



Automated GPC With Inline SPE to Improve Sample Cleanup for PAH Analysis

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PAHs

- Many PAHs have been identified as mutagens and carcinogens
- PAHs are lipophilic and frequently found in challenging matrices like soil and seafood
- The EPA has identified 16 PAHs as Priority Pollutants:

naphthalene

phenanthrene

benzo(a)anthracene

benzo(a)pyrene

acenaphthylene

anthracene

chrysene

indeno(1,2,3-cd)pyrene

acenaphthene

fluoranthene

benzo(b)fluoranthene

dibenzo(a,h)anthracene

fluorene

pyrene

benzo(k)fluoranthene

benzo(ghi)perylene

Sample Cleanup for PAH Analysis

- Sample cleanup is critical prior to analysis for most matrices to minimize interfering peaks and extend the lifetime of the analytical equipment
- SPE requires multiple manual steps and sometimes cannot handle high-lipid samples
- GPC removes lipids, but cleanup may be incomplete
- For problematic matrices, GPC can be combined with traditional SPE methods, but these methods require additional steps that increase the time and solvent required to process each sample

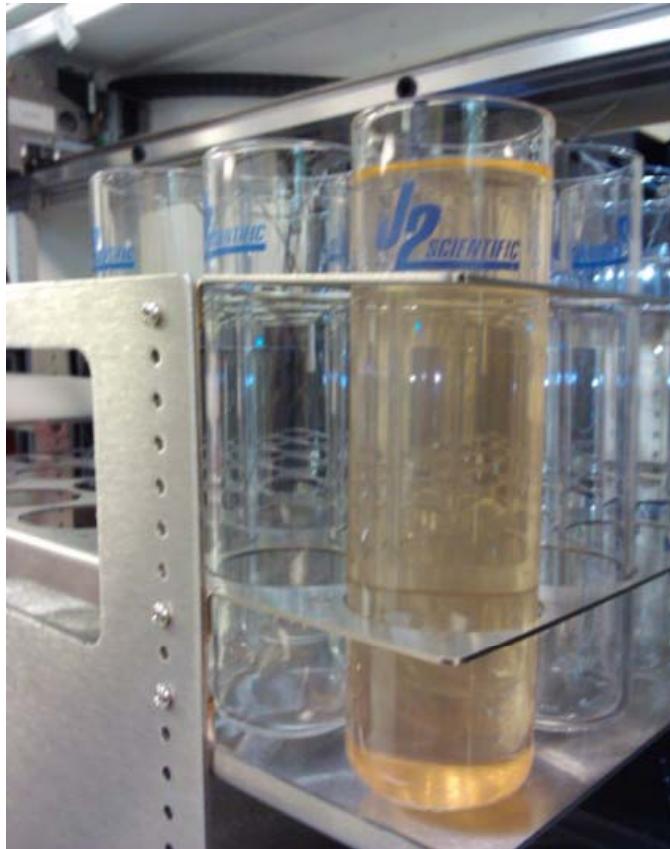
GPC with inline SPE

- Non-retentive SPE for the analytes of interest can remove many matrix interferences
- Adding inline SPE to a GPC method on the PrepLincTM system takes no additional time or solvent per sample
- Common SPE Cartridge Types:
 - Silica
 - FlorisilTM
 - Alumina

Extract Preparation

Matrices Tested:

- Topsoil
- Red Snapper filet (skin on)
- Shucked oysters



- Add methylene chloride (50mL per 10g sample) and blend until homogenous
- Remove suspended solids and water from extract
- Spike extract with PAH Standard prior to loading onto PrepLinc System

Automated Concentration and Cleanup

Live Update

Sample Introduction

Volume (uL) Solvent

Pre-evap Spike 1000 H Accuvap Syringe Valve Dodecane Ke

Time (sec) Combine with Previous Sample

Chamber Dump 5 Combine with Next Sample

Sample Introduction Zone Settings 1 2 3

Heating Rate 20 29 31

Vacuum (Torr) 400 350 300

Endpoint

Level Sensor Dryness

Endpoint Time (min) 10 Cool Time (sec) 5

Endpoint Zone Settings 1 2

Heating Rate 20 30

Vacuum (Torr) 500 250

Chamber Rinses

Time (sec) Heater Repeats

#1 20 50 1

#2 20 50

Transfer Rinse Reps 1

EVS Advance

Sample Rinse

Volume (uL) Solvent Aspirate (uL/min) Dispense (uL)

5000 F Accuvap Syringe Valve DCM 25000 450

Dilutions/Exchanges Stage 1

Reps 1

Volume (uL) 0 Prime Solvent (uL) 0

Solvent F Accuvap Syringe Valve DCM

Exchange Heating Rate 0 Vacuum (Torr) 0

Line Editor New Open Save Save As... Help

Methods

- GPC
 - 10Min DCM Dryness.gmf
 - 10min DCM Level Sensor.gmf
 - 2Min DCM Dryness.gmf
 - 2min DCM Level Sensor.gmf
 - 3Collect.gmf
 - 5 Minute Collect.gmf
 - 5min Collect 70EA30CyH.gmf
 - C0775.gmf
 - C0100 Calibration.gmf
 - C0770 Calibration.gmf
 - DCM Dryness Transfer Test.gmf
 - DCM LS Transfer Test.gmf
 - Frac5 ASM Solvent C.gmf
 - Frac5.gmf
 - GPC AVM Dry Run.gmf
 - GPC dry.gmf
 - GPC SPE AVM Dry Run.gmf
 - GPC to SPE dry.gmf
 - PAHs GPC Inline SPE.gmf
 - PAHs GPC Only.gmf
- SPE
- AVM
 - DCM EVS.evs
 - EVS Fish DCM.evs
 - PAH EVS.evs

Add >>

<< Remove

Move Up

Move Down

EVs PAH EVS.evs

GPC PAHs GPC Inline SPE.gmf

Method Load View

Select Delete

Total Fractions Time

Volume (uL) Aspirate (uL/min) Dispense (uL/min)

Inject 7500 30000 7500

Solvent B DCM Clear

Probe Rinses

Reps Volume (uL) Aspirate (uL/min) Dispense (uL/min)

