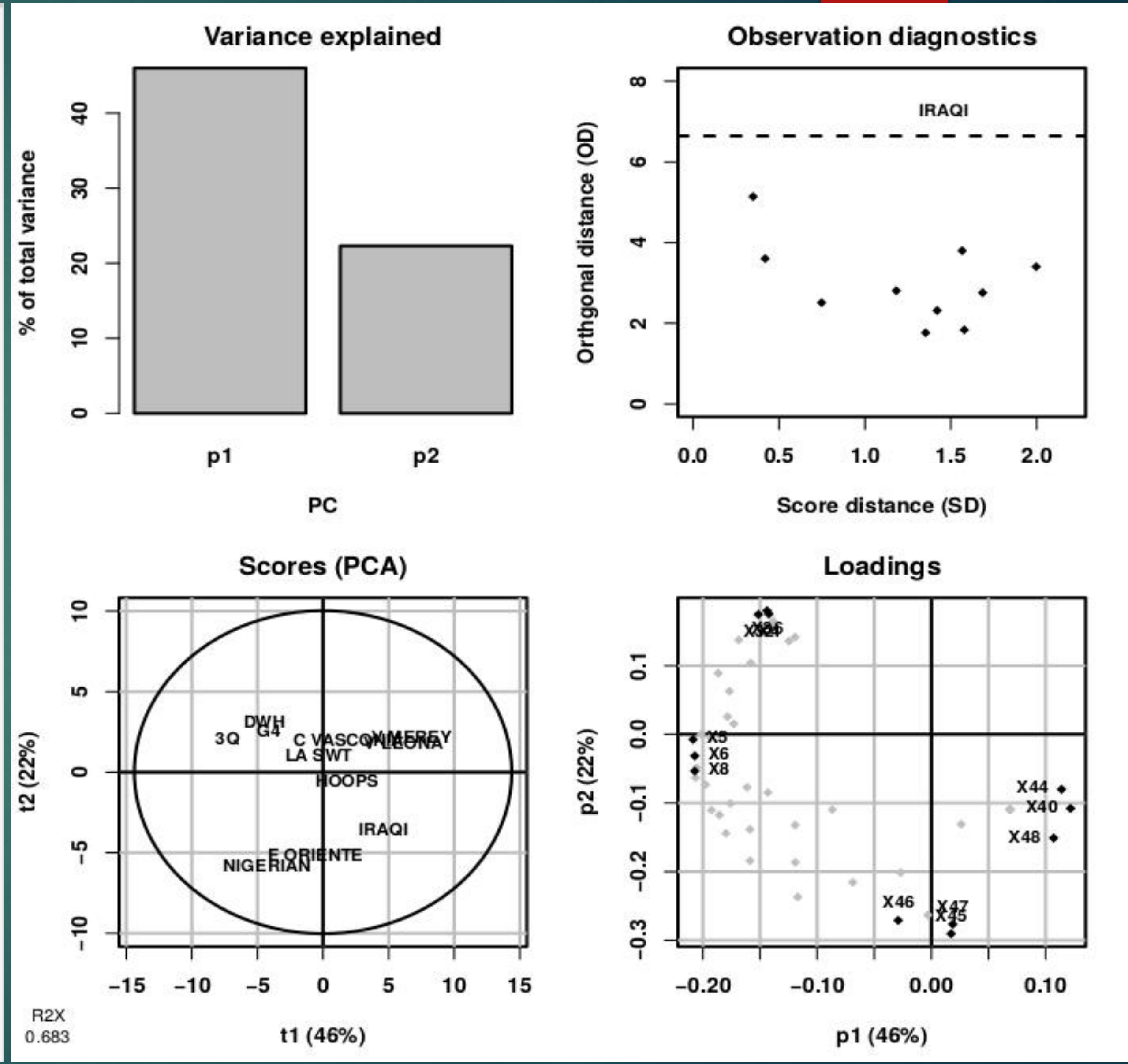




January Weiner (2017). pca3d: Three Dimensional PCA Plots. R package version 0.10. https://CRAN.R-project.org/package=pca3d

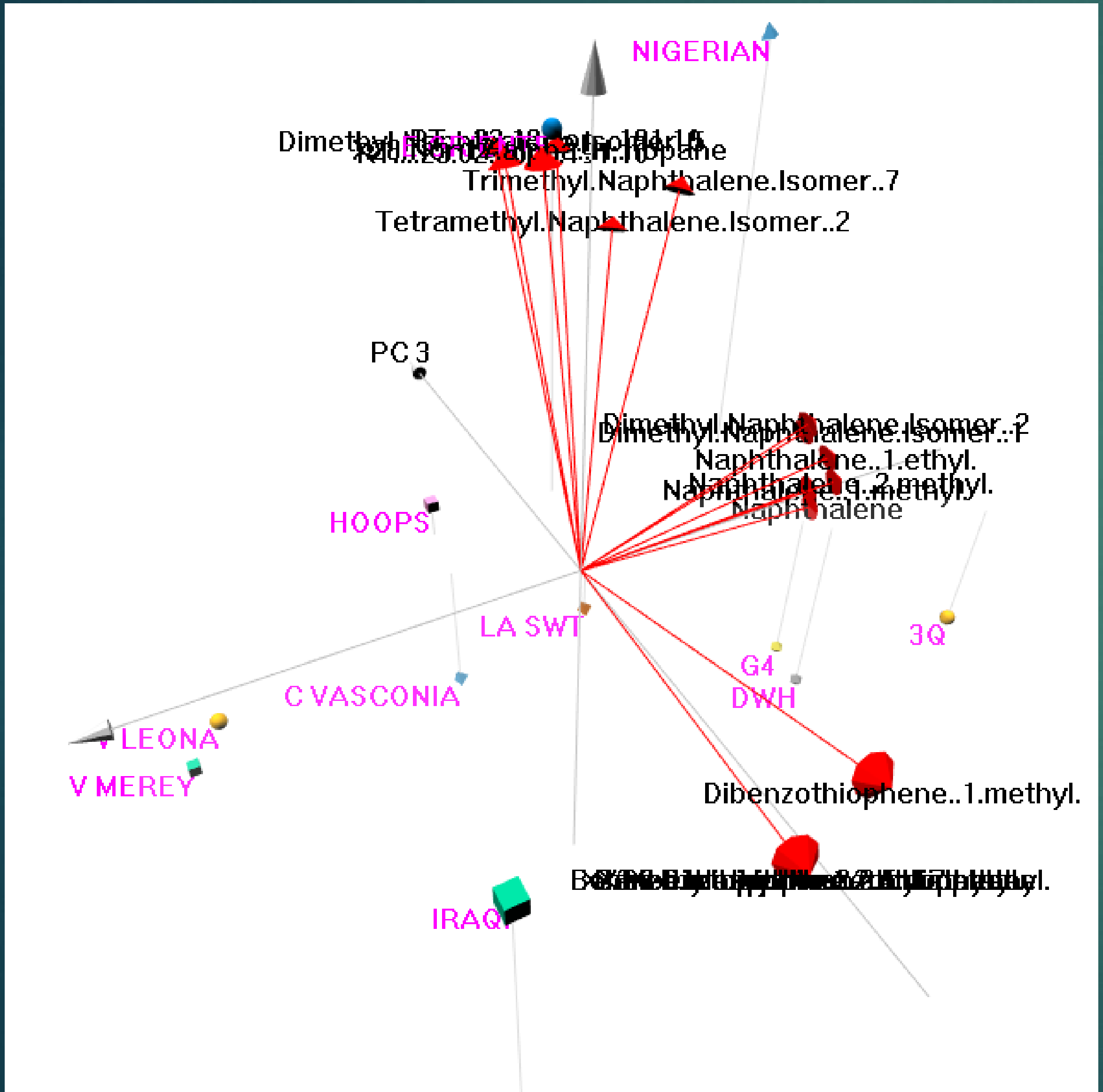
Principal Component Analysis

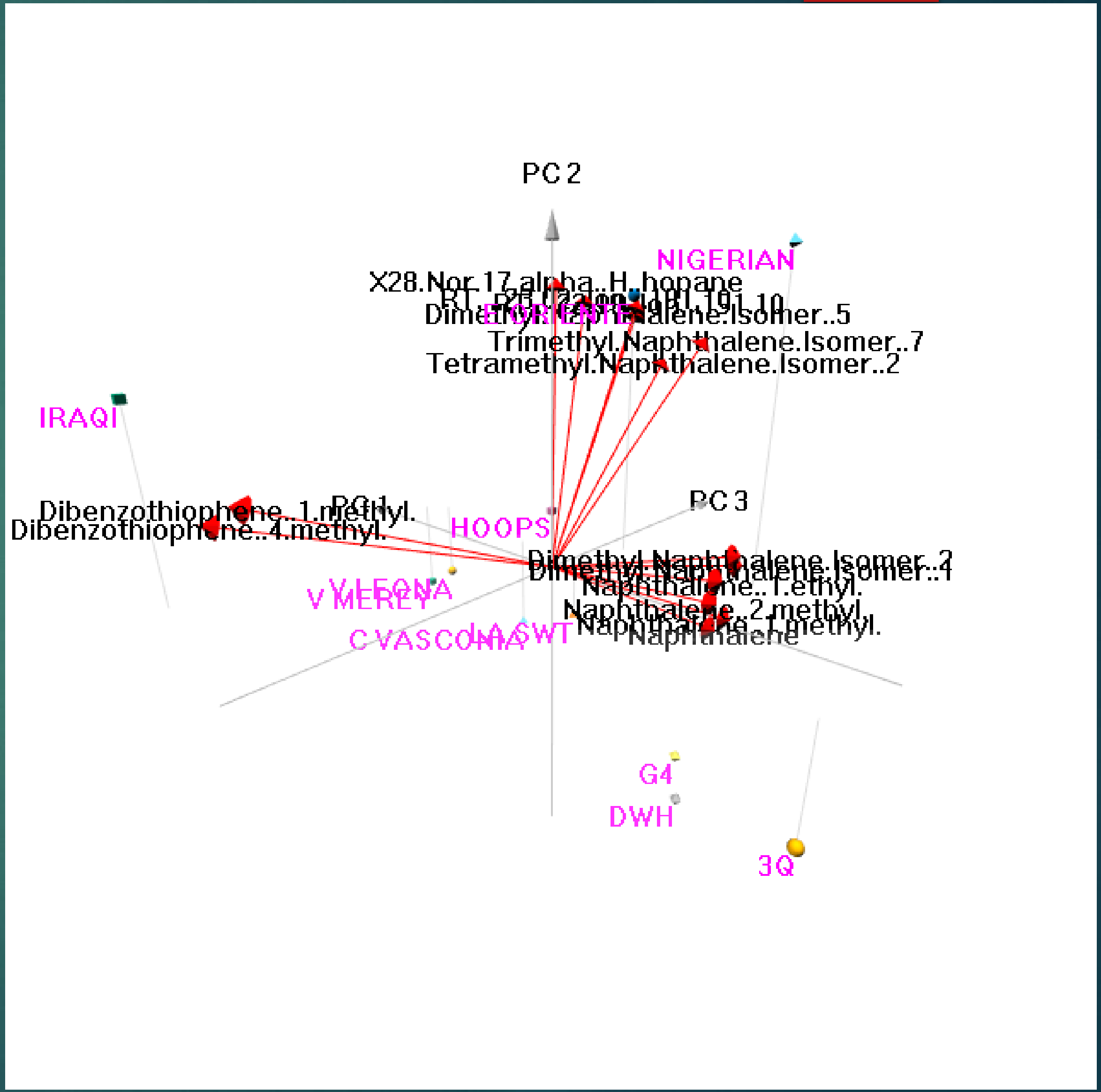




Thevenot, E.A., Roux, A., Xu, Y., Ezan, E., Junot, C. 2015.
http://pubs.acs.org/doi/full/10.1021/acs.jproteome.5b00354

3D PCA of PAH Data (With Variables)

