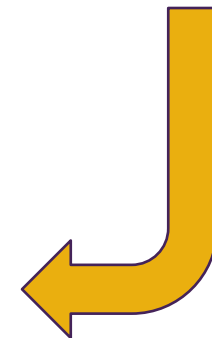
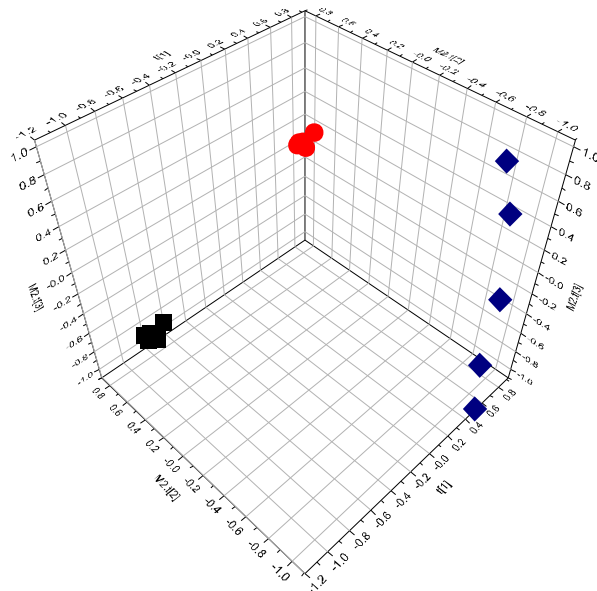
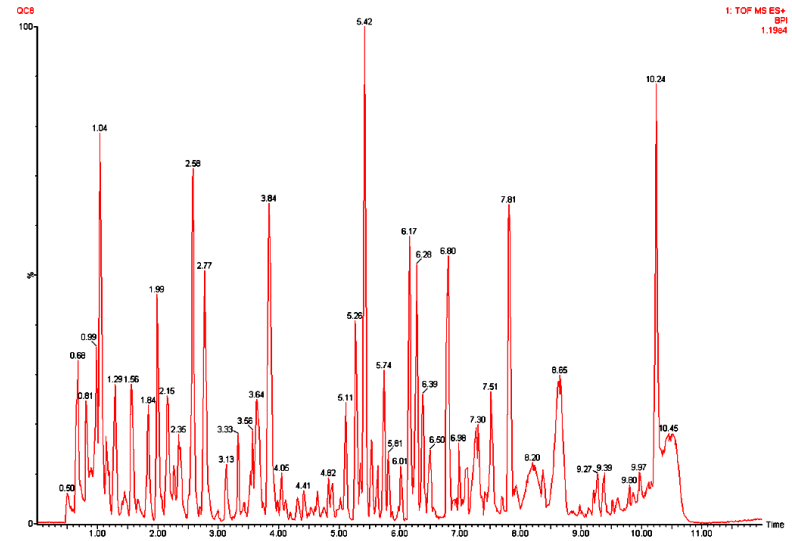
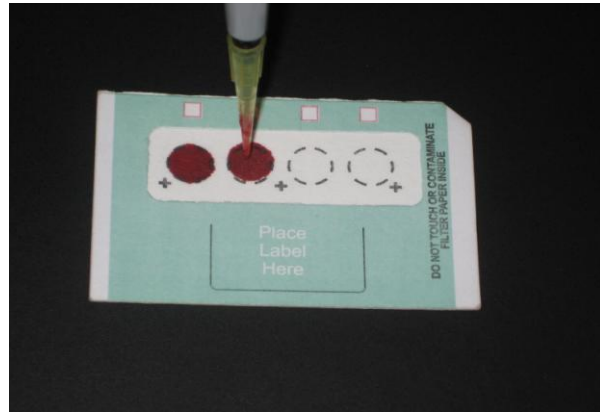


Metabonomic Analysis of Dried Biofluid (Blood, Plasma, Urine) Spots

EBF workshop – Connecting Strategies on Dried Blood Spots
Christopher smith



Bioanalysis card 226



Metabonomics

The study of metabolic responses to drugs, diseases, environmental changes.