Post Inject 1 7500 30000 30000

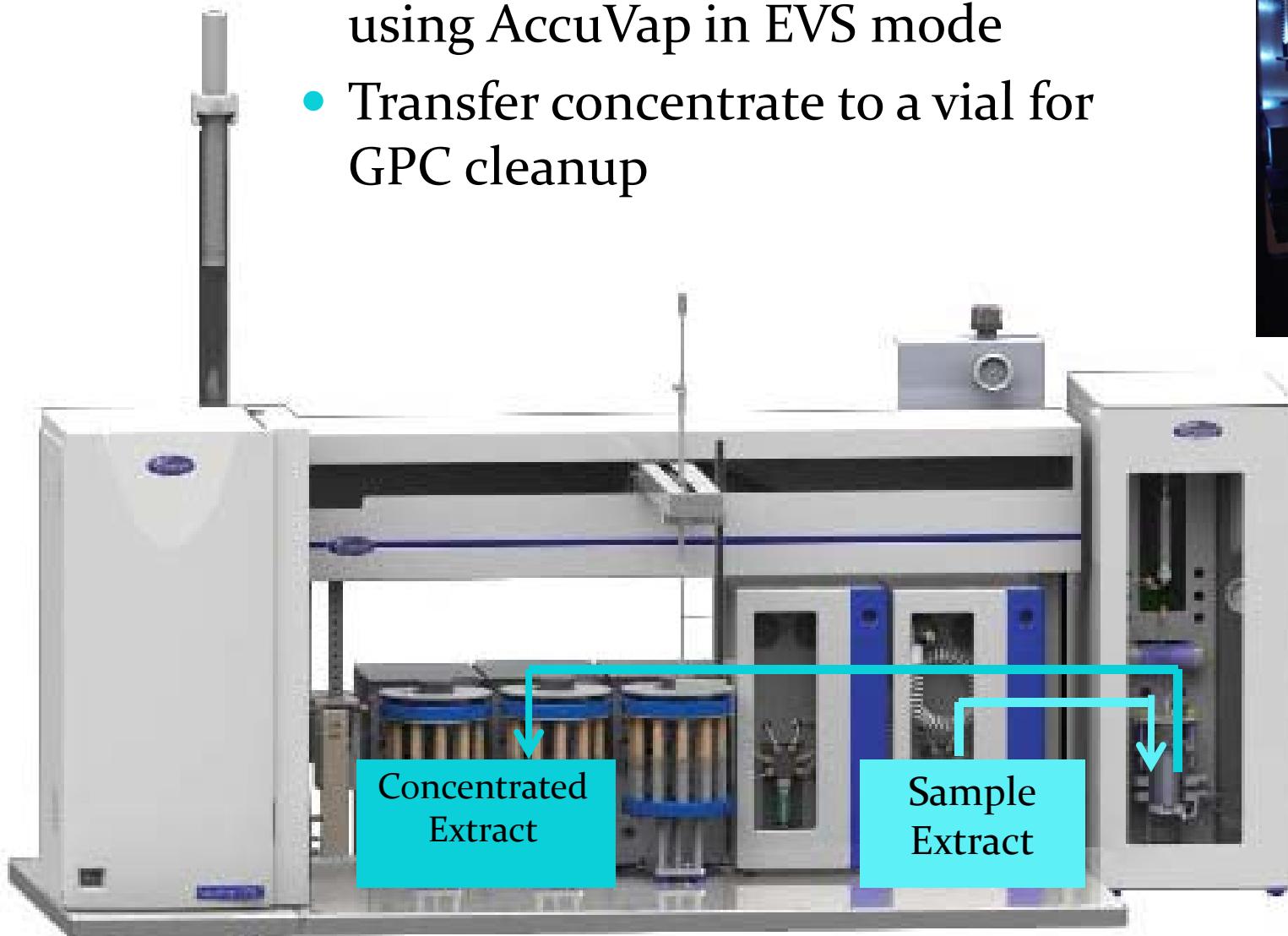
Solvent B DCM Clear

Post Collect 0 0 0 0

Solvent Clear

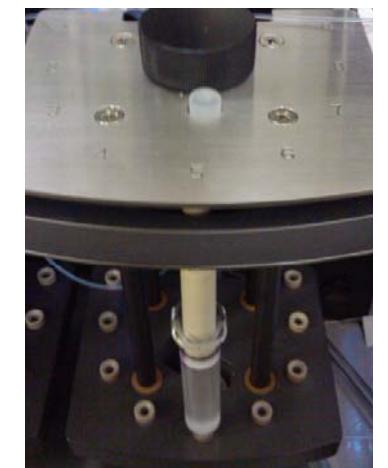
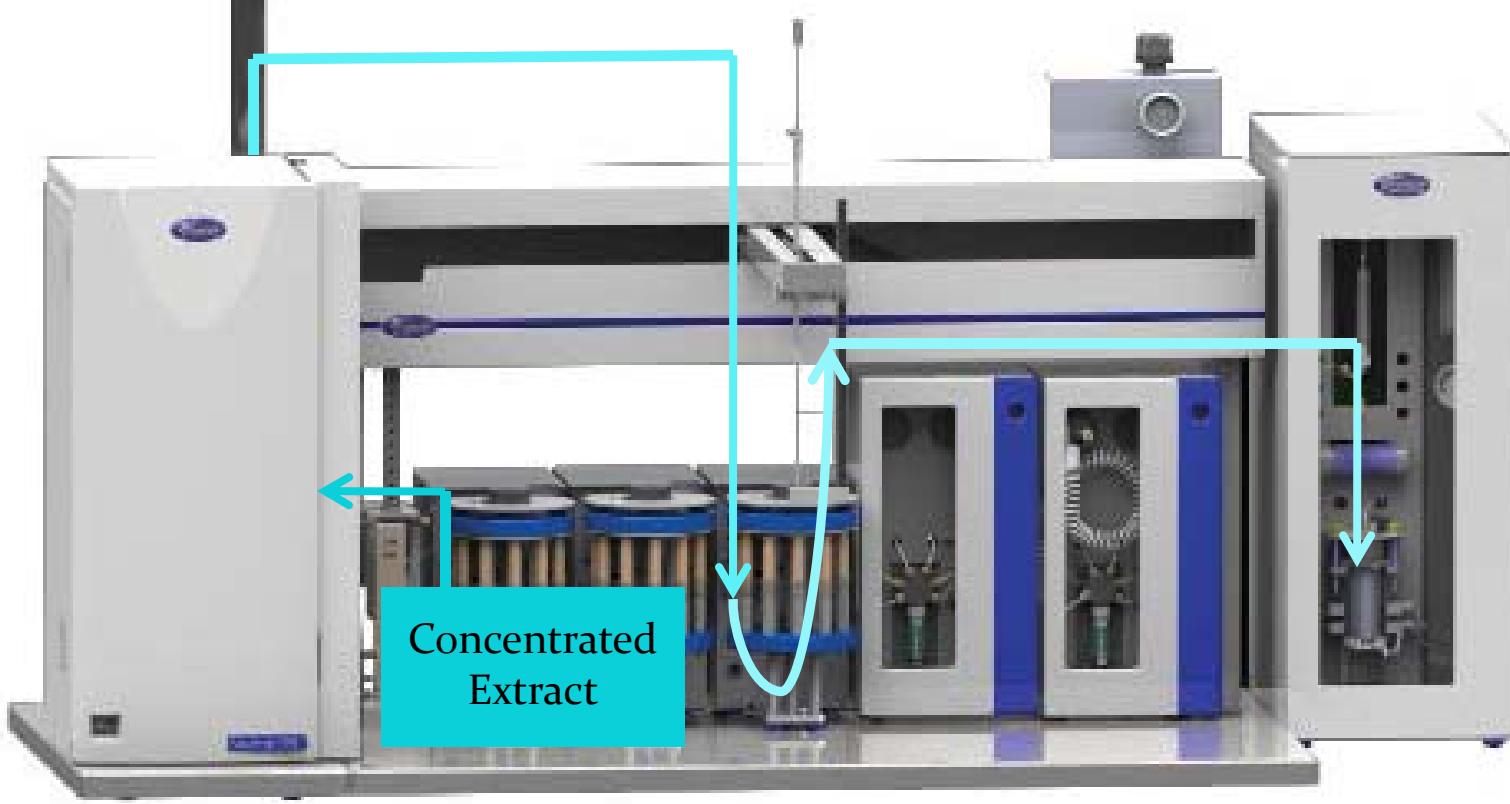
Cleanup – Extract Concentration