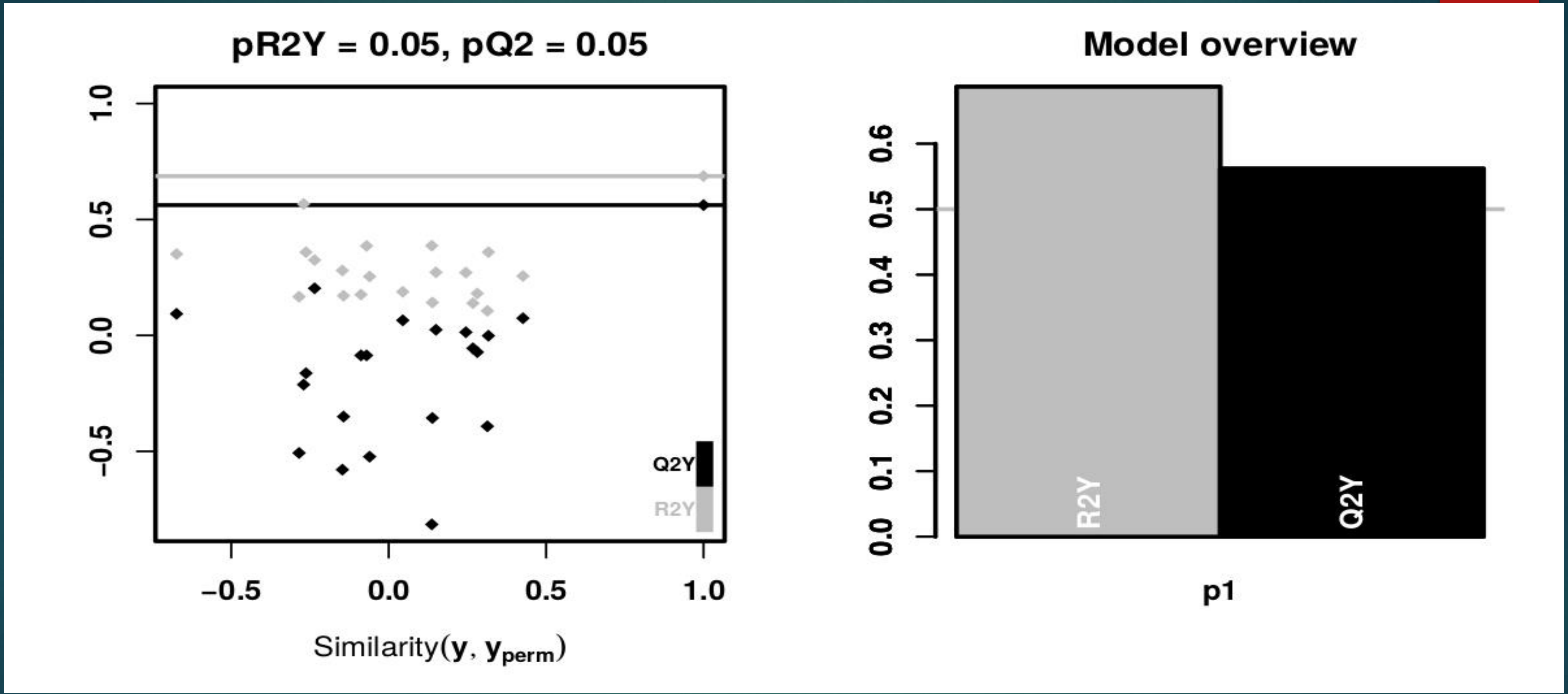




Partial Least Squares Regression

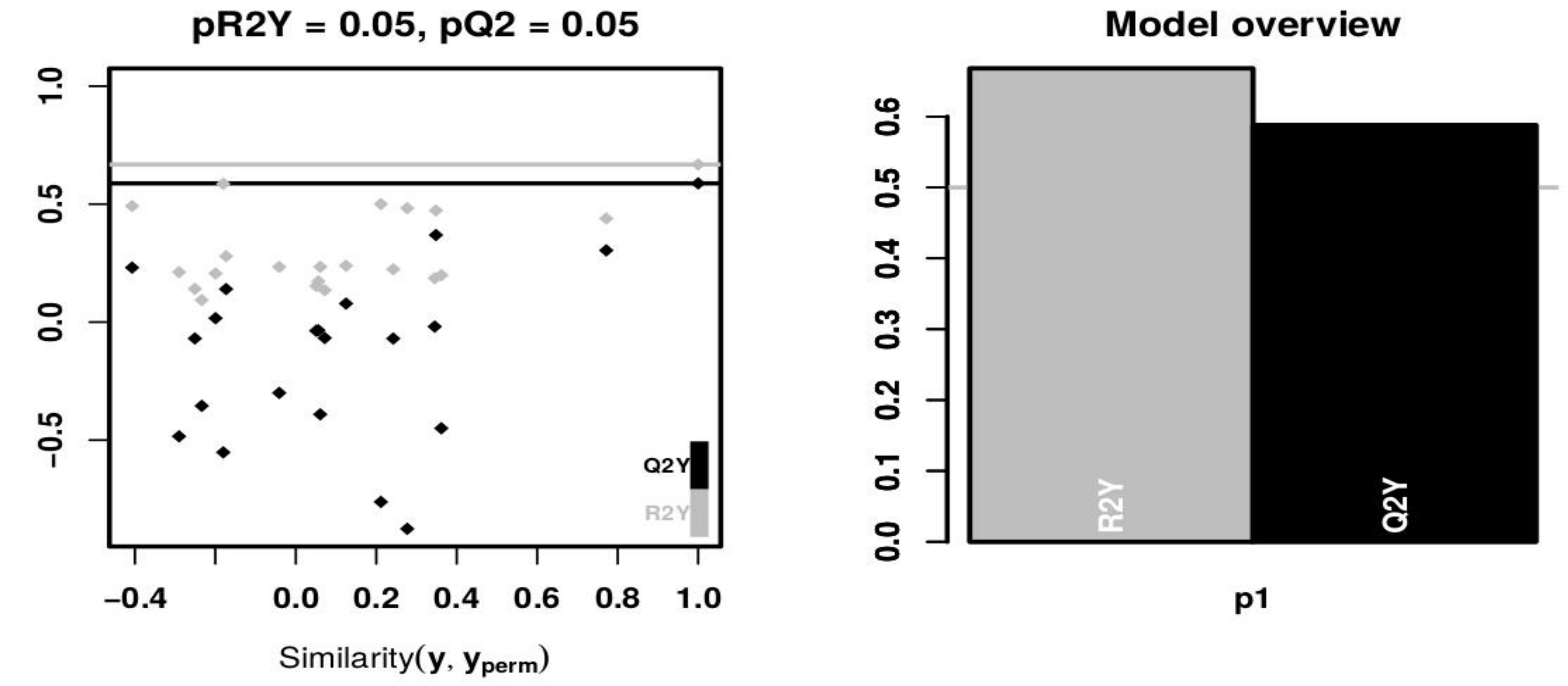
- Determining the Relationship between Predictors and Variables
  - The Regression Coefficient profile VIP gives a more direct indication of which predictors are most useful for predicting the dependent variable.
  - Regression coefficient = importance each predictor has in the prediction in the response
  - VIPs = contribution of each predictor in fitting the PLS model for BOTH predictors and response.

PLSr on 2.5 mL/Kg Relative Spleen Weights(HP-5)



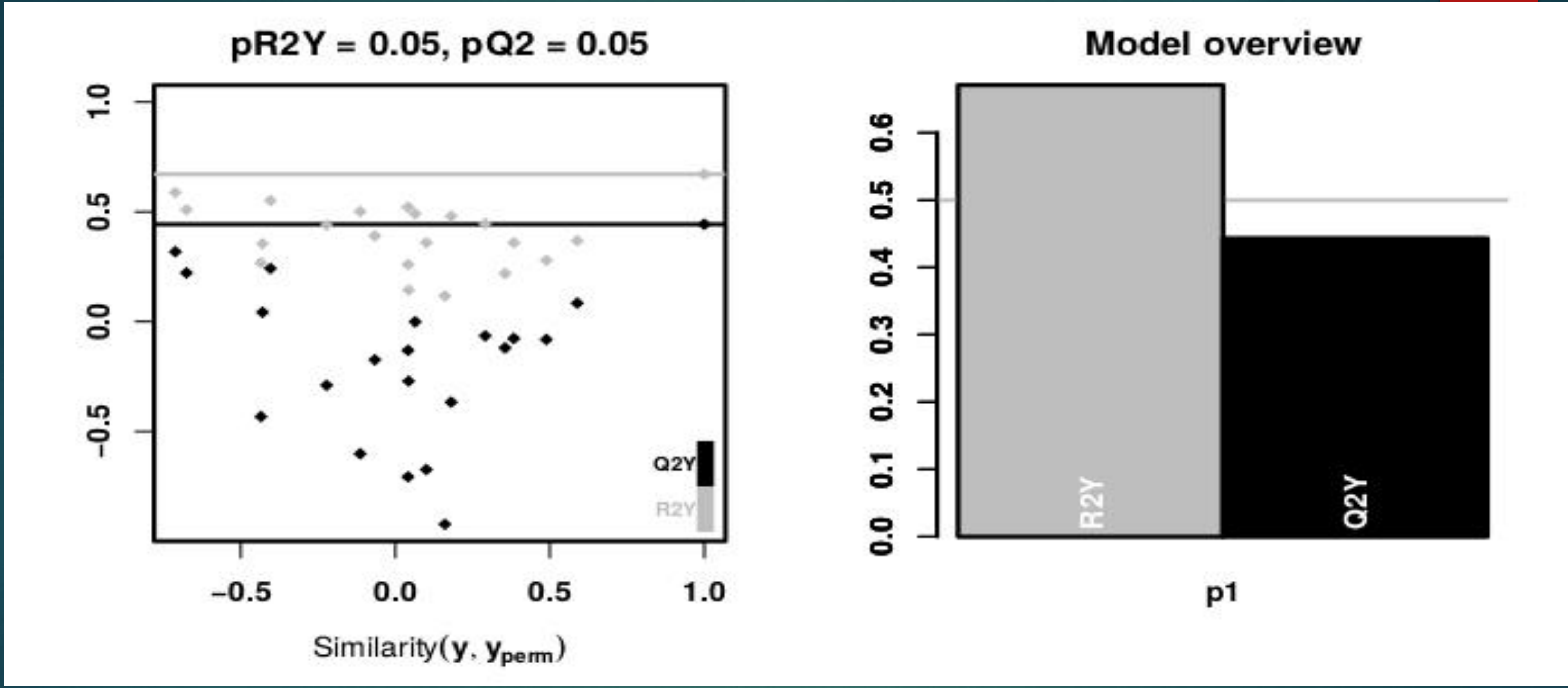
HP-5	R2X(cum)	R2Y(cum)	Q2Y(cum)	RMSEE	pre	Ort	pR2Y	pQ2
Low Dose Spleen	0.693	0.687	0.562	0.0108	1	0	0.05	0.05

PLSr on 5.0 mL/Kg Relative Spleen Weights (HP-5)



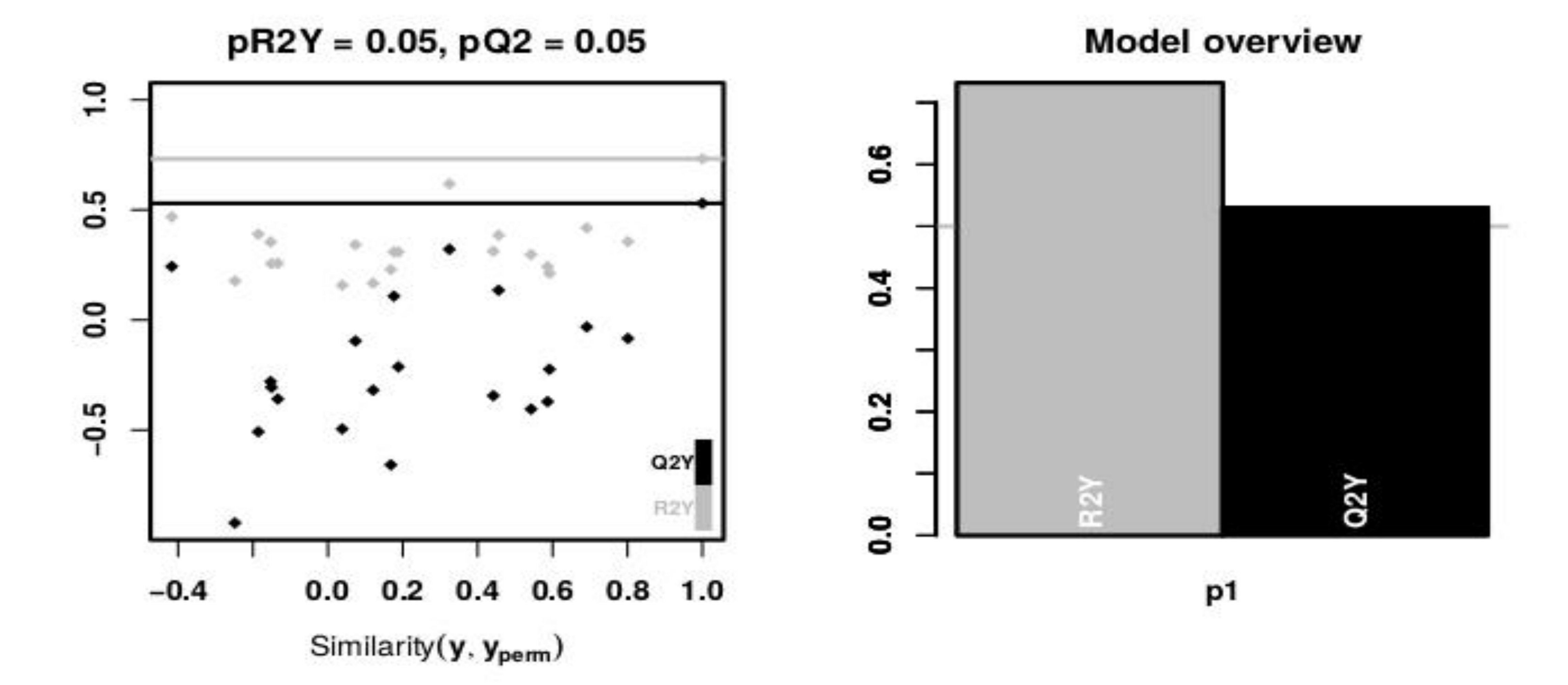
HP-5	R2X(cum)	R2Y(cum)	Q2Y(cum)	RMSEE	pre	Ort	pR2Y	pQ2
High Dose Spleen	0.693	0.668	0.588	0.0175	1	0	0.05	0.05

PLSr on 2.5 mL/Kg Relative Spleen Weights (PAH)



PAH	R2X(cum)	R2Y(cum)	Q2Y(cum)	RMSEE	pre	Ort	pR2Y	pQ2
Low Dose Spleen	0.52	0.687	0.443	0.0116	1	0	0.05	0.05

PLSr on 5.0 mL/Kg Relative Spleen Weights (PAH)



PAH	R2X(cum)	R2Y(cum)	Q2Y(cum)	RMSEE	pre	Ort	pR2Y	pQ2
High Dose Spleen	0.524	0.732	0.53	0.0158	1	0	0.05	0.05

Summary of PLSr Results

HP-5	R2X(cum)	R2Y(cum)	Q2Y(cum)	RMSEE	pre	Ort	pR2Y	pQ2
Low Dose Spleen	0.693	0.687	0.562	0.0108	1	0	0.05	0.05
High Dose Spleen	0.693	0.668	0.588	0.0175	1	0	0.05	0.05
PAH	**R2X(cum)**	**R2Y(cum)**	**Q2Y(cum)**	**RMSEE**	**pre**	**Ort**	**pR2Y**	**pQ2**
Low Dose Spleen	0.52	0.671	0.443	0.0116	1	0	0.05	0.05
High Dose Spleen	0.524	0.732	0.53	0.0158	1	0	0.05	0.05

Score Plots Rel. Spleen Weights L. & H. Dose

Highest 12 VIPs from 110 Peaks (HP-5 Dataset)

Spleen VIPs					
Low Dose			High Dose		
Peak #	VIP	Library/ID	Peak #	VIP	Library/ID
59	1.210	4-methyl-Decane	65	1.308	4-ethyl-1,2-dimethyl-Benzene
32	1.204	2,6-dimethyl-Heptane	57	1.264	2,4,6-trimethyl-Benzene
14	1.201	Ethyl-Cyclopentane	28	1.261	Octane
39	1.195	1-methyl-2-propyl-Cyclopentane	53	1.236	1-ethyl-3-methyl-Benzene
25	1.187	Trans-1-ethyl-3-methyl-Cyclopentane	39	1.231	1-methyl-2-propyl-Cyclopentane
85	1.180	2-methyl-Tridecane	14	1.223	Ethyl-Cyclopentane
26	1.169	Cis-1-ethyl-2-methyl-Cyclopentane	54	1.215	1,2,3-trimethyl-Benzene
38	1.168	1,2,4-trimethyl-Cyclohexane	80	1.196	Tridecane
88	1.168	Tetradecane	32	1.193	2,6-dimethyl-Heptane
15	1.165	1,2,4-trimethyl-Cyclopentane	25	1.188	Trans-1-ethyl-3-methyl-Cyclopentane
75	1.162	2,6-dimethyl-Undecane	88	1.176	Tetradecane
94	1.161	Pentadecane	74	1.171	Dodecane

Score Plots Rel. Spleen Weights L. & H. Dose

Highest 12 VIPs from 43 Peaks (PAH Dataset)

Spleen VIPs					
Low Dose			High Dose		
Peak #	VIP	Library/ID	Peak #	VIP	Library/ID
3	1.5267	Indene Isomer	3	1.4399	Indene Isomer
9	1.3763	Dimethyl-Naphthalene Isomer #2	8	1.3992	Dimethyl-Naphthalene Isomer #1
8	1.3743	Dimethyl-Naphthalene Isomer #1	9	1.3908	Dimethyl-Naphthalene Isomer #2
6	1.3426	Naphthalene, 2-methyl-	6	1.3673	Naphthalene, 2-methyl-
7	1.3331	Naphthalene, 1-ethyl-	5	1.3559	Naphthalene, 1-methyl-
13	1.3320	Naphthalene, 1-propyl-	7	1.3387	Naphthalene, 1-ethyl-
2	1.3295	Naphthalene	19	1.3374	Trimethyl-Naphthalene Isomer #5
39	1.3229	RT: 20.53; Ion: 191.10	16	1.3308	Trimethyl-Naphthalene Isomer #2
17	1.3176	Trimethyl-Naphthalene Isomer #3	17	1.3239	Trimethyl-Naphthalene Isomer #3
5	1.3175	Naphthalene, 1-methyl-	2	1.3147	Naphthalene
19	1.3175	Trimethyl-Naphthalene Isomer #5	13	1.2790	Naphthalene, 1-propyl-
20	1.3040	Trimethyl-Naphthalene Isomer #6	10	1.2494	Dimethyl-Naphthalene Isomer #3

VIP to Spleen weight analysis by Crude Oil

- Determining the Relationship between selected constituents via VIPs, Area Response, and Endpoints.