Untargeted Approach

Profiling subjects with complementary analytical platforms to obtain comprehensive data

- LC-MS
 - a) Reverse Phase
 - b) Ion Exchange
 - c) HILIC
 - d) (+), (-)
- GC-MS
- NMR

Targeted Approach

Metabolic responses are related to certain classes of metabolites e.g sugars, aminoacids, flavonoids etc

Extraction Protocol

4 tubes of 5x3.2mm blood cores
2 tubes of 4x4.7 and 1x3.2mm plasma cores
5 tubes of 8x4.7mm urine cores



20min extraction
with 160/200 μ L 25% (v/v)
aqueous MeOH



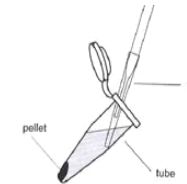
10 min at 20800g



Reconstitution in 240 μ L
50% aqueous MeOH
or 200 μ L H₂O



Evaporation at 40 $^{\circ}$ C

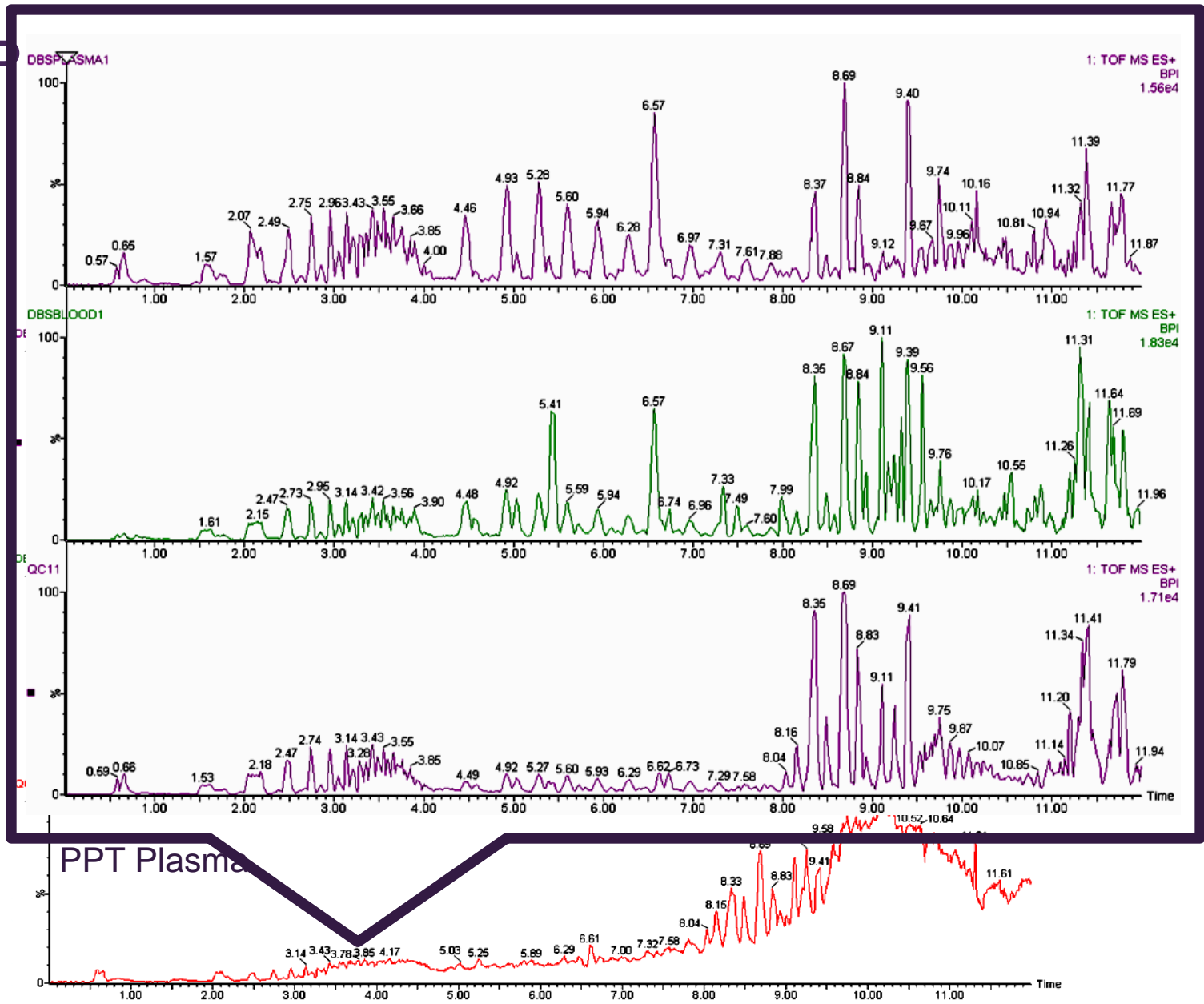


Supernatant transferred to
HPLC vial

15 min Spin at 2500g/10 $^{\circ}$ C
Prior LC-MS analysis



UP



UPLC-MS/MS Blood derived Negative Data

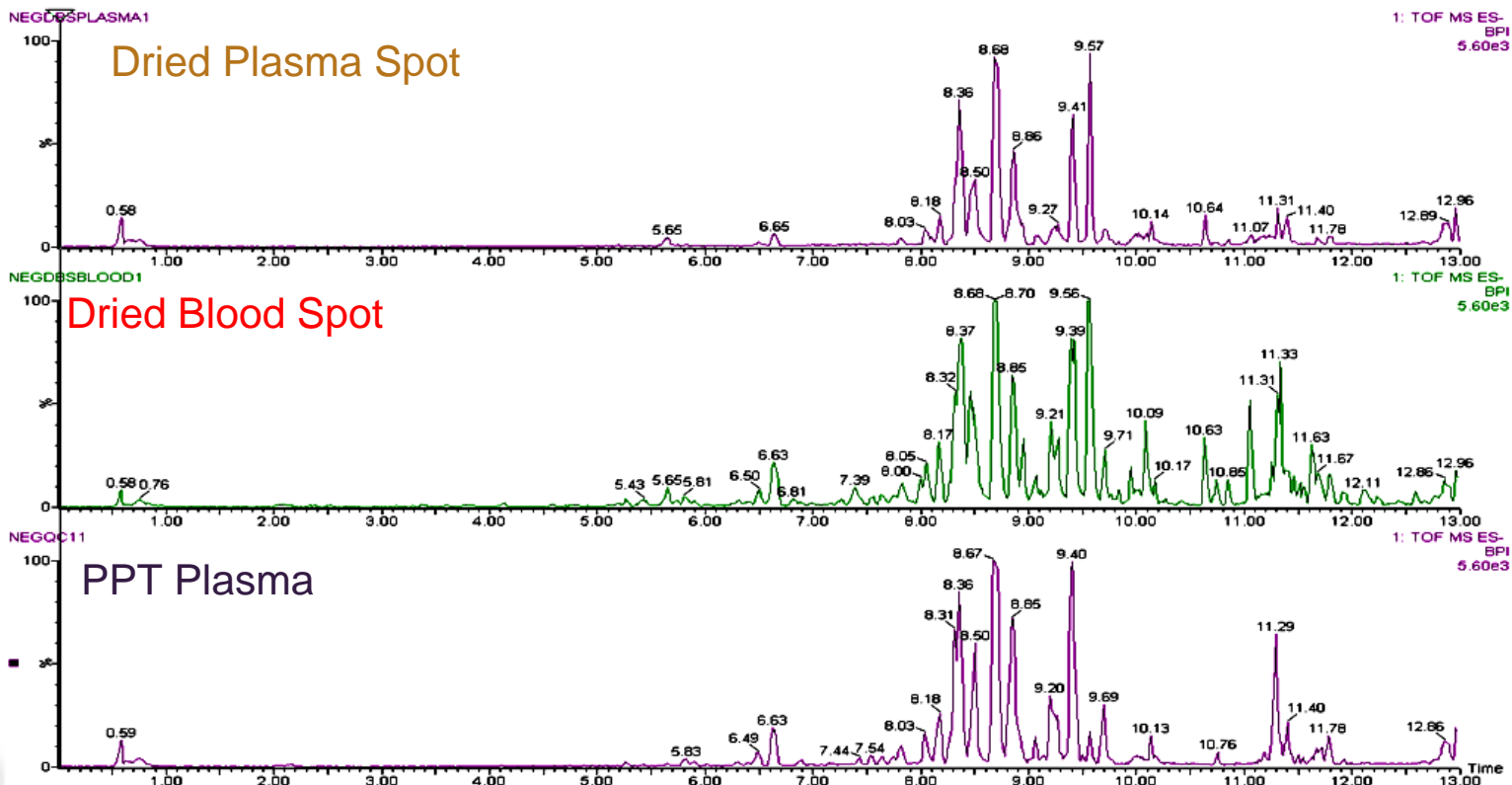
Column: Acquity BEH C18 2.1×100mm 1.7µM

Column temperature: 50°C

Solvent A: H₂O 0.1%F.A

Solvent B: MeOH 0.1%F.A

Mass Range: 100-800amu (-)