- Concentrate 50mL extract to 2mL using AccuVap in EVS mode
- Transfer concentrate to a vial for GPC cleanup



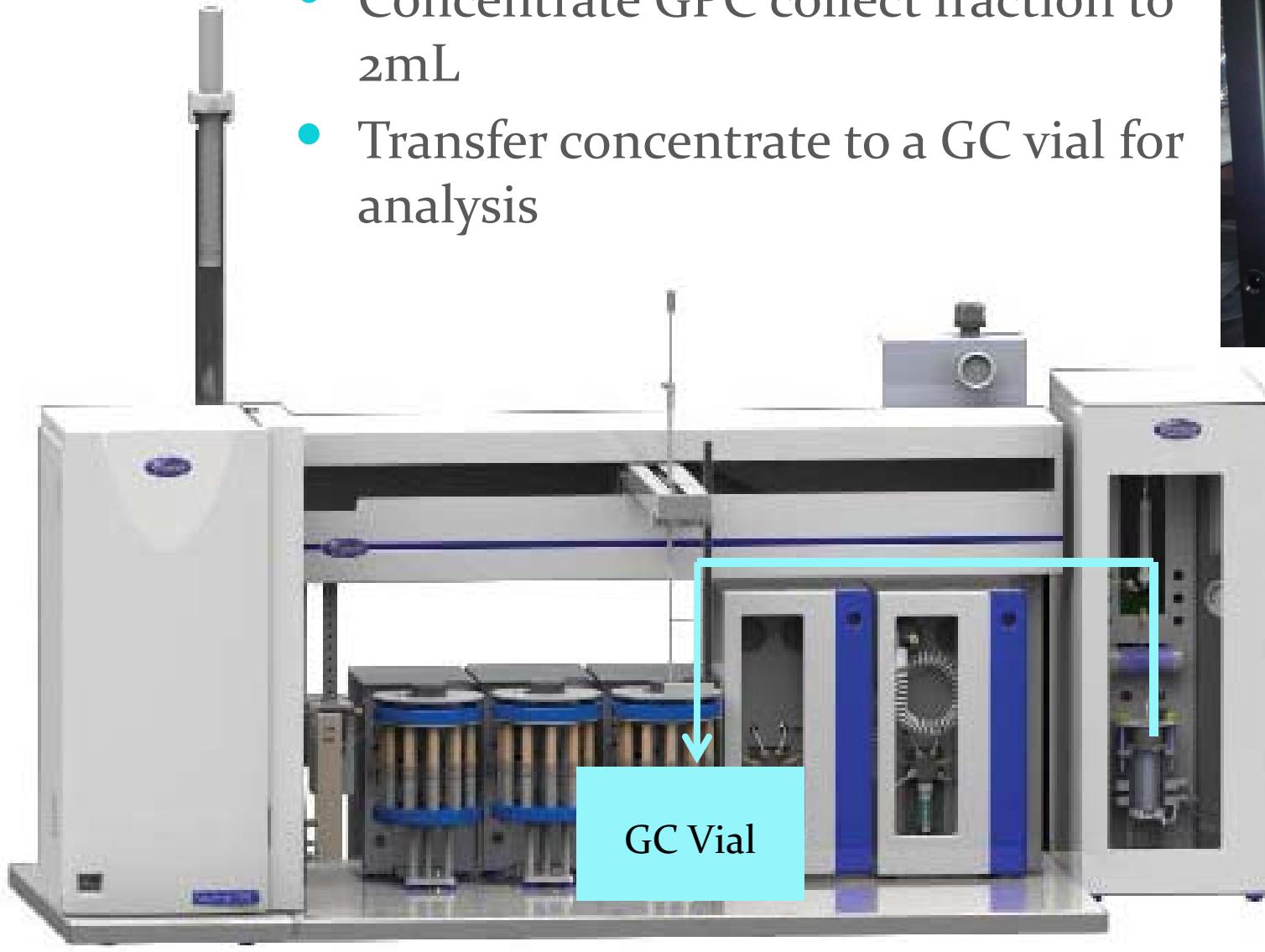
Cleanup – GPC-SPE

- Load concentrated sample onto Express GPC Column
- Collect PAH fraction through SPE cartridge and into AccuVap for concentration



Cleanup – Final Concentration

- Concentrate GPC collect fraction to 2mL
- Transfer concentrate to a GC vial for analysis



Automated Concentration and Cleanup

PrepLinc 1.2.0.66 [System] - Sequence Editor - 201184_1.seq

File View Tools Help

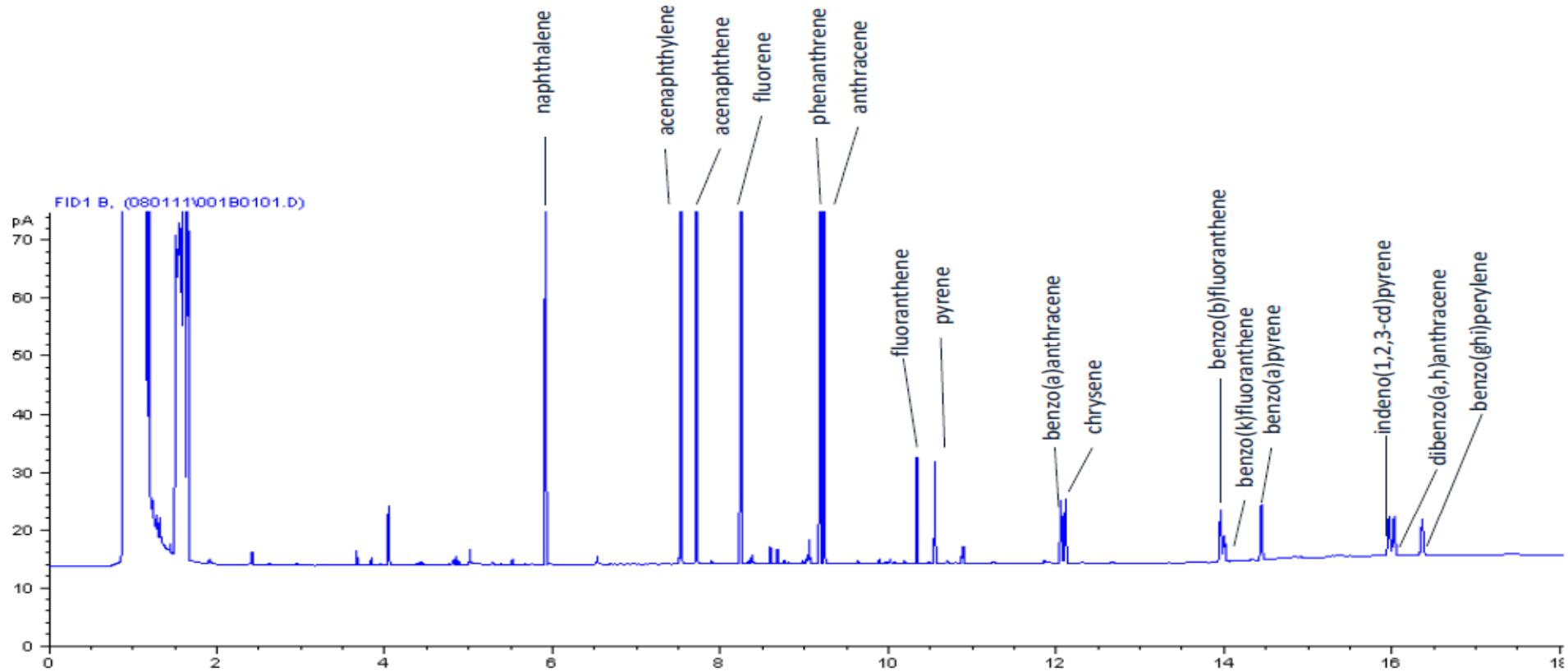
Autosampler Accuvap GPC SPEi Navigate to Sequence Editor Method Explorer Reporting Start Pause Terminate Resume