LA Sweet		Nigerian		Iraqi	
Library/ID	Area	Library/ID	Area	Library/ID	Area
Decane, 4-m	148105	Decane, 4-m	143471.653	Decane, 4-m	114143.935
Heptane, 2,6	113935	Heptane, 2,6	91455.1492	Heptane, 2,6	99770.2076
Cyclopentan	27361	Cyclopentan	51619.6328	Cyclopentan	26068.828
Cyclopentan	28905	Cyclopentan	38884.497	Cyclopentan	36482.5745
Cyclopentan	12395	Cyclopentan	27869.1337	Cyclopentan	13646.8952
Tridecane, 2	84695	Tridecane, 2	74154.5319	Tridecane, 2	67607.8968
Cyclopentane	22669	Cyclopentane	51881.2734	Cyclopentane	26742.4007
Cyclohexane	23448	Cyclohexane	28887.7682	Cyclohexane	12127.4406
Tetradecane	553266	Tetradecane	538515.153	Tetradecane	517485.504
Cyclopentan	67730	Cyclopentan	135121.202	Cyclopentan	31003.1393
Undecane, 2	245717	Undecane, 2	220971.656	Undecane, 2	142922.718
Pentadecane	526115	Pentadecane	568423.025	Pentadecane	466823.442

- Etc.

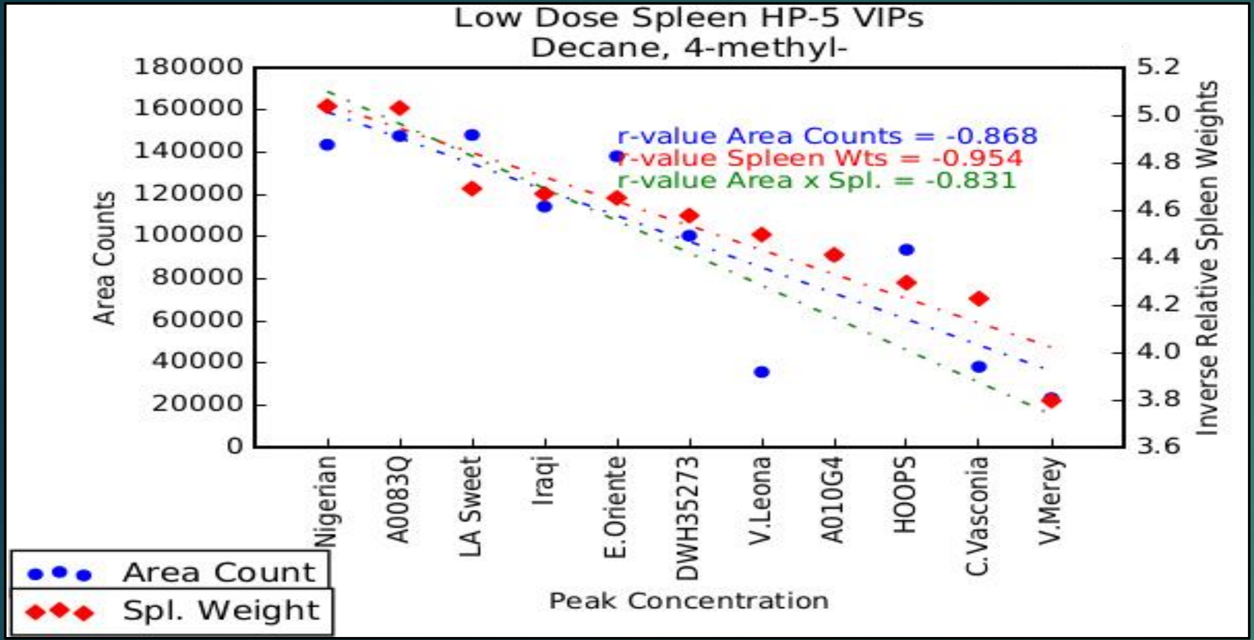
VIP to Spleen weight analysis by Crude Oil

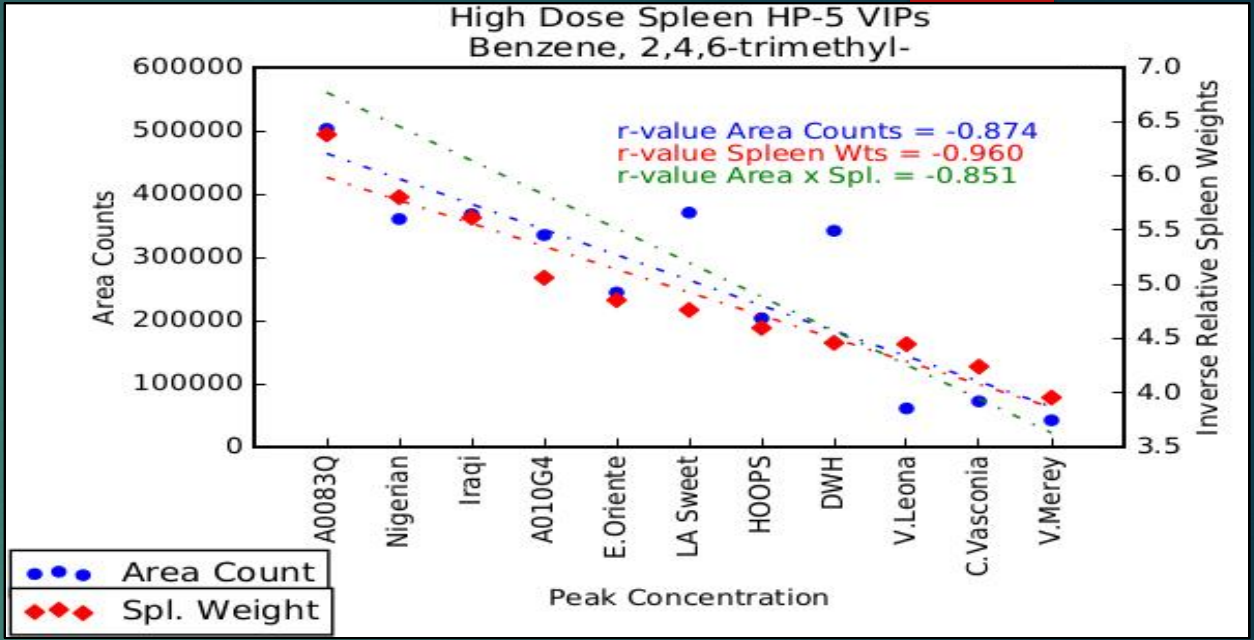
- Python script compiled VIP selected peaks with Area.

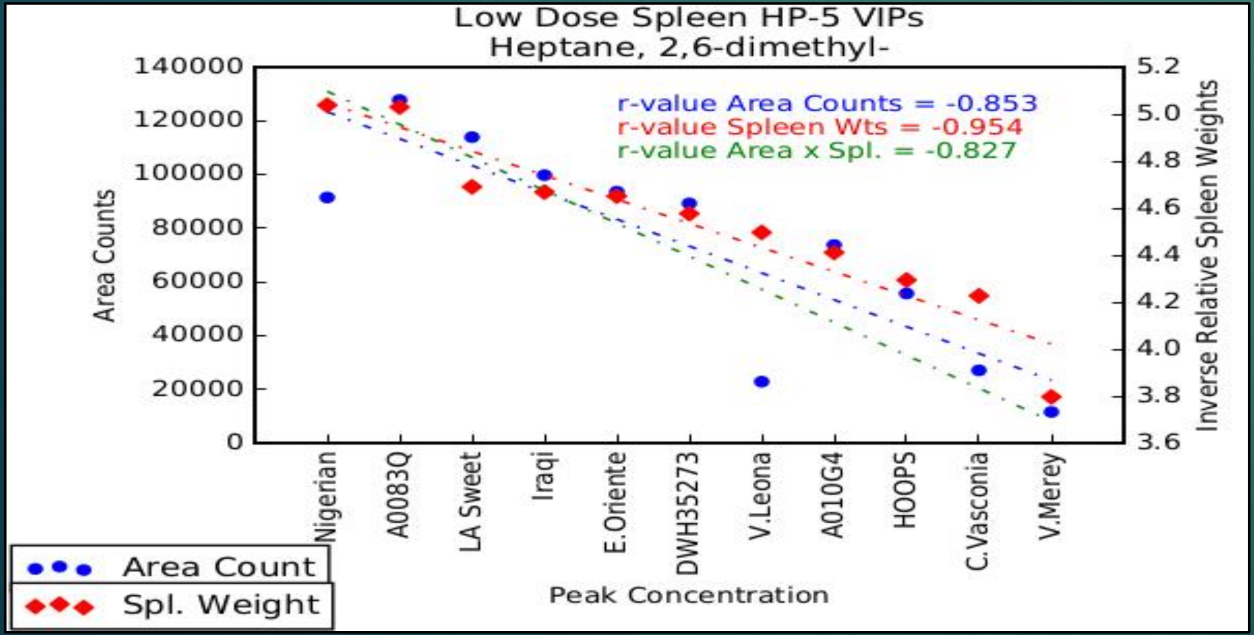
	Library/ID	Area_1	Area_2	Area_3	Area_4	Area_5	Area_6	Area_7	Area_8	Area_9	Area_10	Area
0	Decane, 4	143471.7	147649.1	148105	114143.9	138015.4	100317.9	35687.76	90964.36	93683.28	38147.67	23255.4
1	Heptane,	91455.15	127753.4	113935	99770.21	93643.6	89244.27	22848.45	73746.18	55693.44	27065.87	11587.03
2	Cyclopent	51619.63	48670.81	27361	26068.83	27843.88	35170.73	0	29802.48	6187.356	5579.422	0
3	Cyclopent	38884.5	38484.93	28905	36482.57	38140.08	26930.54	6844.726	20190.2	23721.57	9934.29	5251.662
4	Cyclopent	27869.13	17098.46	12395	13646.9	17500.25	11581	0	9759.753	4125.869	0	0
5	Tridecane	74154.53	74380.99	84695	67607.9	55248.12	52927.33	10915.59	53543.23	21311.2	19773.29	7837.355
6	Cyclopent	51881.27	33256.6	22669	26742.4	40723.25	23673.6	2693.93	16288	12305.22	9717.357	1560.077
7	Cyclohexa	28887.77	24383.02	23448	12127.44	23381.71	16806.08	6448.72	15173.49	16520.85	10327.61	4137.321
8	Tetradeca	538515.2	590602.2	553266	517485.5	412776.5	450832.8	79061.3	455990.2	89531.36	175488.2	56857.79
9	Cyclopent	135121.2	101260.6	67730	31003.14	77369.25	74093.22	10285.25	60305.43	25810.57	20063.21	4680.232
10	Undecane	220971.7	206163.5	245717	142922.7	225795.8	151587.4	57055.73	144687.7	111268.2	66460.4	38944.89
11	Pentadeca	568423	531206.9	526115	466823.4	430891.5	432661	73499.06	463684	74756.4	187577.6	53608.78

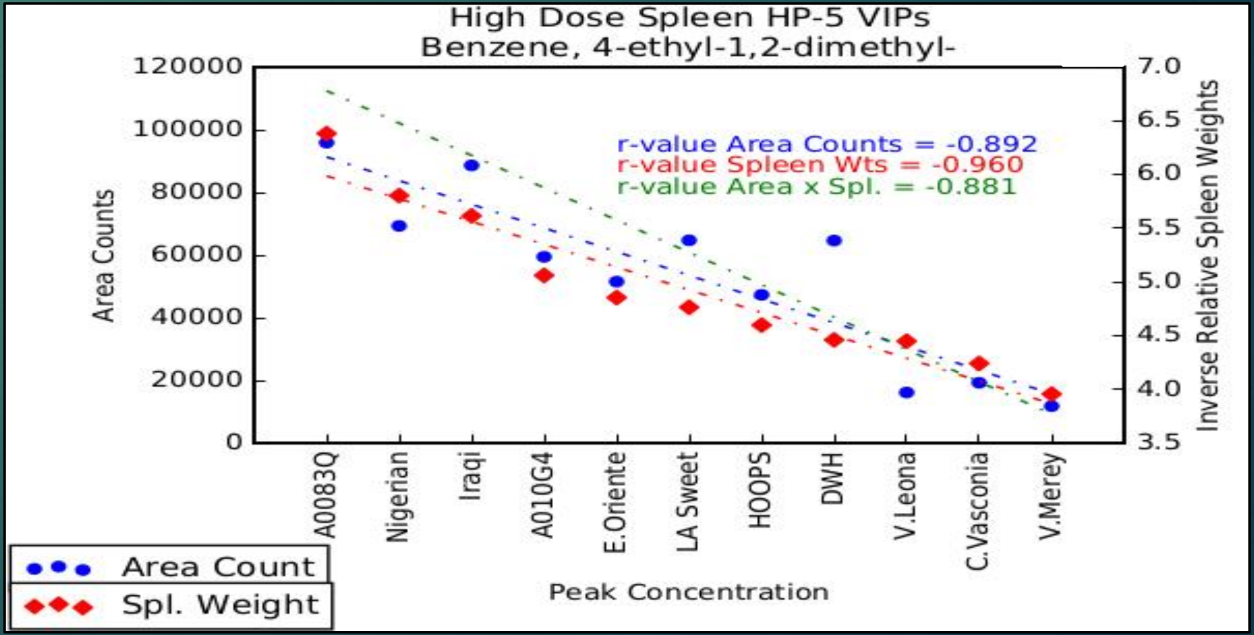
Spleen Weight (g)		LA Sweet	Nigerian	Iraqi	G4	3Q	E. Oriente	C. Vasconia	V. Merey	V. Leona	DWH	HOOPS
	Low Dose	0.2132	0.1985	0.2142	0.2267	0.1988	0.215	0.2366	0.2634	0.2224	0.2245	0.2393
	High Dose	0.2099	0.1723	0.1781	0.1976	0.1567	0.206	0.2358	0.2528	0.2248	0.2304	0.2235