UPLC-oaQTOF Urine derived Positive Data

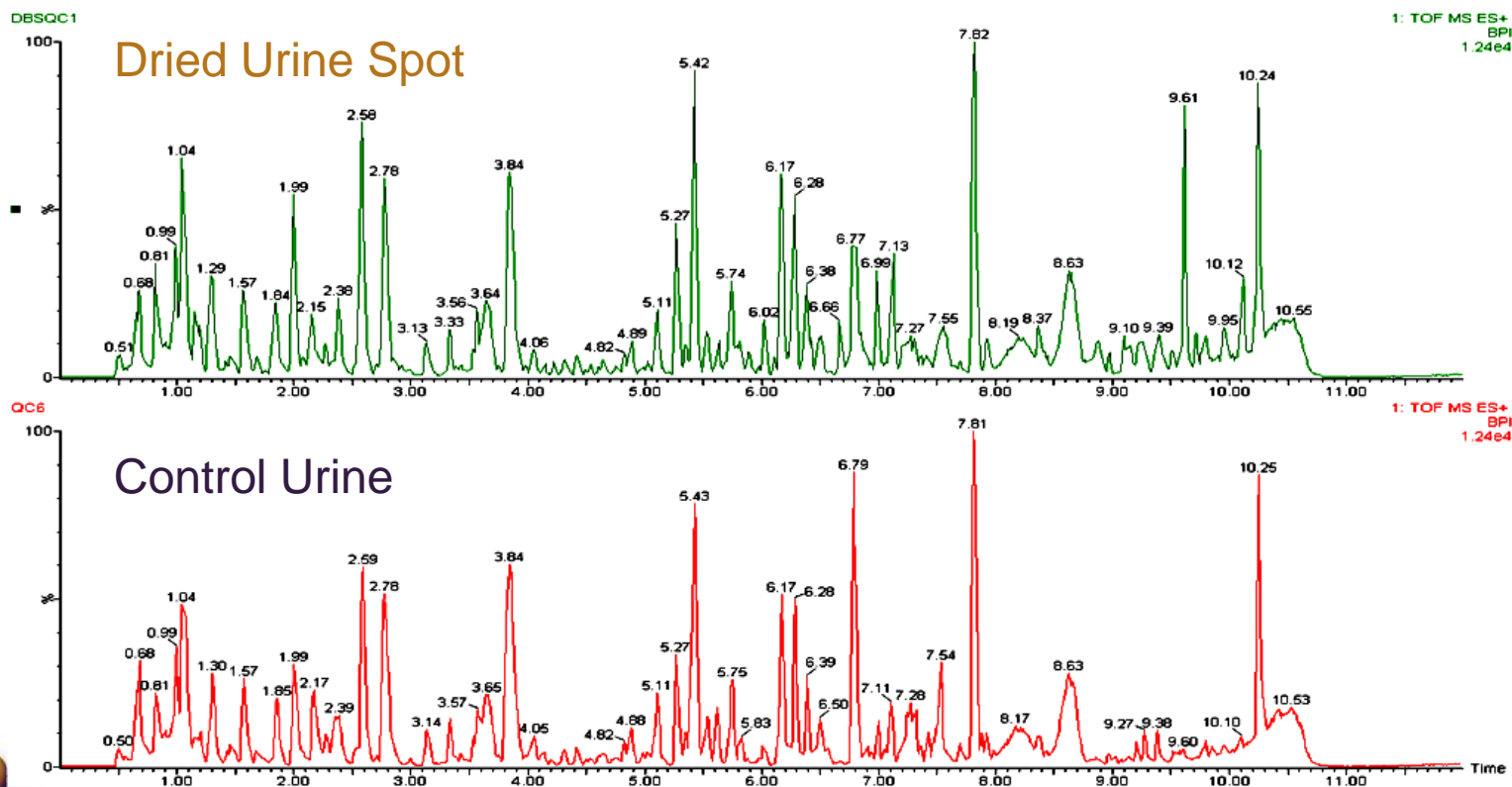
Column: Acquity BEH C18 2.1×100mm 1.7µM

Column temperature: 40°C

Solvent A: H₂O 0.1% F.A

Solvent B: ACN 0.1% F.A

Mass Range: 100-800amu (+)