HUB	Autosampler	Accuvap	GPC	SPE	Sequence Editor
Status RUN Operation Duration [] Disconnect Reset	Status IDLE Operation Evaporation Heat Rate Set 29.0 Current 29.3 Vacuum (Tor) 00275 00276 EVS Sensor	Status RUN Operation Heat Rate Set 29.0 Current 29.3 Vacuum (Tor) 00275 00276 EVS Sensor	Status IDLE Operation Duration [] Pressure -1 Detector 1 79 2 2481 Flow Rate [] (mL/min) Column Position: Bypass	Status IDLE Operation Duration [] Pressure 0	Line # 5 Method PAH EVS.evs
<p>Sequence Editor New Open Save Print... Settings... Current Mat: The Mat.m2k</p> <p>Start Pause Terminate Resume</p>					

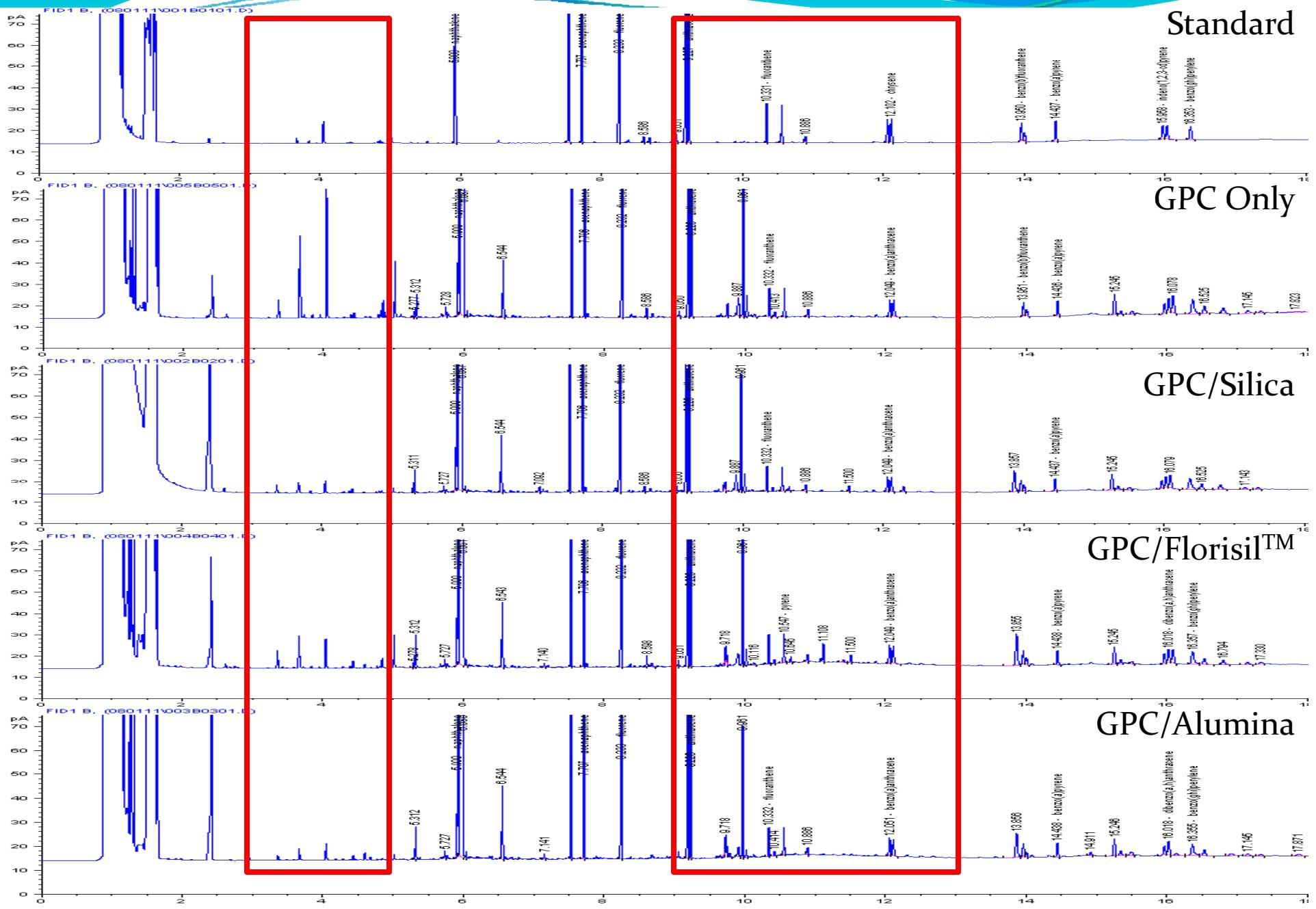
GC Analysis

Detector	FID, 350°C
Injection	1µl, splitless, 330°C
Column	Restek 12723: RTX®-5Sil MS 30m x 0.25mm x 0.25µm
Flow	He, 4 mL/min