VIP Correlation Coefficient Graphs for Rel. Spleen Weights





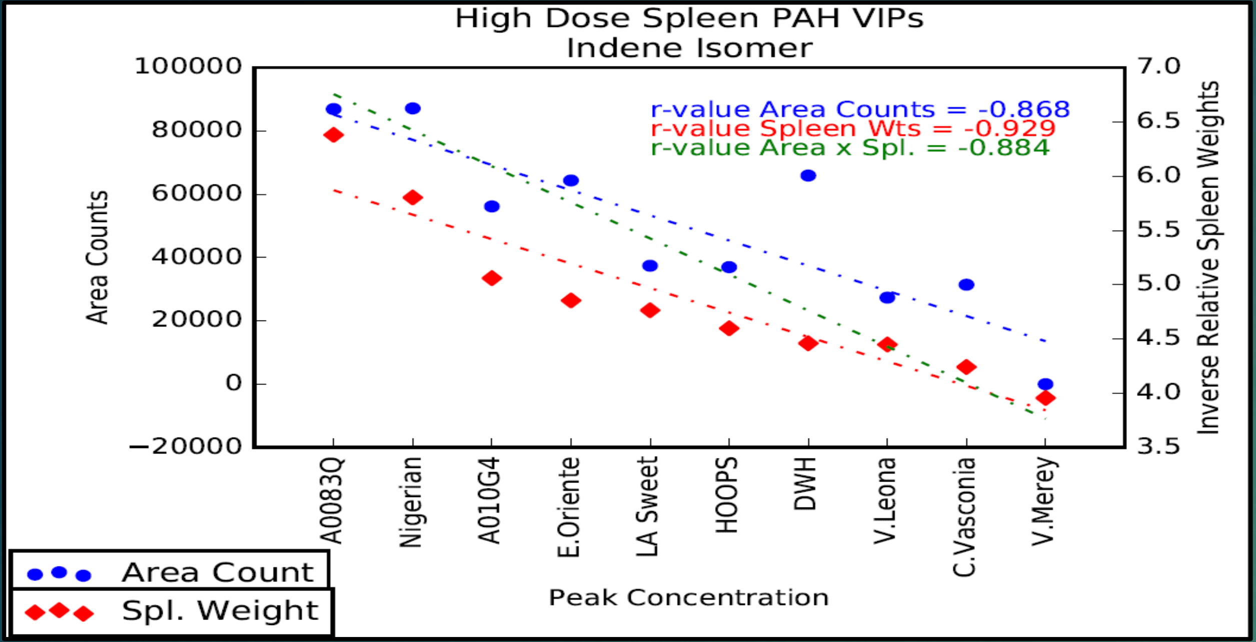


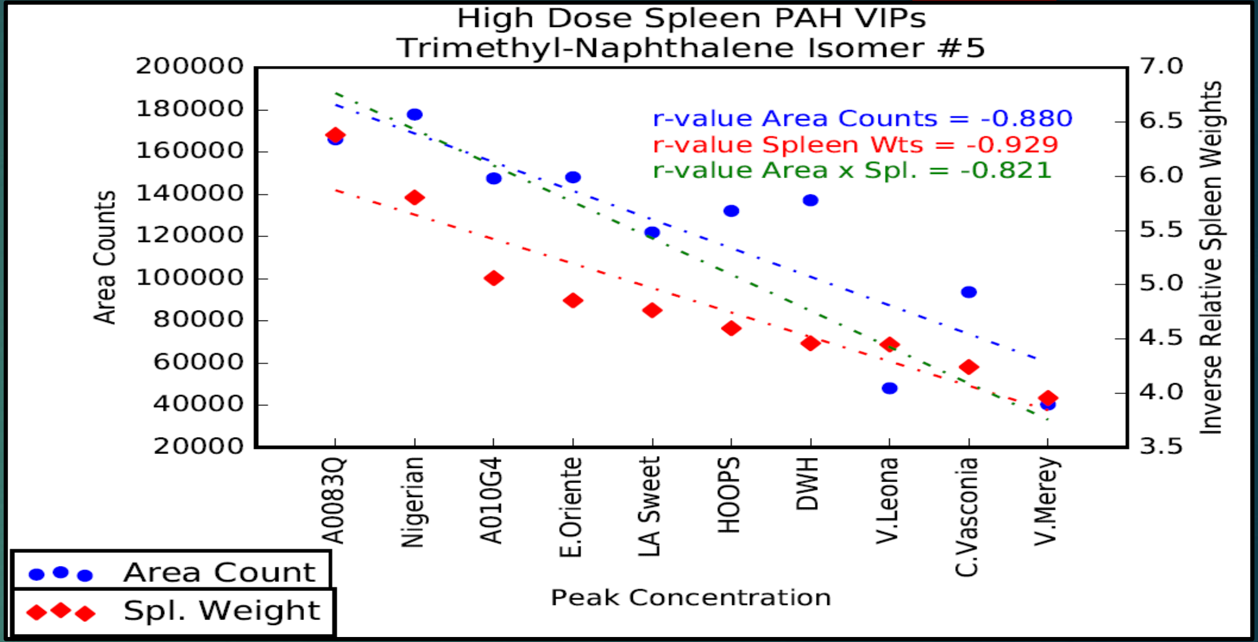


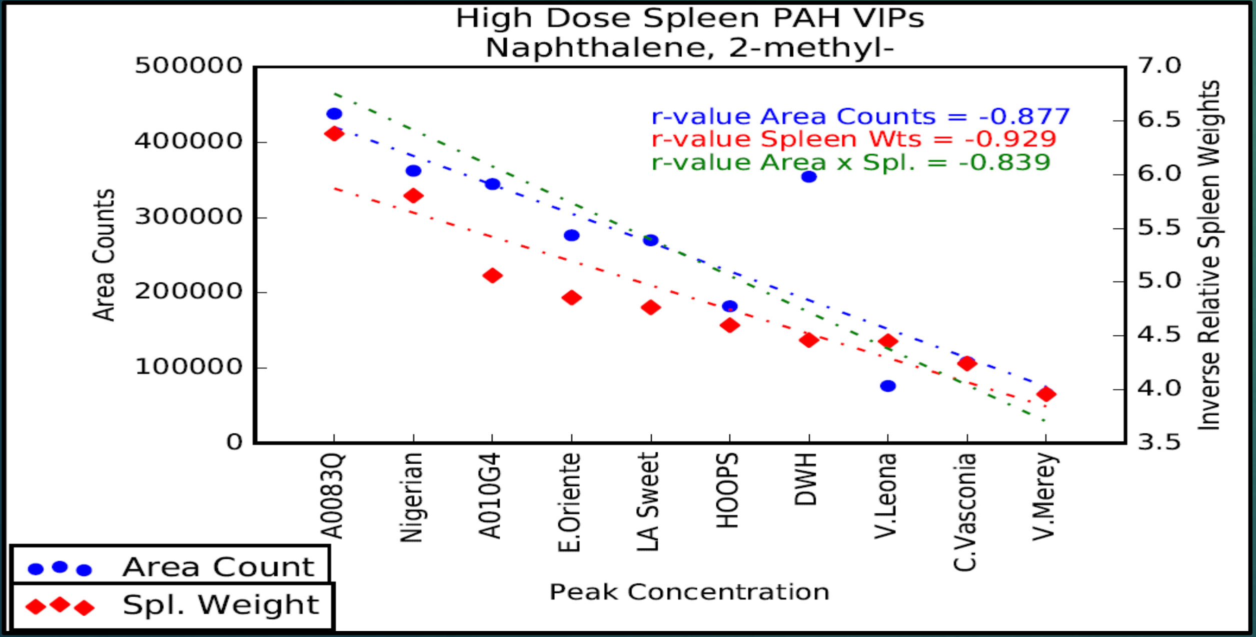
Correlation Coefficient Values for Selected VIPs

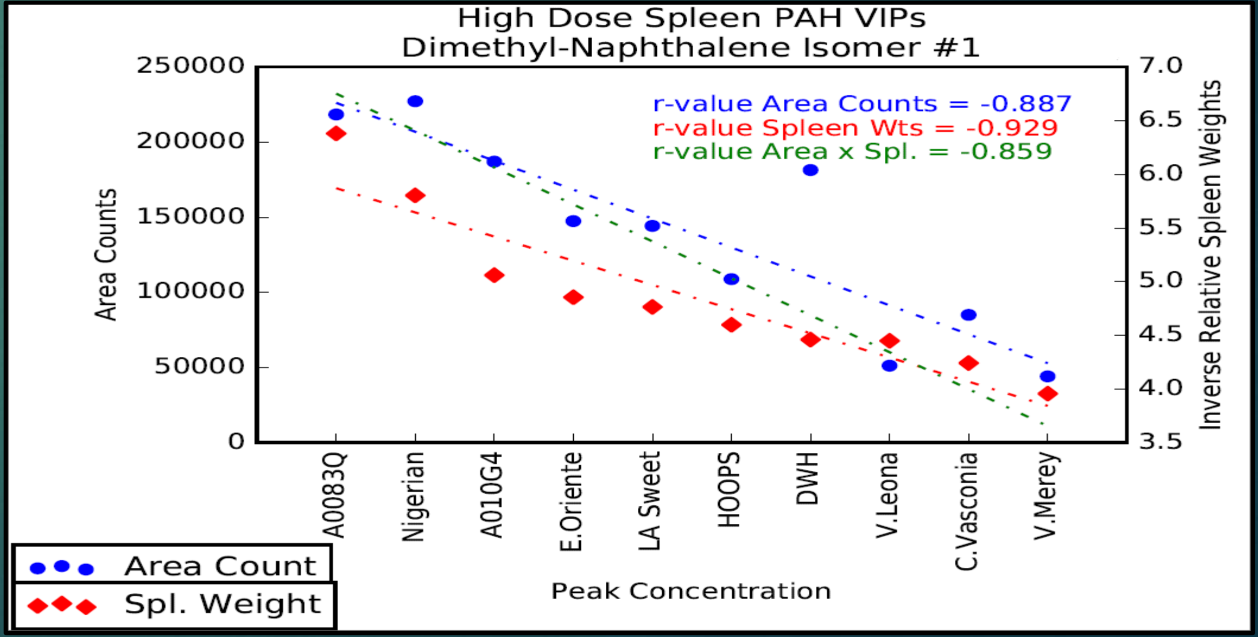
Top 12 Low Dose Spleen HP-5 VIPs (X>1.0)			Top 12 High Dose Spleen HP-5 VIPs (X>1.0)		
Library/ID	*VIP*	*r value*	*Library/ID*	*VIP*	*r value*
Decane, 4-methyl-	1.2099	-0.831	Benzene, 4-ethyl-1,2-dimethyl-	1.3085	-0.881
Heptane, 2,6-dimethyl-	1.2038	-0.827	Benzene, 2,4,6-trimethyl-	1.2635	-0.851
Cyclopentane, ethyl-	1.2008	-0.825	Octane	1.2605	-0.736
Cyclopentane, 1-methyl-2-propyl-	1.1946	-0.821	Benzene, 1-ethyl-3-methyl-	1.2357	-0.832
Cyclopentane, 1-ethyl-3-methyl-, trans-	1.1874	-0.816	Cyclopentane, 1-methyl-2-propyl-	1.2312	-0.829
Tridecane, 2-methyl-	1.1802	-0.811	Cyclopentane, ethyl-	1.2235	-0.824
Cyclopentane, 1-ethyl-2-methyl-, cis-	1.1685	-0.803	Benzene, 1,2,3-trimethyl-	1.2146	-0.818
Cyclohexane, 1,2,4-trimethyl-	1.1684	-0.803	Tridecane	1.1960	-0.806
Tetradecane	1.1679	-0.802	Heptane, 2,6-dimethyl	1.1925	-0.803
Cyclopentane, 1,2,4-trimethyl-	1.1647	-0.800	Cyclopentane, 1-ethyl-3-methyl-, trans-	1.1879	-0.800
Undecane, 2,6-dimethyl-	1.1623	-0.799	Tetradecane	1.1764	-0.792
Pentadecane	1.1614	-0.798	Dodecane	1.1708	-0.789

VIP Correlation Coefficient Graphs for Rel. Spleen Weights





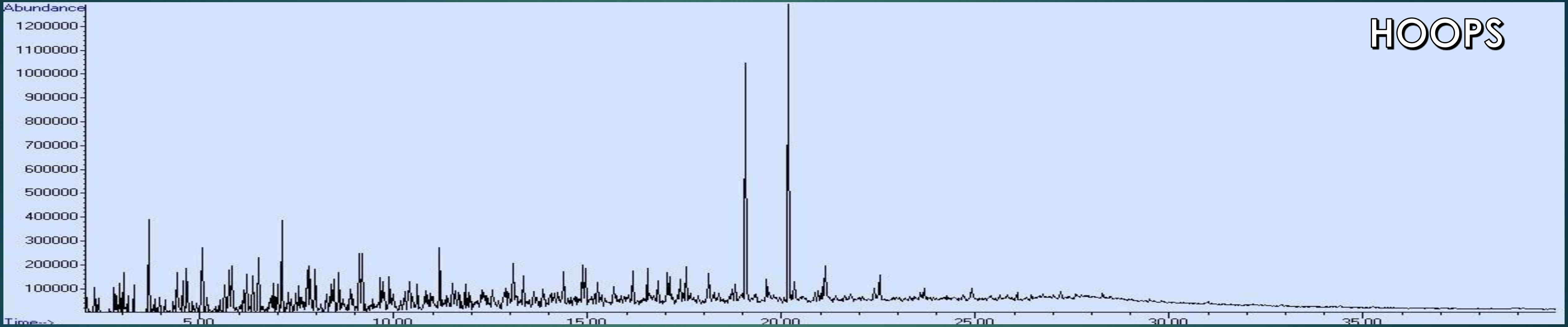


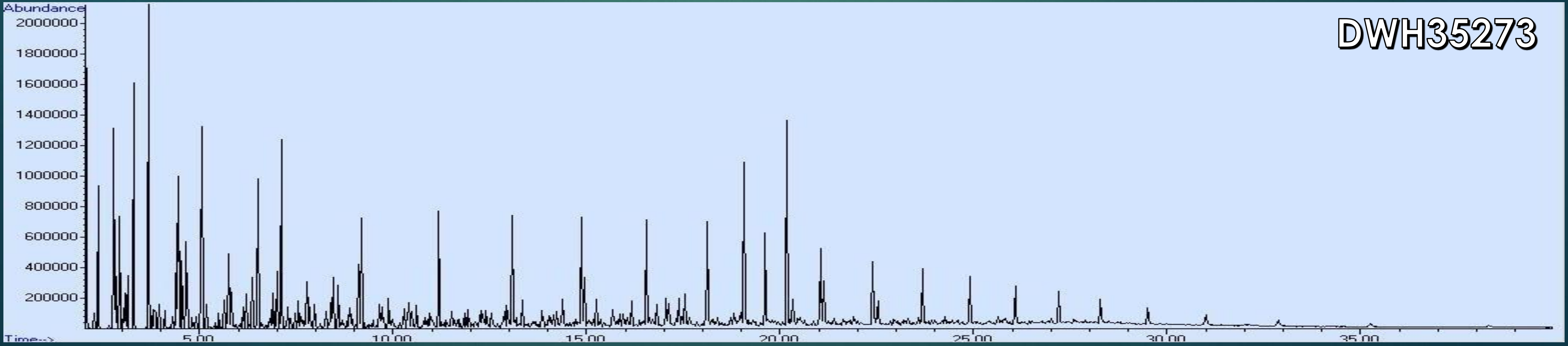


Correlation Coefficient Values for Selected VIPs

Top 12 Low Dose Spleen PAH VIPs (X>1.0)			Top 12 High Dose Spleen PAH VIPs (X>1.0)		
Library/ID	*VIP*	*r value*	*Library/ID*	*VIP*	*r value*
Indene Isomer	1.5267	-0.887	Indene Isomer	1.4399	-0.884
Dimethyl-Naphthalene Isomer #2	1.3763	-0.799	Dimethyl-Naphthalene Isomer #1	1.3992	-0.859
Dimethyl-Naphthalene Isomer #1	1.3743	-0.798	Dimethyl-Naphthalene Isomer #2	1.3908	-0.854
Naphthalene, 2-methyl-	1.3426	-0.780	Naphthalene, 2-methyl-	1.3673	-0.839
Naphthalene, 1-ethyl-	1.3331	-0.774	Naphthalene, 1-methyl-	1.3559	-0.832
Naphthalene, 1-propyl-	1.3320	-0.774	Naphthalene, 1-ethyl-	1.3387	-0.822
Naphthalene	1.3295	-0.772	Trimethyl-Naphthalene Isomer #5	1.3374	-0.821
RT: 20.53; Ion: 191.10	1.3229	-0.768	Trimethyl-Naphthalene Isomer #2	1.3308	-0.817
Trimethyl-Naphthalene Isomer #3	1.3176	-0.765	Trimethyl-Naphthalene Isomer #3	1.3239	-0.813
Naphthalene, 1-methyl-	1.3175	-0.765	Naphthalene	1.3147	-0.807
Trimethyl-Naphthalene Isomer #5	1.3175	-0.765	Naphthalene, 1-propyl-	1.2790	-0.785
Trimethyl-Naphthalene Isomer #6	1.3040	-0.758	Dimethyl-Naphthalene Isomer #3	1.2494	-0.767