UPLC-oaQTOF Urine derived Negative Data

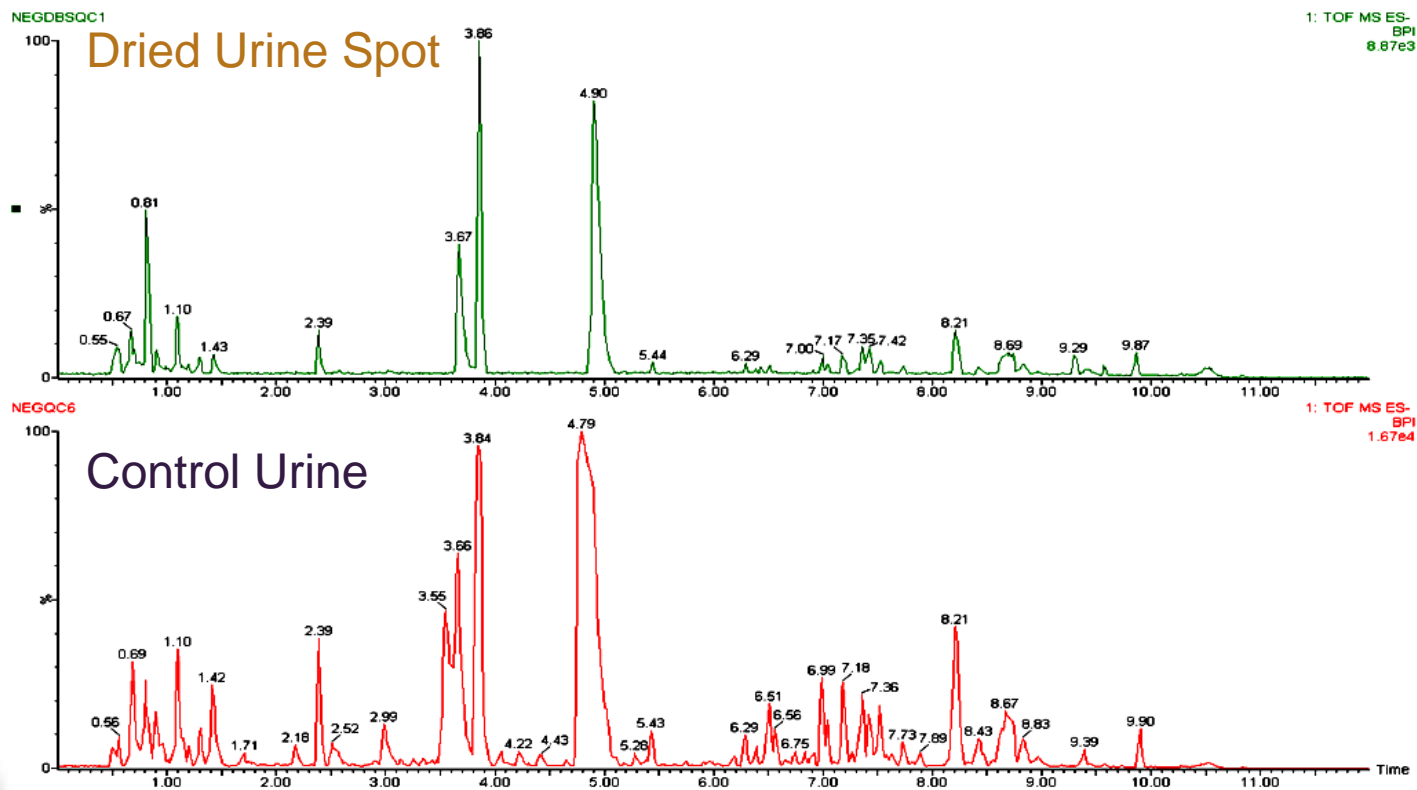
Column: Acquity BEH C18 2.1×100mm 1.7µM