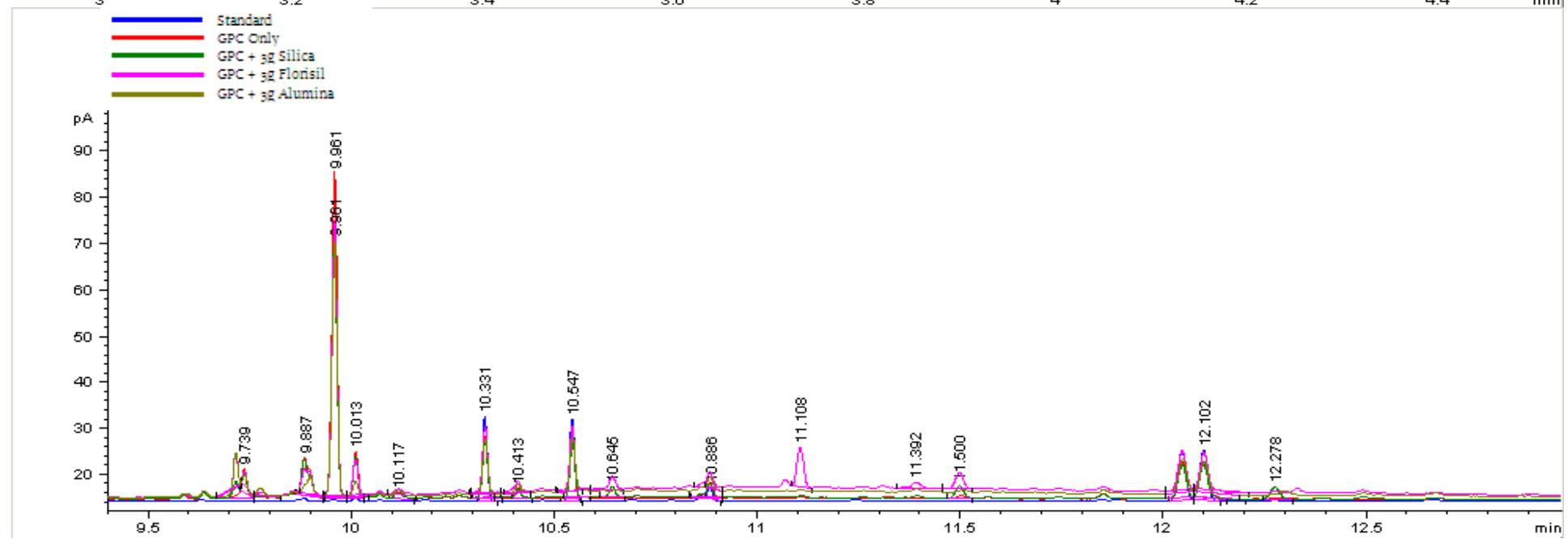
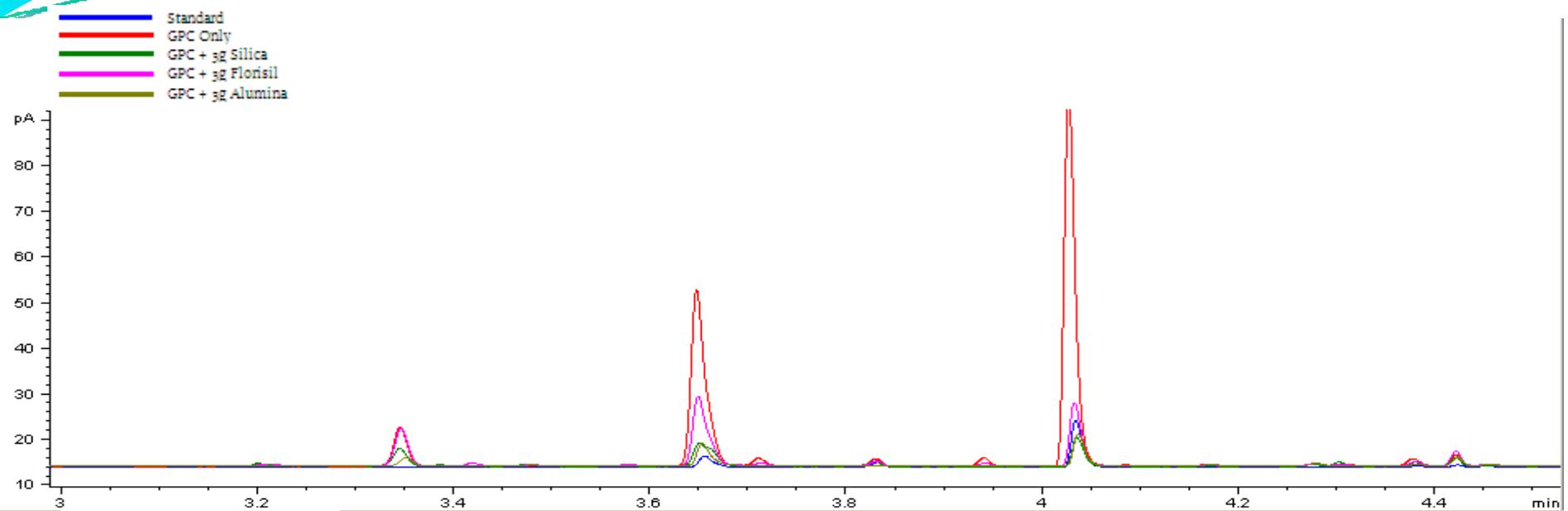
Oven Temperature	Ramp Rate (°C/min)	Temperature (°C)	Hold Time (minutes)
		40	2
	25	250	0
	5	265	0
	25	300	4