Using the PLSr to Predict the Endpoints of Crude Oils in situ: DWH35273 and HOOPS Blend





Peek at the Past: Comparing of PLSr Results

PLSr on 9 Crude Oils: Relative Spleen Weights

	R2X(cum)	R2Y(cum)	Q2Y(cum)	RMSEE	pre	Ort	pR2Y	pQ2
Low Dose Spleen	0.706	0.68	0.534	0.0121	1	0	0.15	0.05
High Dose Spleen	**0.706**	**0.775**	**0.715**	**0.0158**	**1**	**0**	**0.1**	**0.1**

PLSr on 11 Crude Oils: Relative Spleen Weights

	R2X(cum)	R2Y(cum)	Q2Y(cum)	RMSEE	pre	Ort	pR2Y	pQ2
Low Dose Spleen	0.693	0.687	0.562	0.0108	1	0	0.05	0.05
High Dose Spleen	**0.693**	**0.668**	**0.588**	**0.0175**	**1**	**0**	**0.05**	**0.05**

PLSr for Predicting Outcomes

		Tested Crude Oils									Predicted Oils		
Spleen Weight (g)		LA Sweet	Nigerian	Iraqi	G4	3Q	E. Orient	C. Vascon	V. Merey	V. Leona	DWH	HOOPS	
	Low Dose	0.2132	0.1985	0.2142	0.2267	0.1988	0.215	0.2366	0.2634	0.2224	0.2125	0.2313	1st Fac
	High Dose	0.2099	0.1723	0.1781	0.1976	0.1567	0.206	0.2358	0.2528	0.2248	0.1892	0.2212	1st Fac
Liver Weight (g)													
	Low Dose	4.8241	5.4201	6.1107	5.4993	5.4811	5.1424	4.8277	4.7683	5.7081	5.3774	5.1651	2nd Fac
	High Dose	5.3753	6.0749	6.5267	6.0115	5.7619	5.965	6.1944	6.1283	5.4432	5.9693	5.6914	4th Fac
ALP													
	Low Dose	310	519.75	543	552.2	696	415	370.75	426	471	553.56	440.49	2nd Fac
	High Dose	444	598.5	645.75	759.75	398.6	499.5	418.25	566.75	478	557.42	371.77	4th Fac
Albumin													
	Low Dose	2.81141	3.12379	3.12379	3.03429	3.18857	3.275	3.25	3.85714	3.72857	3.2589	3.4986	3rd Fac
	High Dose	2.7767	2.9676	3.07172	2.95714	2.82857	3.13333	3.25	3.53571	3.79286	3.0534	3.3598	3rd Fac
Glucose													
	Low Dose	189	180.25	240.33	177.8	205.4	207	244.75	253	180.75	199.39	219.21	2nd Fac
	High Dose	199.67	193	209.25	156	170.4	196	239.25	167.5	206.5	175.36	208.21	4th Fac

PLSr for Predicting Outcomes Chi-Square Test for DWH35273

		DWH35273						
Spleen Weight (g)		Predicted	Actual	Residual		Residual^2/Predicted	Chi-Square	p-value
	Low Dose	0.21246	0.2245	0.0120		0.000681674	0.00964113	0.92178201
	High Dose	0.1892	0.2304	0.0412		0.008959457		
Liver Weight (g)								
	Low Dose	5.37743	5.369	-0.0080		1.20358E-05	0.02468023	0.87516649
	High Dose	5.96931	6.353	0.3837		0.024668192		
ALP								
	Low Dose	553.561	358.25	-195.3110		68.91090001	69.0509343	9.5953E-17
	High Dose	557.415	566.25	8.8350		0.14003431		
Albumin								
	Low Dose	3.25887	3.025	-0.2339		0.016783479	0.02162938	0.88307731
	High Dose	3.05336	3.175	0.1216		0.004845904		
Glucose								
	Low Dose	199.394	219.25	19.8560		1.977294883	8.0534372	0.00454174
	High Dose	175.358	208	32.6420		6.076142315		

PLSr for Predicting Outcomes Chi-Square Test for HOOPS Blend

		HOOPS Blend					
Spleen Weight (g)		Predicted	Actual	Residual	$Residual^2$/Predicted	Chi-Square	p-value
	Low Dose	0.23131	0.2393	0.0080	0.000277961	0.00030242	0.98612533
	High Dose	0.22118	0.2235	0.0023	2.44581E-05		
Liver Weight (g)							
	Low Dose	5.16507	5.002	-0.1626	0.005119403	0.04207528	0.83747633
	High Dose	5.69141	6.150	0.4586	0.036955881		
ALP							
	Low Dose	440.49	374.75	-65.7400	9.811227497	33.0267525	9.0899E-09
	High Dose	371.765	464.666667	92.9017	23.21552505		
Albumin							
	Low Dose	3.49861	3.275	-0.2236	0.014291799	0.03438259	0.85289537
	High Dose	3.35981	3.1	-0.2598	0.02009079		
Glucose							
	Low Dose	219.213	233	13.7870	0.867108105	7.51639758	0.00611398
	High Dose	208.208	171	-37.2080	6.64928948		

Conclusion

- SD rats that were administered an acute exposure to various crude oils exhibited a wide variety of toxicological effects.
- We identified significant alterations in clinical chemistry parameters, and observed morphophysiological and organ weight changes of the liver and spleen.
- Finally, we employed chemometrics and PLSr to identify key toxic constituents responsible for affecting an endpoint, and successfully predicted specific endpoints of new crude oils' toxicity in situ using previously analyzed and tested crude oils.

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Future Prospects

- Incorporate more medium and heavy crude oils into analysis.
- Expand on lower dosages for hepatotoxic endpoints.
- Determine amount of key constituents within crude oils that adversely effect relative spleen weights.
  - Test PLSr VIP-determined constituents in-vivo.
- Incorporate histopathology & Image-J software analysis.
- Explore and expand in R, Python, and Multivariate Analyses.

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Questions?

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Extra Slides – Other Robust Endpoints

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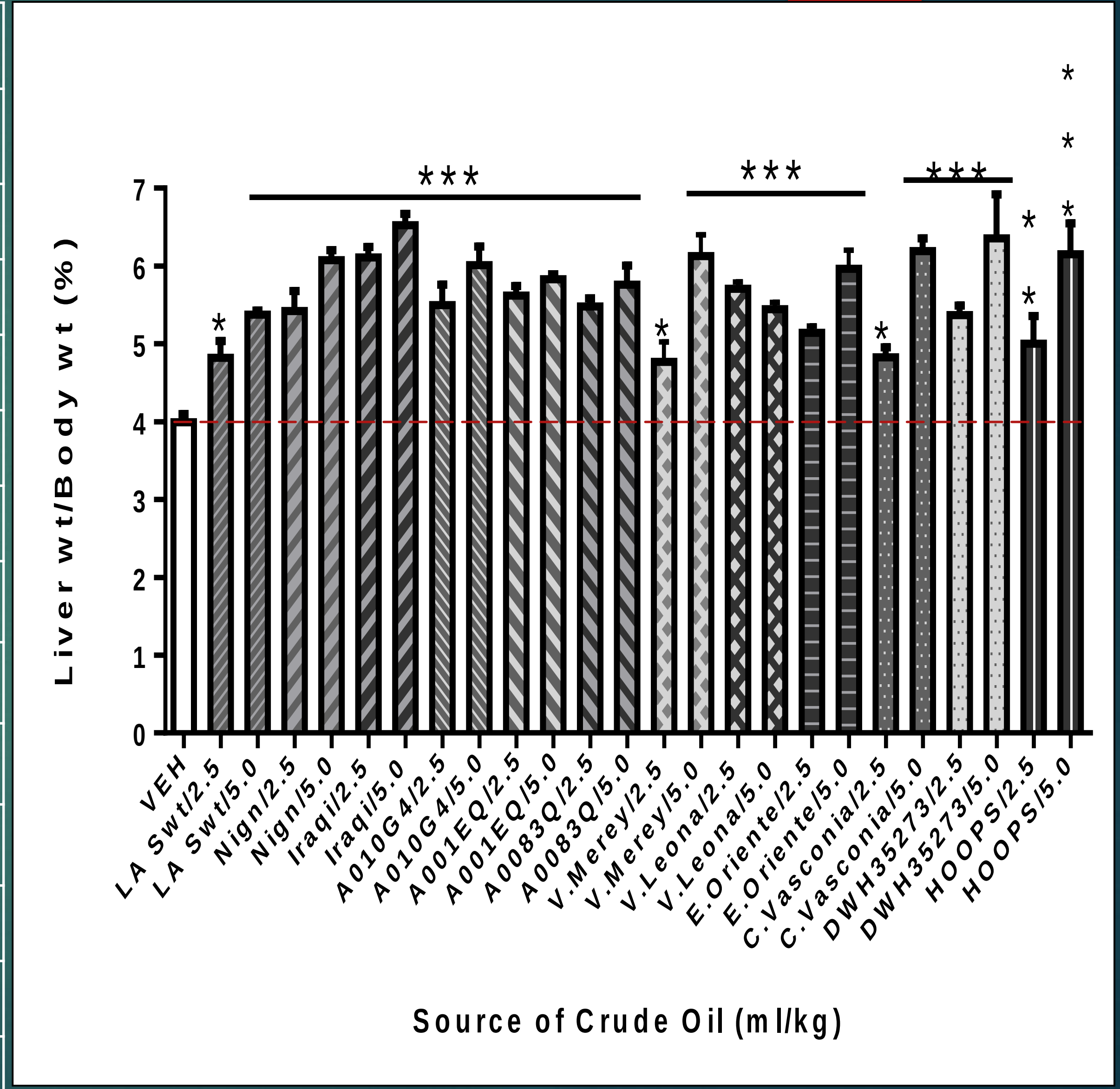
Extra Slides – Liver Histology

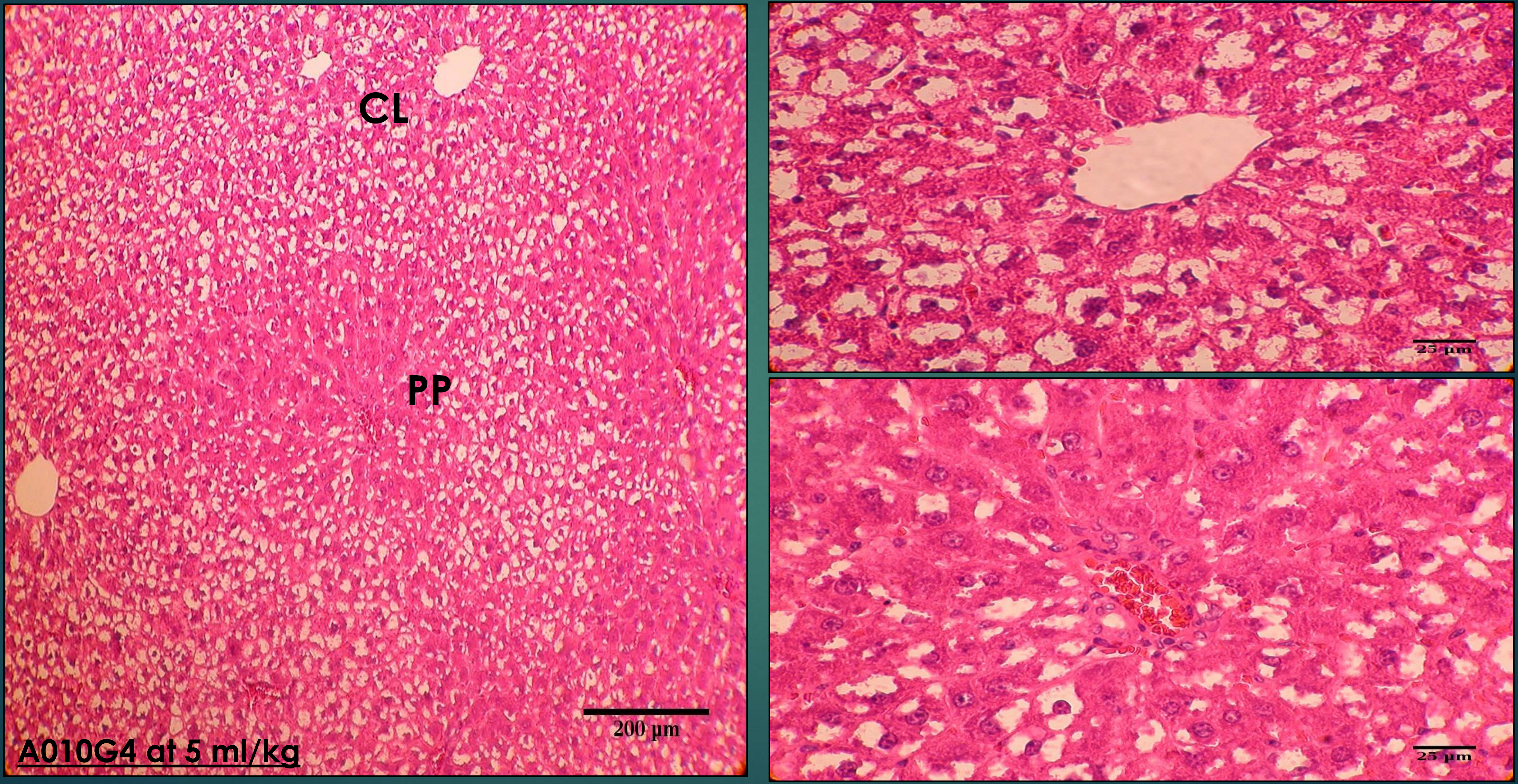
- HISTOPATHOLOGY: Liver and spleen sections (5 um) were cut from formalin-fixed, paraffin-embedded tissues and stained with H&E, PAS, PAS w/ Diastase, or Prussian Blue using standard procedures.