Column temperature: 40°C

Solvent A: H₂O 0.1% F.A

Solvent B: ACN 0.1% F.A

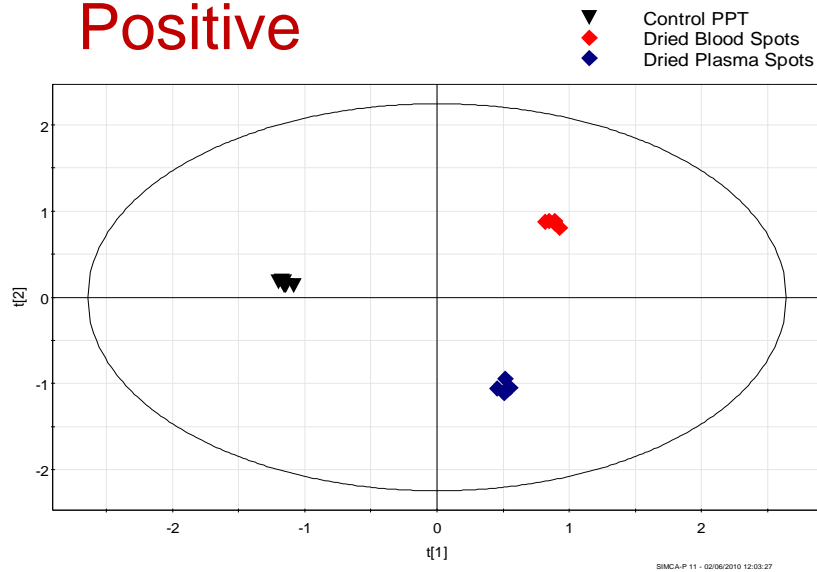
Mass Range: 100-800amu (-)