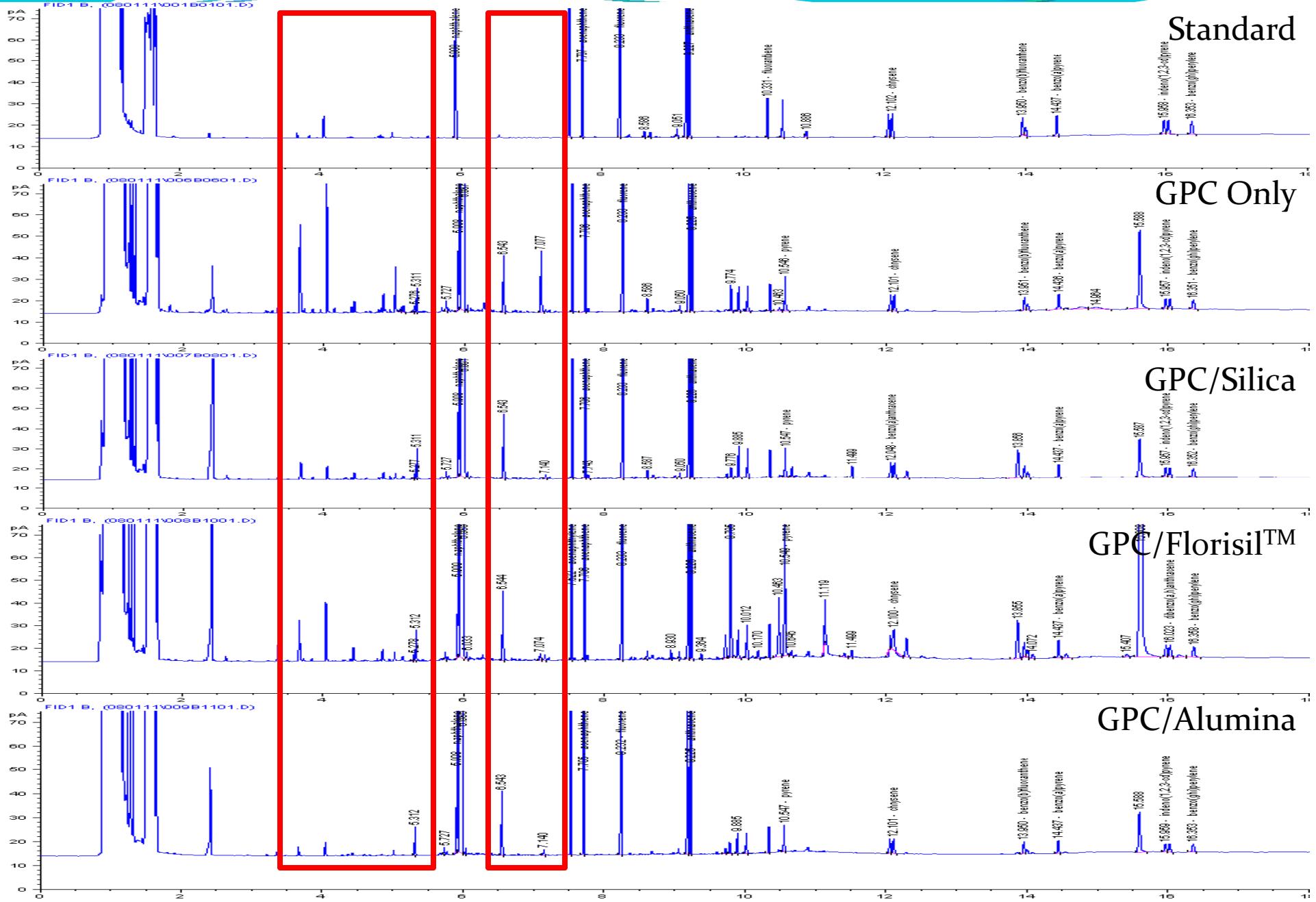
Soil Extract



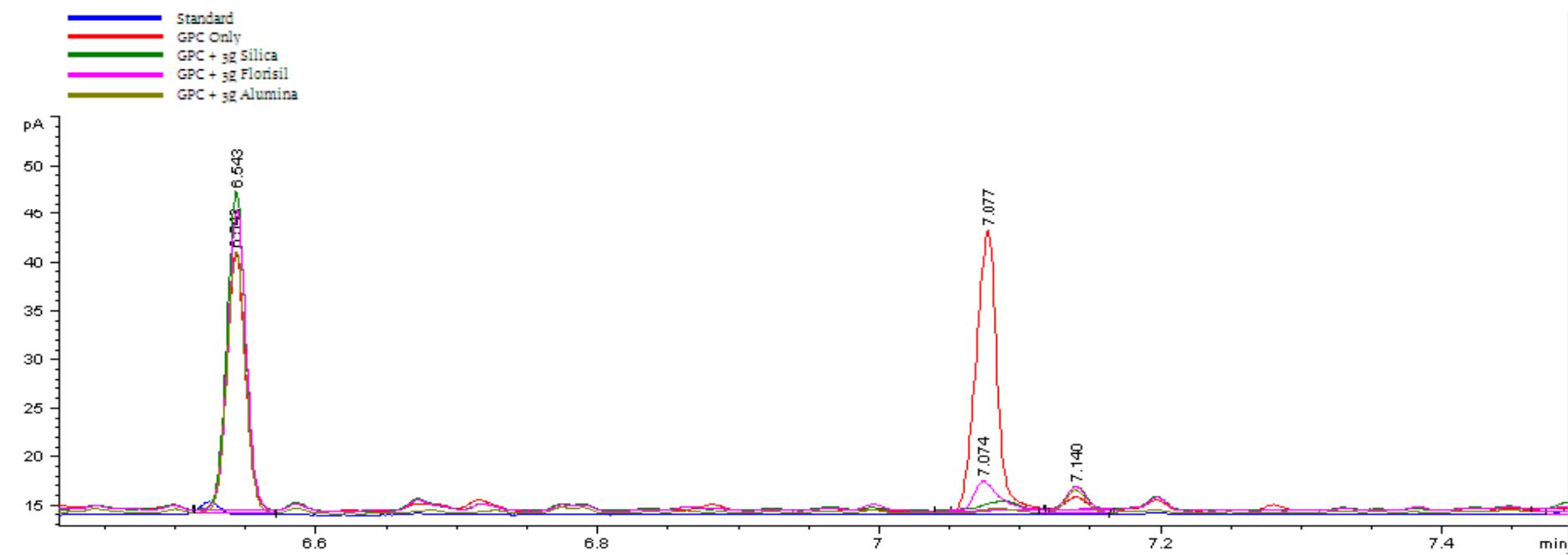
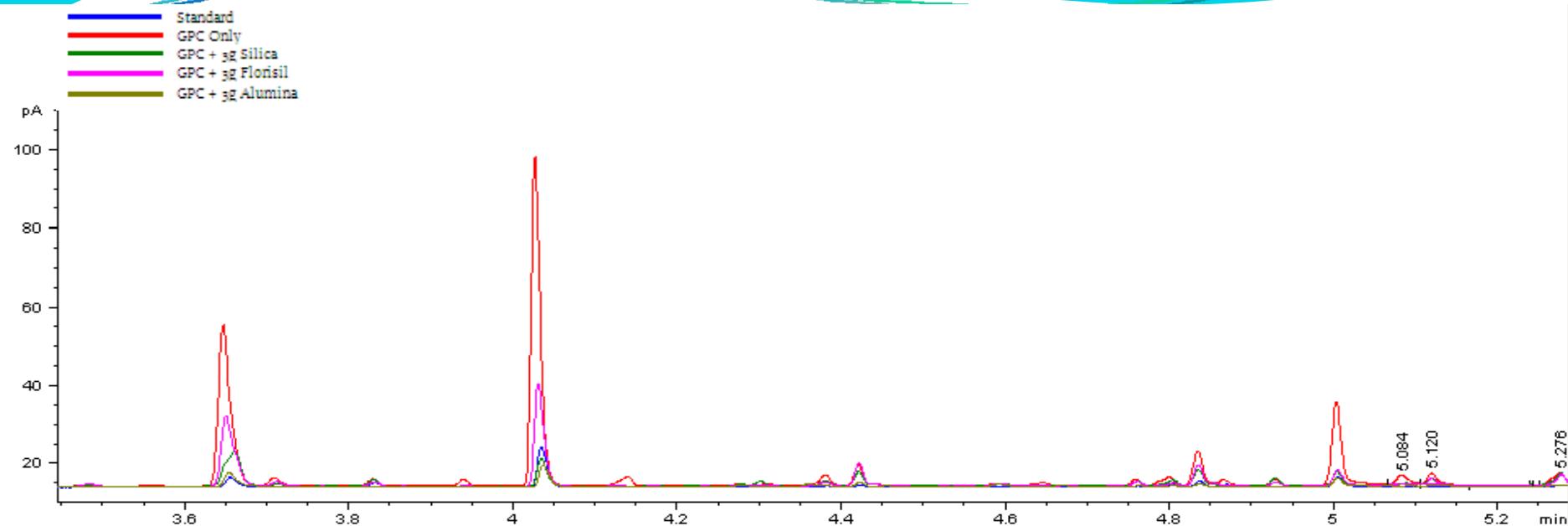
Soil Extract