Composite of all Crude Oil Studies with Comparison of Significant Endpoints to Contemporaneous Control

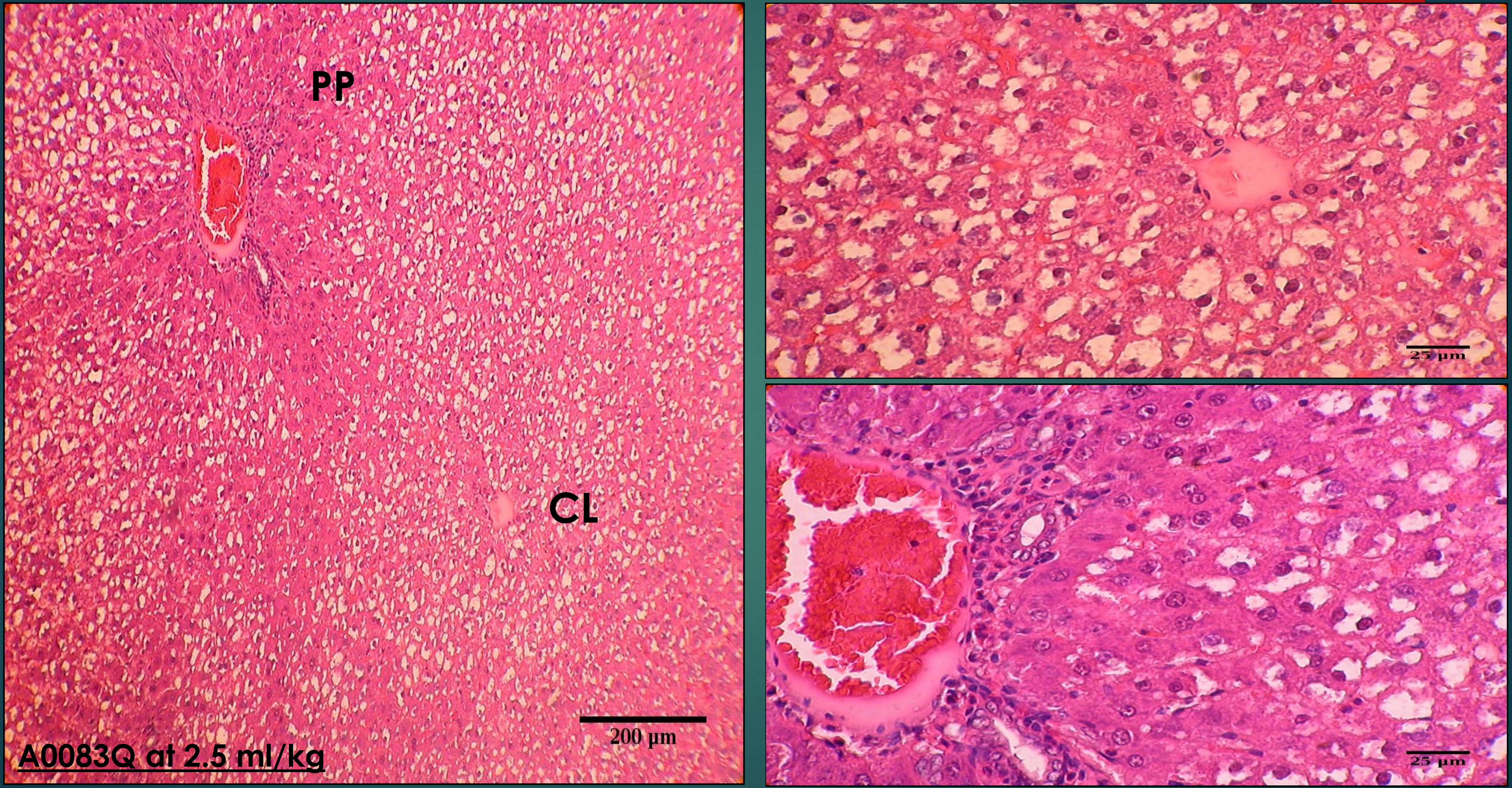
Rel. Liver Wt (g)	Dose			
Oil Data	Cont. Control	2.5 mL/Kg	5.0 mL/Kg	Significance
LA Sweet	3.874	4.824	5.375	p<0.05; p<0.001
Nigerian	-	5.420	6.074	p<0.001
Iraqi	-	6.110	6.526	p<0.001
A010G4	-	5.499	6.011	p<0.001
A001EQ	-	5.619	5.828	p<0.001
A0083Q	-	5.481	5.762	p<0.001
V. Merey	-	4.768	6.128	p<0.05; p<0.001
V. Leona	-	5.708	5.443	p<0.001
E. Oriente	-	5.141	5.965	p<0.001
C. Vasconia	-	4.827	6.194	p<0.001
DWH35273	-	3.353	5.369	p<0.001
HOOPS	-	5.002	6.150	p<0.01; p<0.001



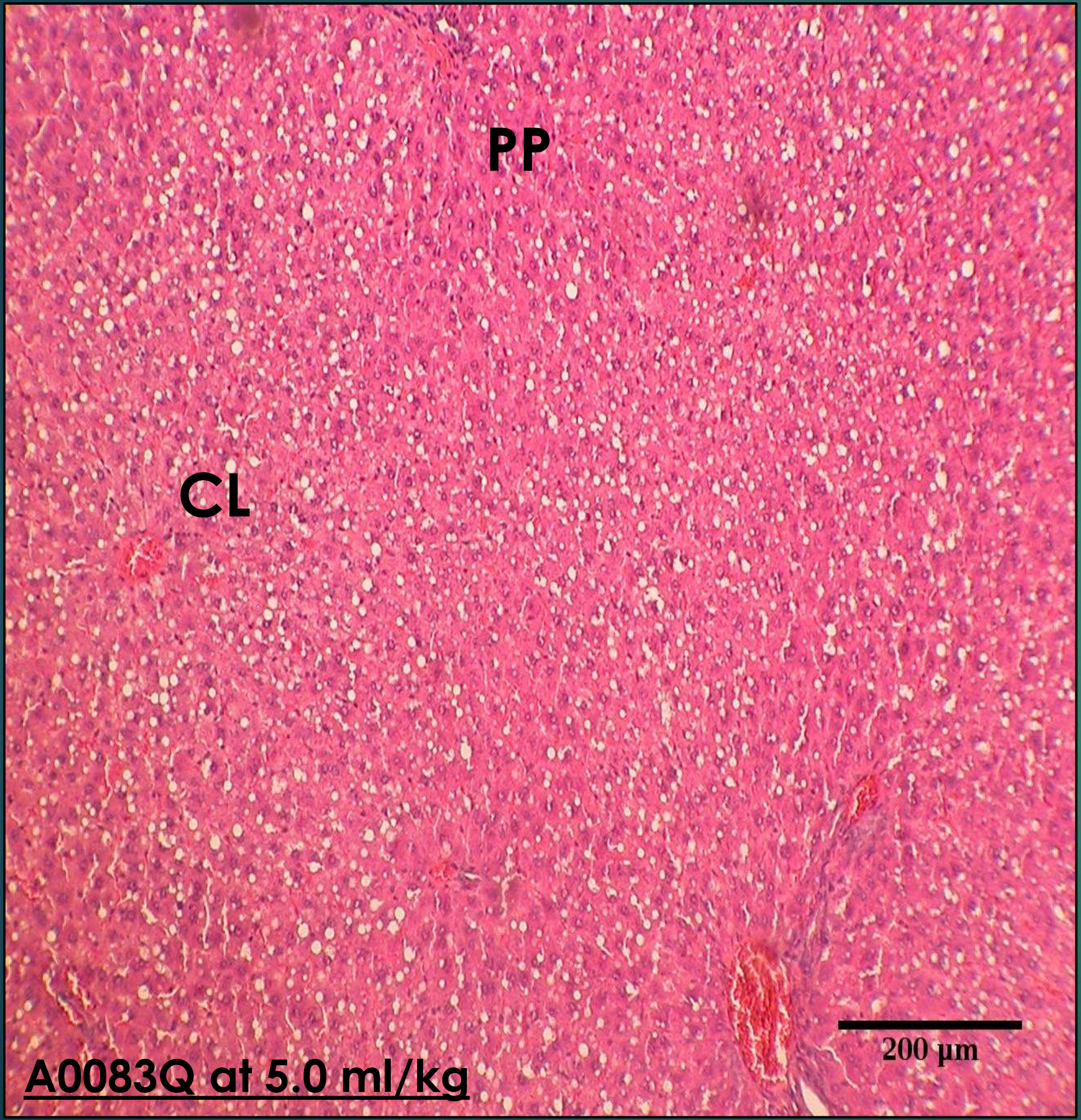
Liver Histology: Cytoplasmic rarefaction

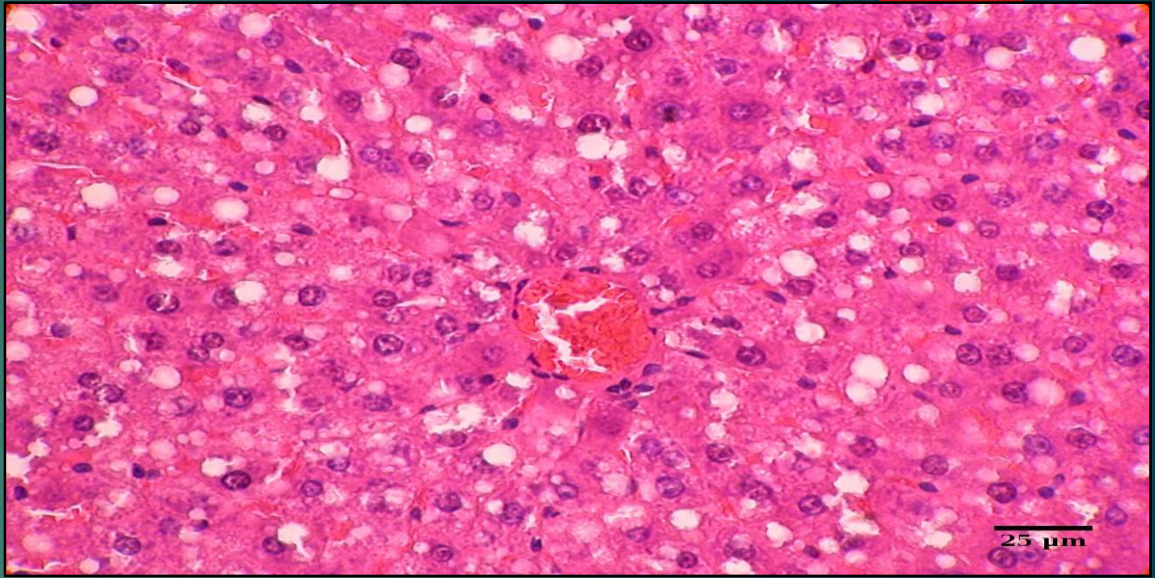


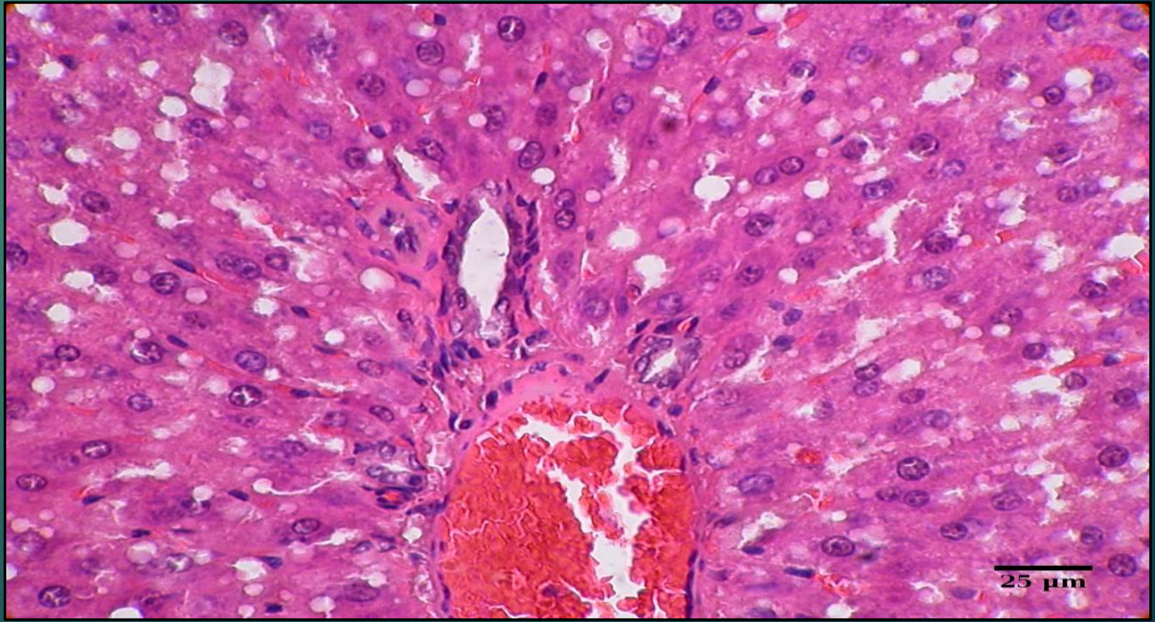
Liver Histology: Cytoplasmic rarefaction