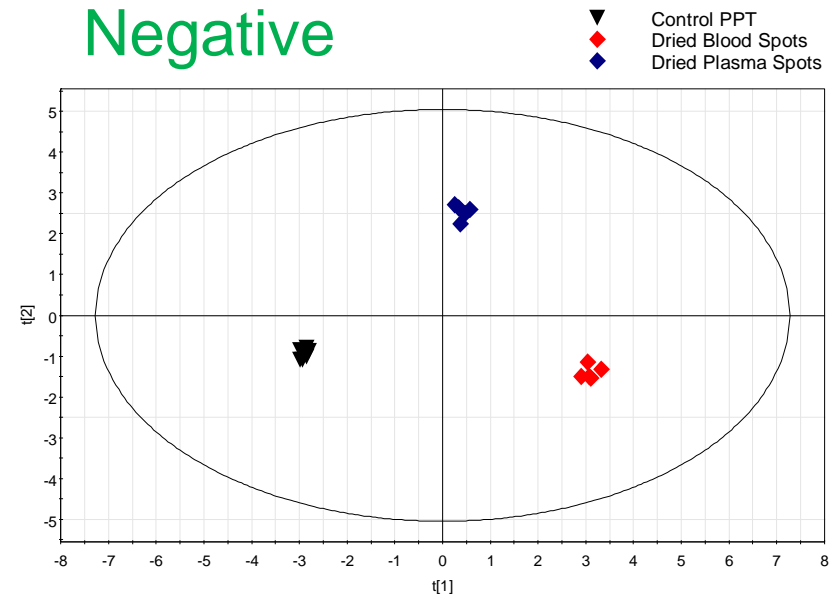
Principal Component Analysis (PCA)

Blood Derived Data

Positive



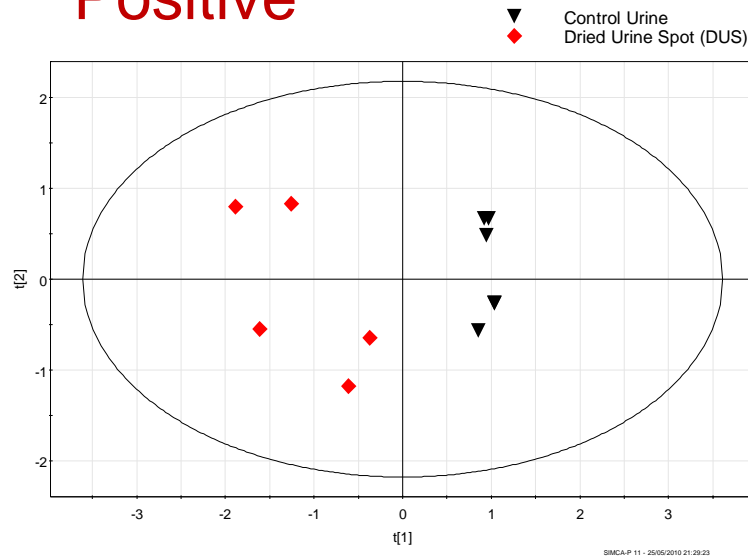
Negative



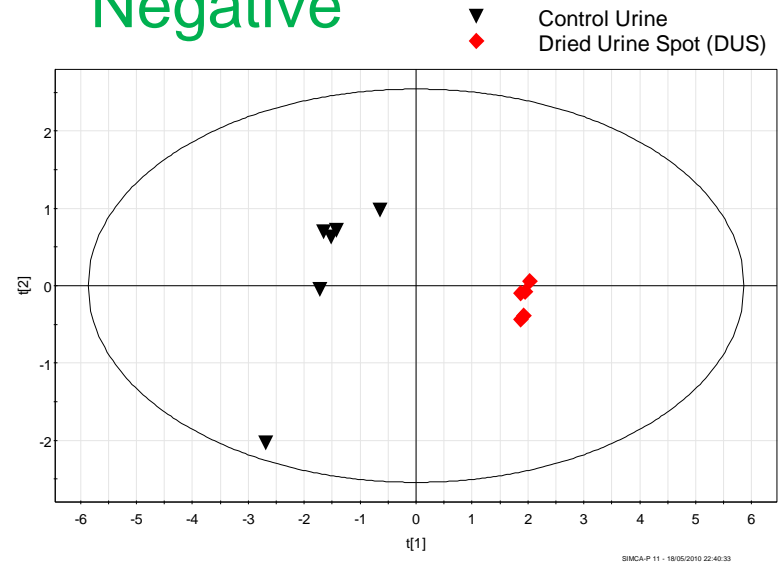
Principal Component Analysis (PCA)