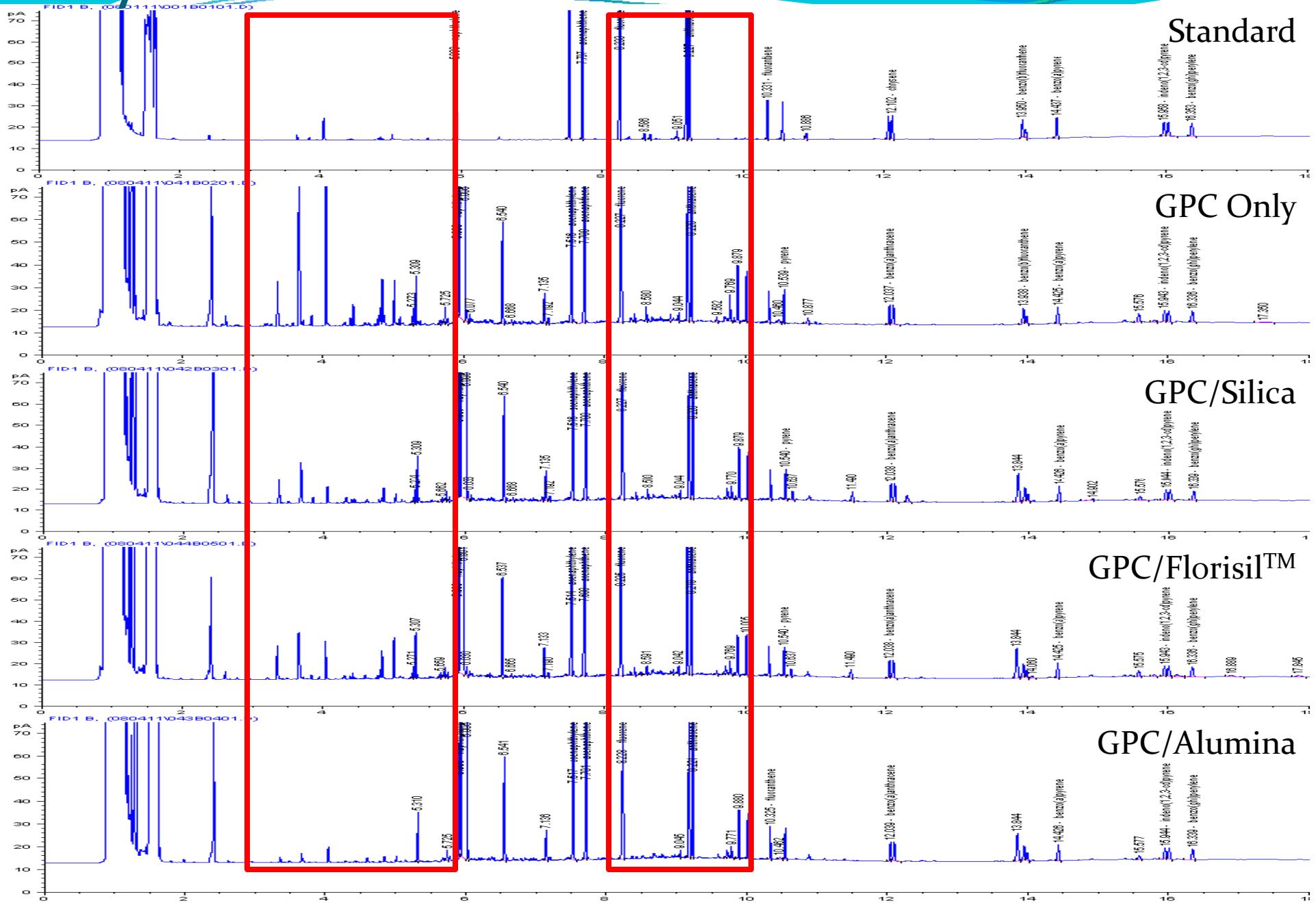
Fish Extract



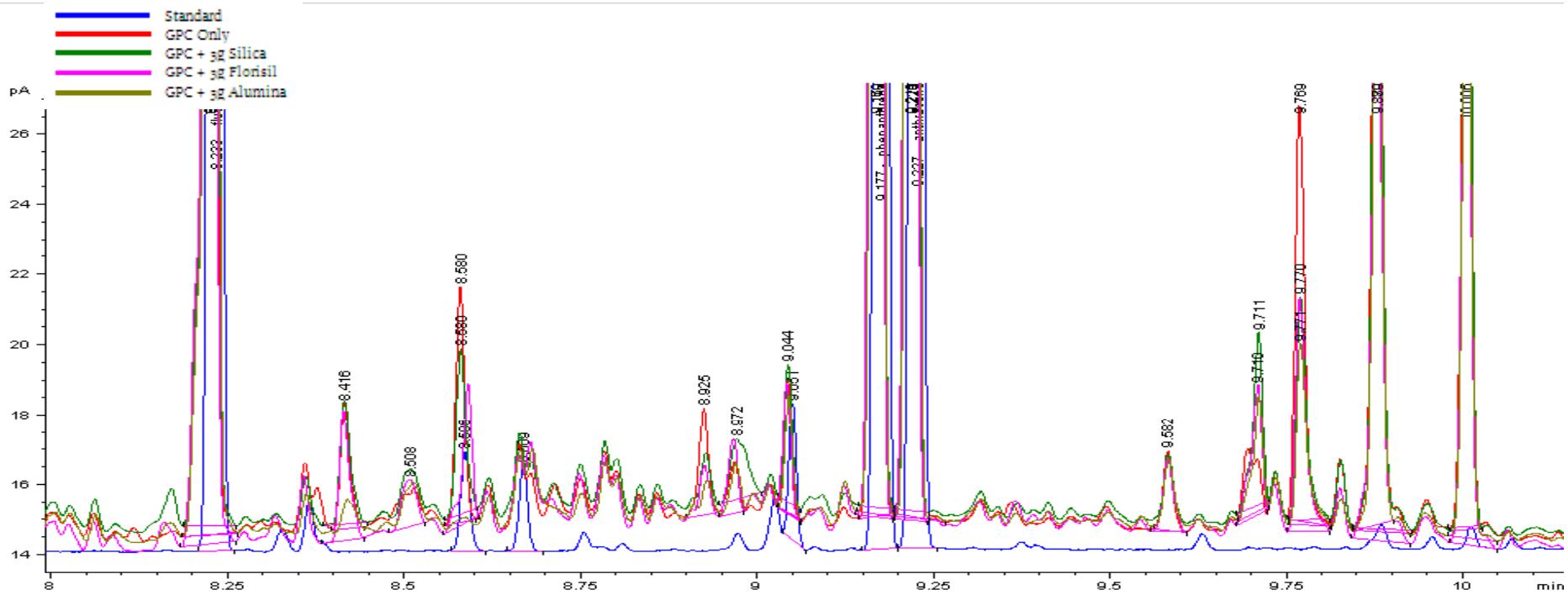
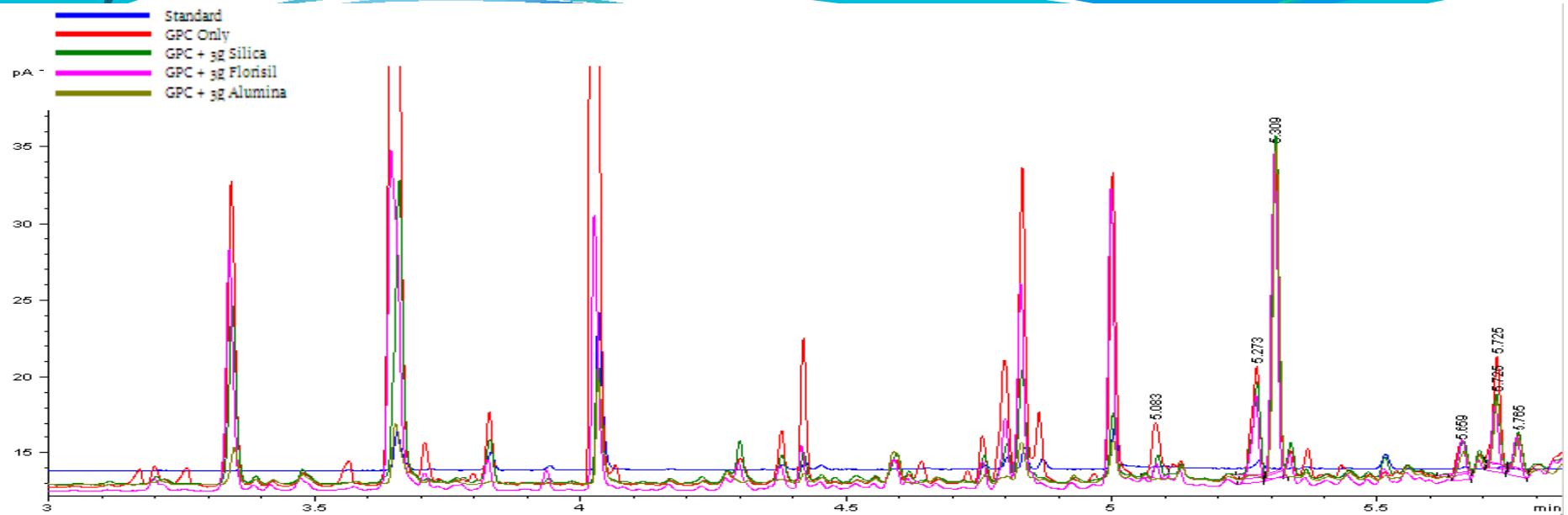
Fish Extract