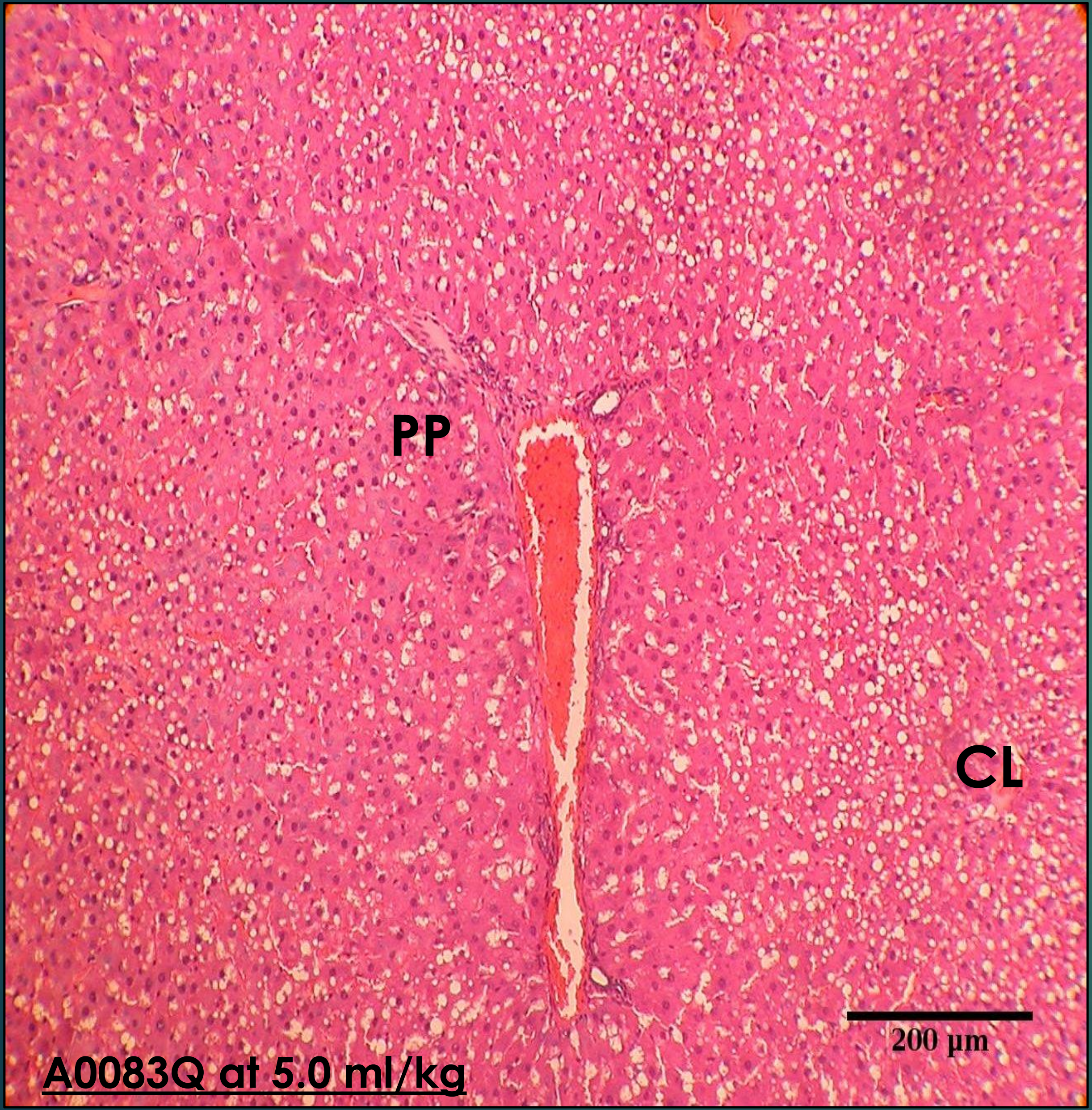
Liver Histology: Macrovesicular vacuolation

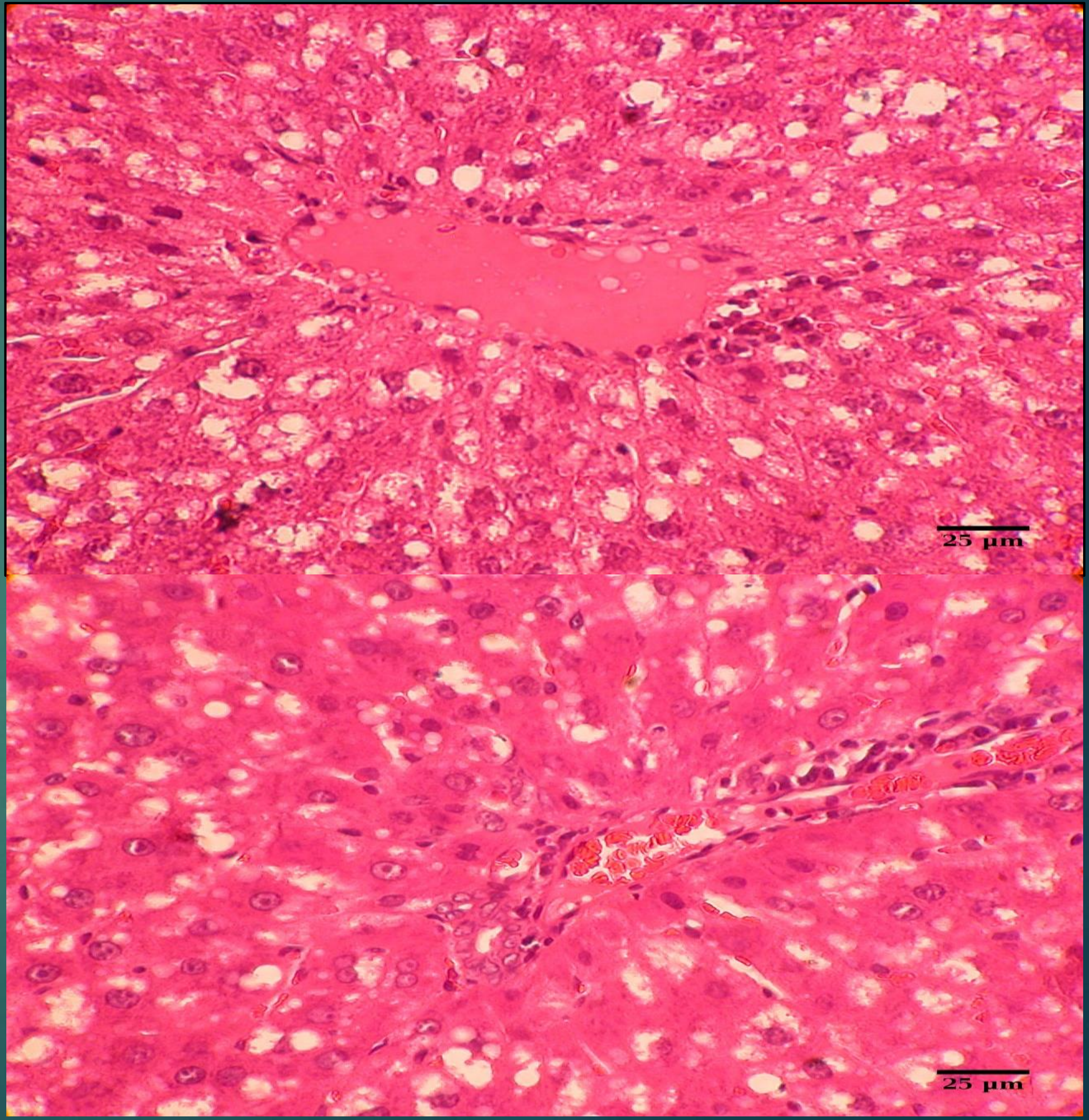






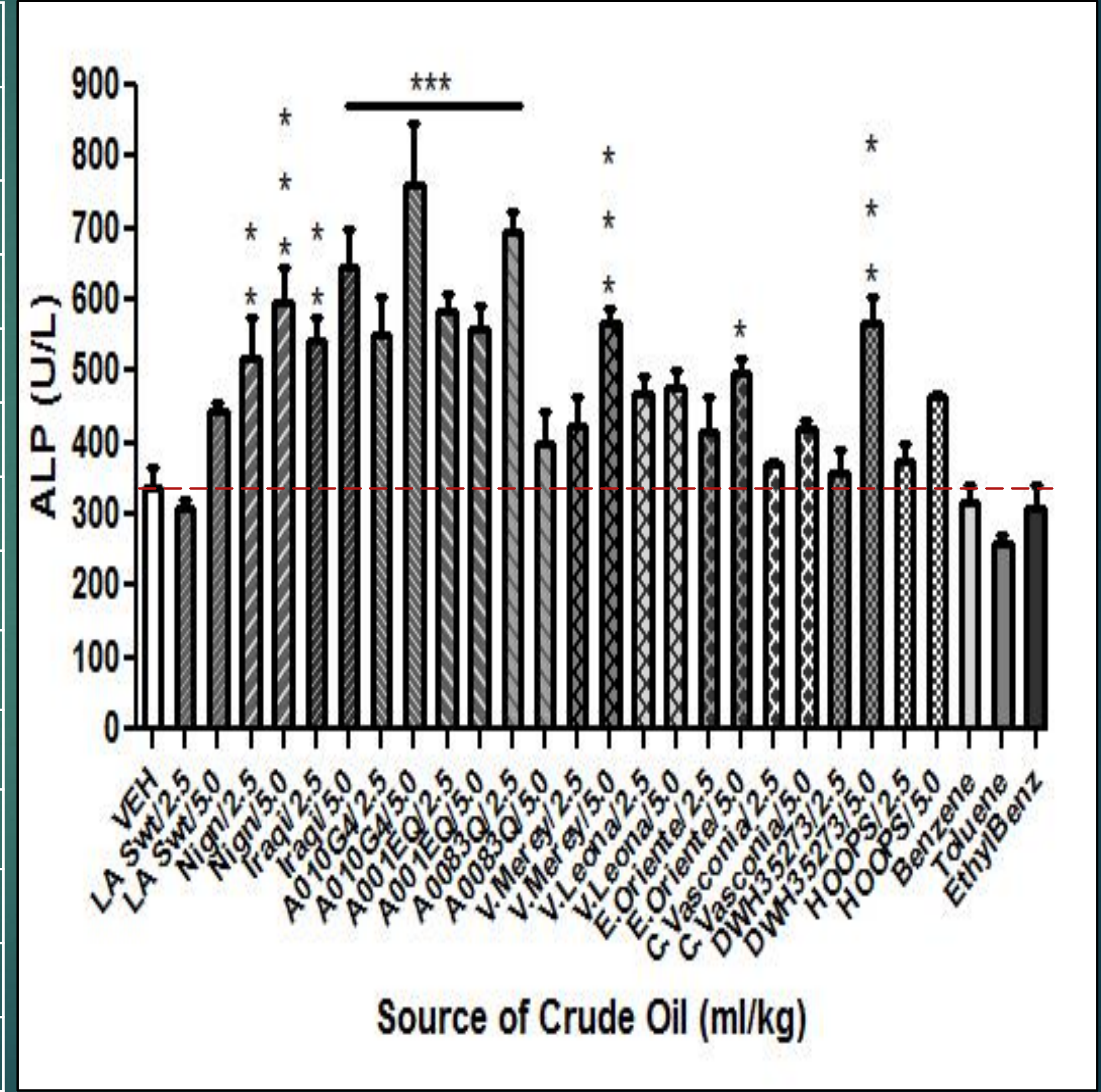
Liver Histology: Macrovesicular vacuolation





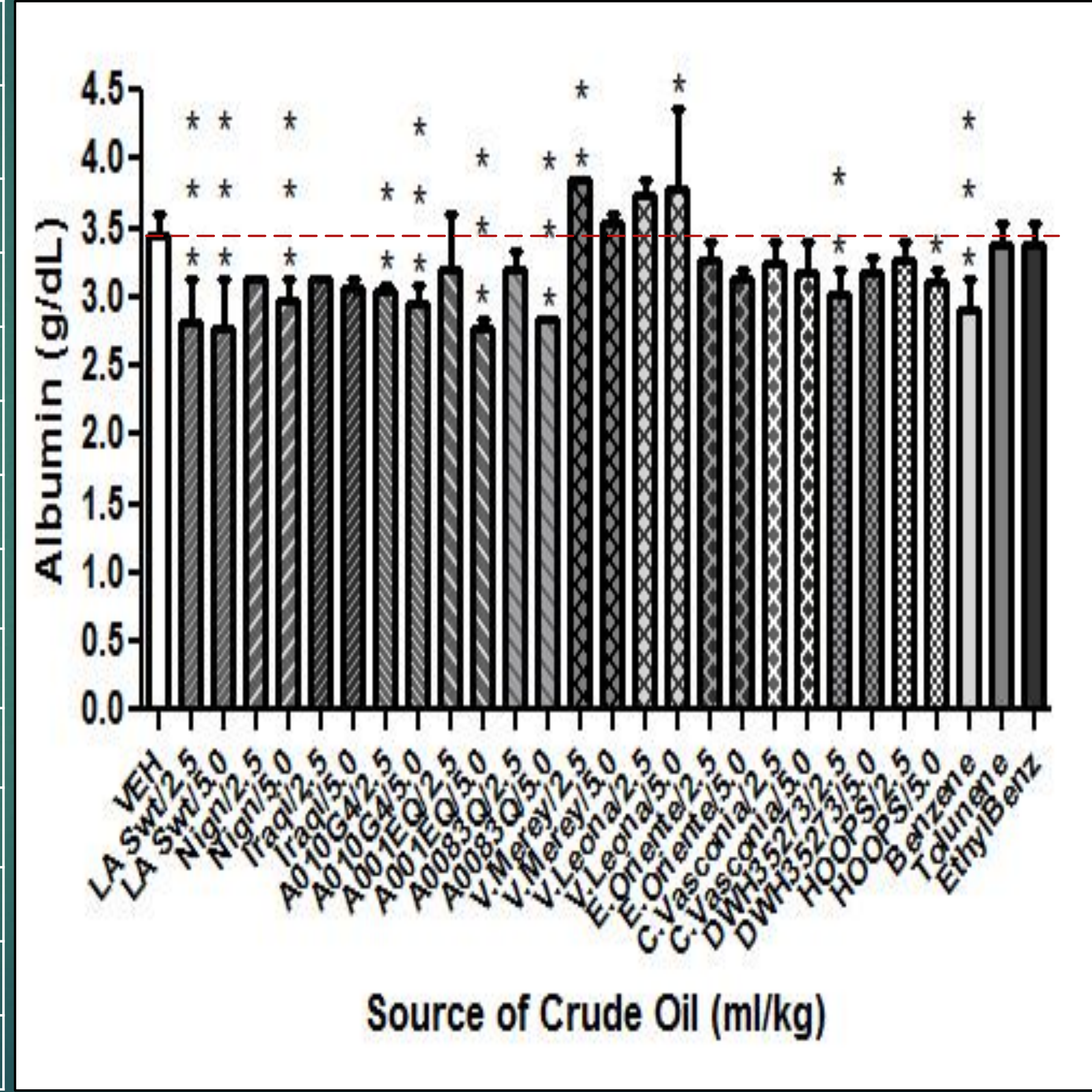
Composite of all Crude Oil Studies with Comparison of Significant Endpoints to Contemporaneous Control

ALP(U/L)	Dose			
Oil Data	Cont. Control	2.5 mL/Kg	5.0 mL/Kg	Significance
LA Sweet	339	310	444	No
Nigerian	-	519	598	p<0.01; <0.001
Iraqi	-	543	645	p<0.01; <0.001
G4	-	552	759	p<0.001
EQ	-	584	559	p<0.001
3Q	-	696	398.6	p<0.001/No
Merey	-	426	566	No/p<0.001
Leona	-	471	478	No
Oriente	-	415	499	No; p<0.05
C. Vasconia	-	370	418.3	No/No
DWH35273	-	358.3	566.3	No; p<0.001
HOOPS	-	374.8	464.7	No



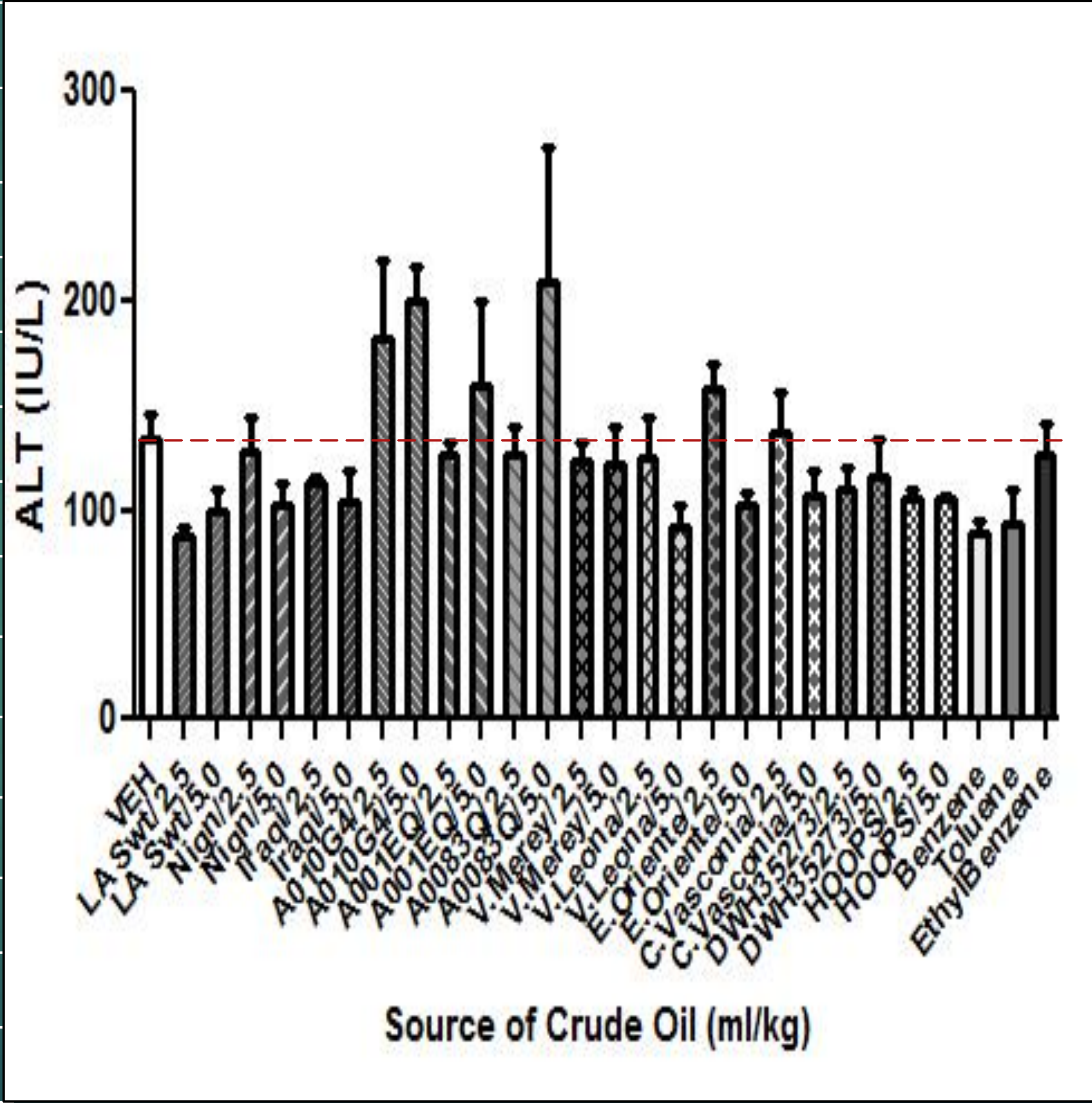
Composite of all Crude Oil Studies with Comparison of Significant Endpoints to Contemporaneous Control

Albumin(g/dL)	Dose			
Oil Data	Cont. Control	2.5 mL/Kg	5.0 mL/Kg	Significance
LA Sweet	3.3	2.8114	2.774	p<0.001
Nigerian	-	3.123	2.967	No; p<0.001
Iraqi	-	3.123	3.071	No; p<0.05
G4	-	3.034	2.957	p<0.01; p<0.001
EQ	-	3.188	2.777	No; p<0.001
3Q	-	3.188	2.828	No; p<0.001
Merey	-	3.857	3.535	p<0.01; No
Leona	-	3.728	3.792	No; p<0.05
Oriente	-	3.275	3.133	No
C. Vasconia	-	3.25	3.18	No
DWH35273	-	3.025	3.175	P<0.01; No
HOOPS	-	3.275	3.1	No; p<0.05



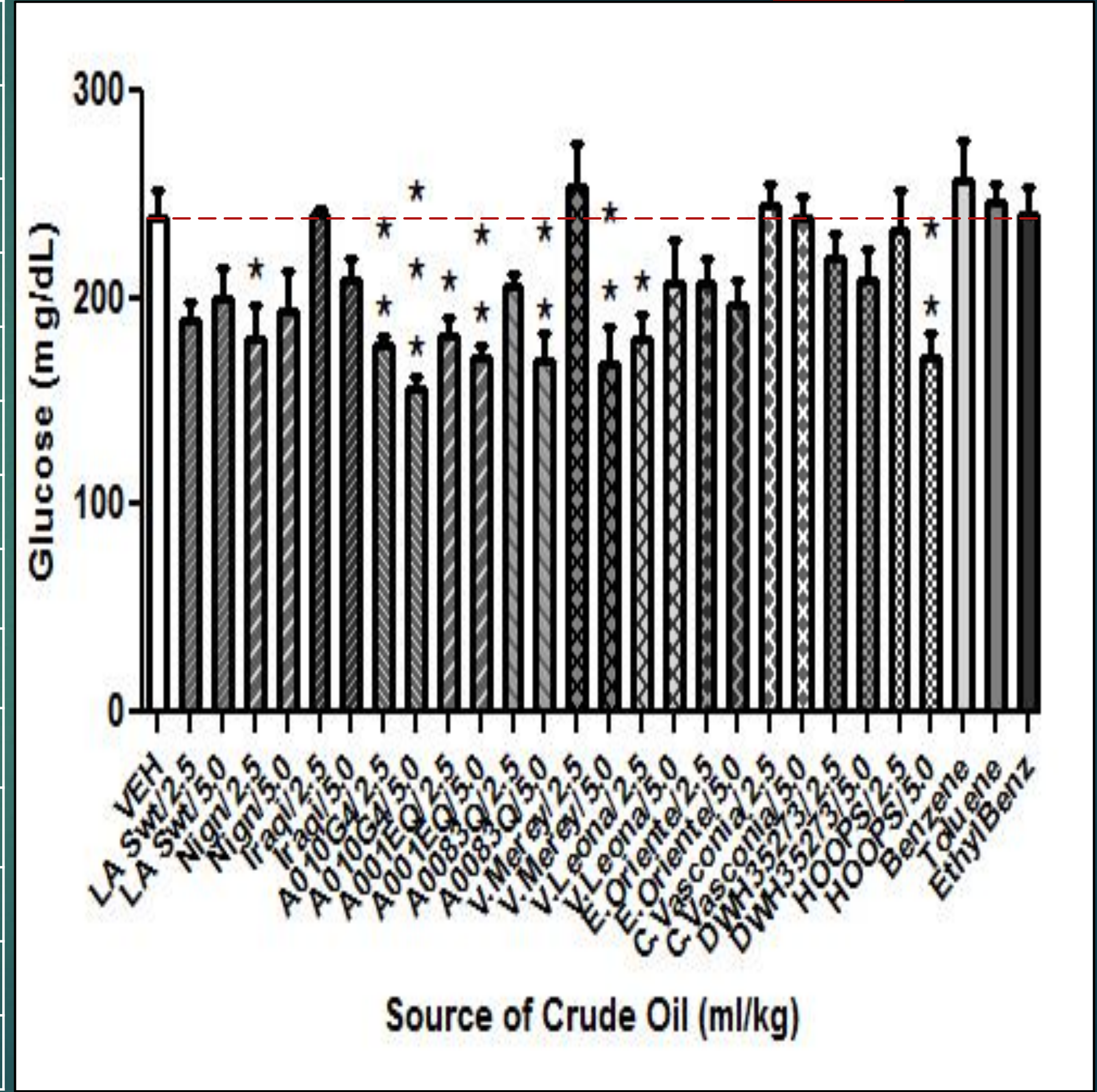
Composite of all Crude Oil Studies with Comparison of Significant Endpoints to Contemporaneous Control

ALT(U/L)	Dose			
Oil Data	Cont. Control	2.5 mL/Kg	5.0 mL/Kg	Significance
LA Sweet	133.5	87.0	99.0	No
Nigerian	-	128.8	102.5	No
Iraqi	-	113.8	103.5	No
G4	-	182.0	199.5	No
EQ	-	126.4	160.4	No
3Q	-	126.4	209.0	No
Merey	-	124.0	122.0	No
Leona	-	125.0	92.75	No
Oriente	-	158.0	102.5	No
C. Vasconia	-	137.3	107.0	No
DWH35273	-	110.0	116.0	No
HOOPS	-	105.75	105.7	No



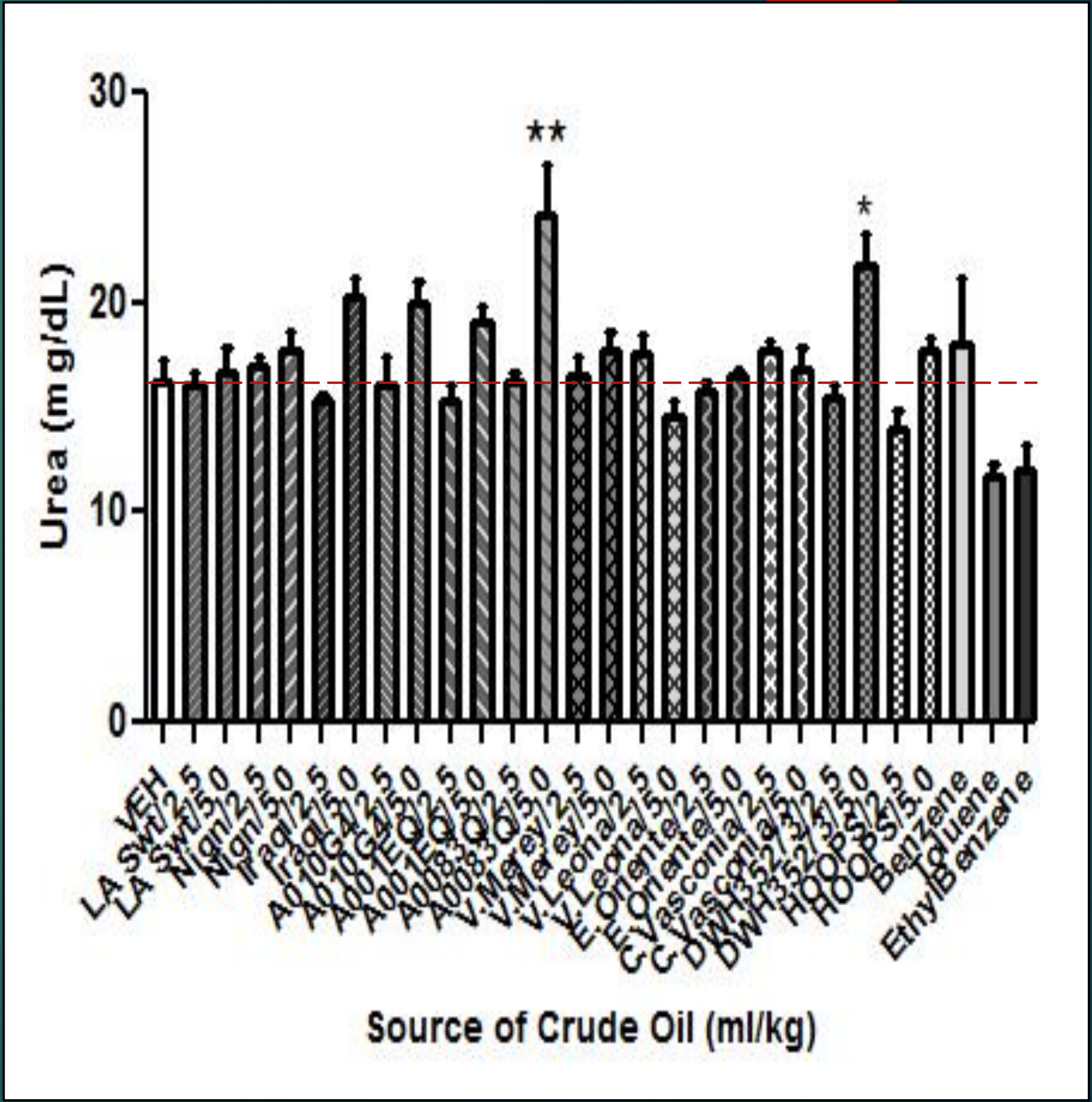
Composite of all Crude Oil Studies with Comparison of Significant Endpoints to Contemporaneous Control

Glucose(mg/dL)	Dose			
Oil Data	Cont. Control	2.5 mL/Kg	5.0 mL/Kg	Significance
LA Sweet	238.33	189.0	199.8	No
Nigerian	-	180.3	193.0	$p<0.05$; No
Iraqi	-	240.3	209.3	No
G4	-	177.8	156.0	$p<0.01$; $p<0.001$
EQ	-	181.2	171.8	$p<0.05$; $p<0.01$
3Q	-	205.4	170.4	No; $p<0.01$
Merey	-	253.0	167.5	No; $p<0.01$
Leona	-	180.8	206.5	$p<0.05$; No
Oriente	-	207.0	196.0	No
C. Vasconia	-	244.8	239.3	No
DWH35273	-	219.3	208.0	No
HOOPS	-	233.0	171.0	No; $p<0.01$



Composite of all Crude Oil Studies with Comparison of Significant Endpoints to Contemporaneous Control

Urea(mg/dL)	Dose			
Oil Data	Cont. Control	2.5 mL/Kg	5.0 mL/Kg	Significance
LA Sweet	16.17	16.0	16.7	No
Nigerian	-	17.0	17.8	No
Iraqi	-	15.33	20.3	No
G4	-	16.0	20.0	No
EQ	-	15.4	19.0	No
3Q	-	16.2	24.2	No; p<0.01
Merey	-	16.5	17.8	No
Leona	-	17.5	14.5	No
Oriente	-	15.8	16.5	No
C. Vasconia	-	17.8	16.8	No
DWH35273	-	15.5	21.8	No; p<0.05
HOOPS	-	14.0	17.7	No



Assessment of Low Dose Nigerian Crude Oil Toxicity

Nigerian Qua Iboe Light/Sweet Crude Oil: Organ Weights

Organ Weight Summary	Vehicle	0.2 mL/Kg	0.5 mL/Kg	1.0 mL/Kg	2.5 mL/Kg	5.0 mL/Kg
Liver Weights (g)	3.942	4.022	4.425	**4.692 ***	**6.008 ****	**6.734 ****
Spleen Weights (g)	0.239	0.2267	0.2544	0.2369	0.221	**0.192 ***