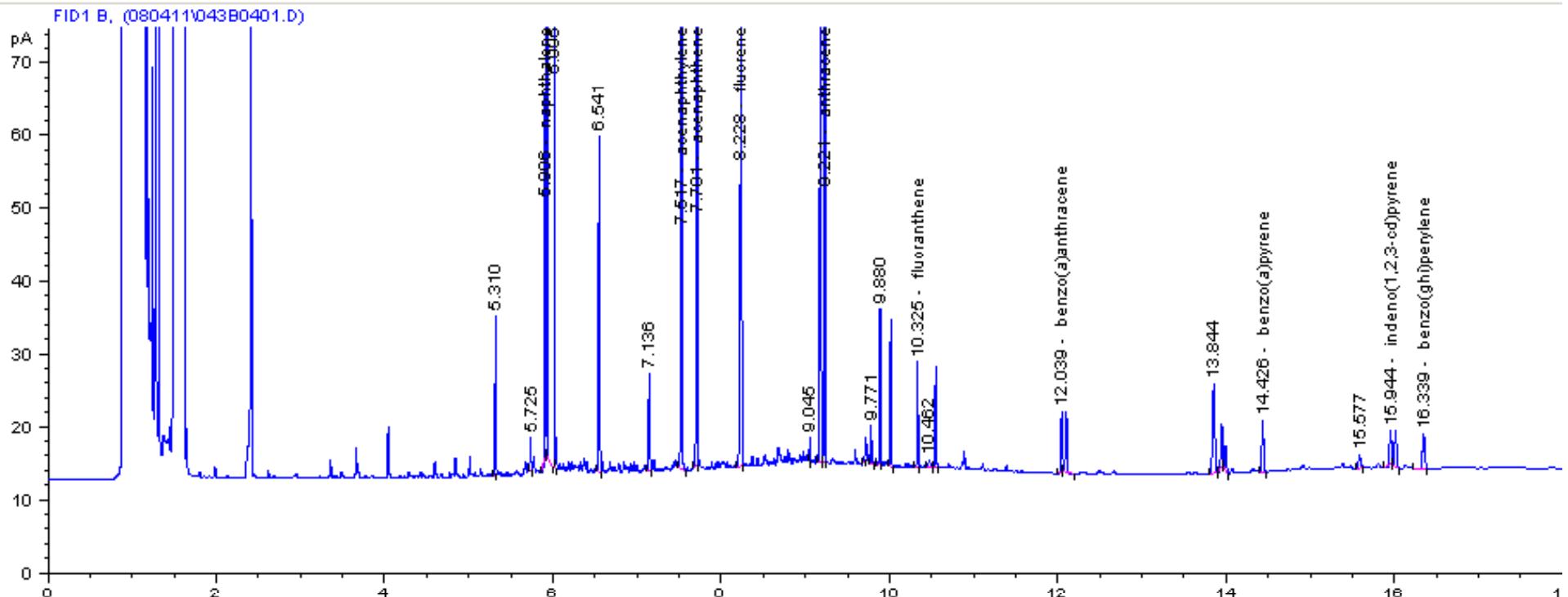
Oyster Extract



Oyster Extract



Oyster Extract Cleanup



GPC with Inline Alumina

Recovery



Analyte	50mL Extract (ppb)	GC Analysis (ppm)	Average (n=3)	RSD	Average (n=3)	RSD	Average (n=3)	RSD
naphthalene	401.6	10.04	80.5	3.0	82.7	2.6	79.8	2.6
acenaphthylene	401.6	10.04	85.6	2.6	87.9	3.9	79.9	5.5
acenaphthene	401.6	10.04	87.9	2.8	91.6	3.4	91.2	4.3
fluorene	401.6	10.04	90.6	3.1	92.8	5.8	91.0	4.1
phenanthrene	401.6	10.04	91.0	2.9	94.5	3.9	87.2	3.6
anthracene	401.6	10.04	90.1	2.9	93.7	3.4	85.9	3.6
fluoranthene	40.16	1.004	94.5	6.4	95.2	3.8	88.5	4.0
pyrene	40.16	1.004	93.0	3.1	103.8	3.4	91.4	5.1
benzo(a)anthracene	40.16	1.004	93.4	2.5	94.9	4.4	88.4	4.0
chrysene	40.16	1.004	93.4	1.9	90.9	5.1	84.0	2.7
benzo(b)fluoranthene	40.16	1.004	89.7	1.3	93.6	3.5	84.4	1.8
benzo(k)fluoranthene	20.00	0.500	96.5	1.5	102.6	4.7	86.5	7.4
benzo(a)pyrene	40.16	1.004	90.5	2.0	96.3	2.0	84.1	3.9
indeno(1,2,3-cd)pyrene	40.16	1.004	105.0	1.6	96.0	1.1	77.6	5.6
dibenzo(a,h)anthracene	40.16	1.004	115.1	1.6	96.9	3.9	78.9	7.5
benzo(ghi)perylene	40.16	1.004	133.1	2.7	94.4	3.6	84.8	5.0

Conclusions

- Inline SPE can provide additional cleanup at a low cost, with no additional time or solvent requirements
- Additional cleanup can improve accuracy and precision and extend the life of the analytical instrument
- Future Studies:
 - GPC with multiple columns inline (silica then alumina, for example) to remove additional interferences
 - SPE to separate two groups of compounds (GPC with inline carbon column to separate PCBs from dioxins)



Thank You

- Thank you co-authors Tom Dobbs, Jeff Wiseman and Jennifer Salmons
- Thank you everyone else at J2 Scientific
- Thank you Restek for supplying the GC Column used in this study