Urine Derived Data

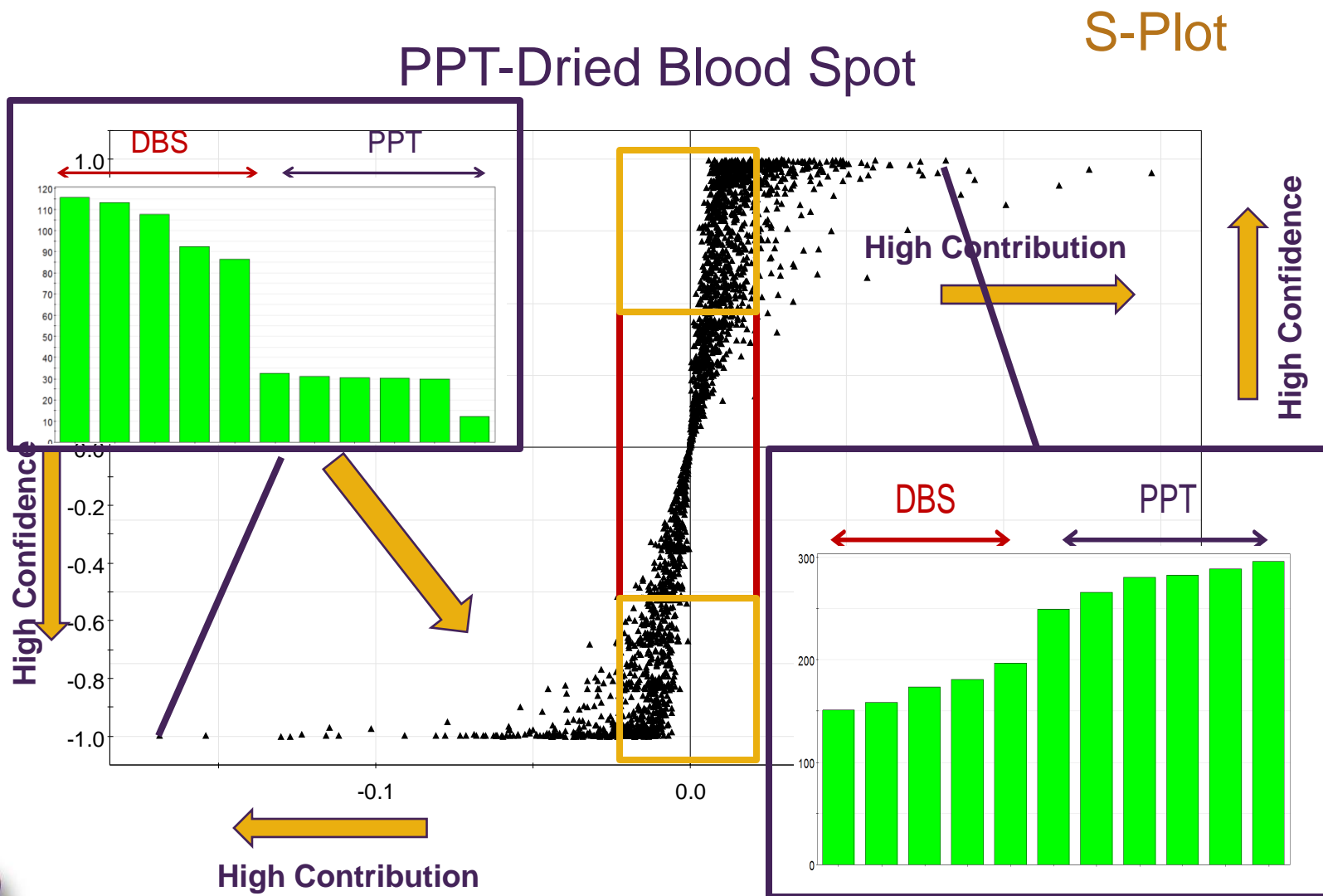
Positive



Negative

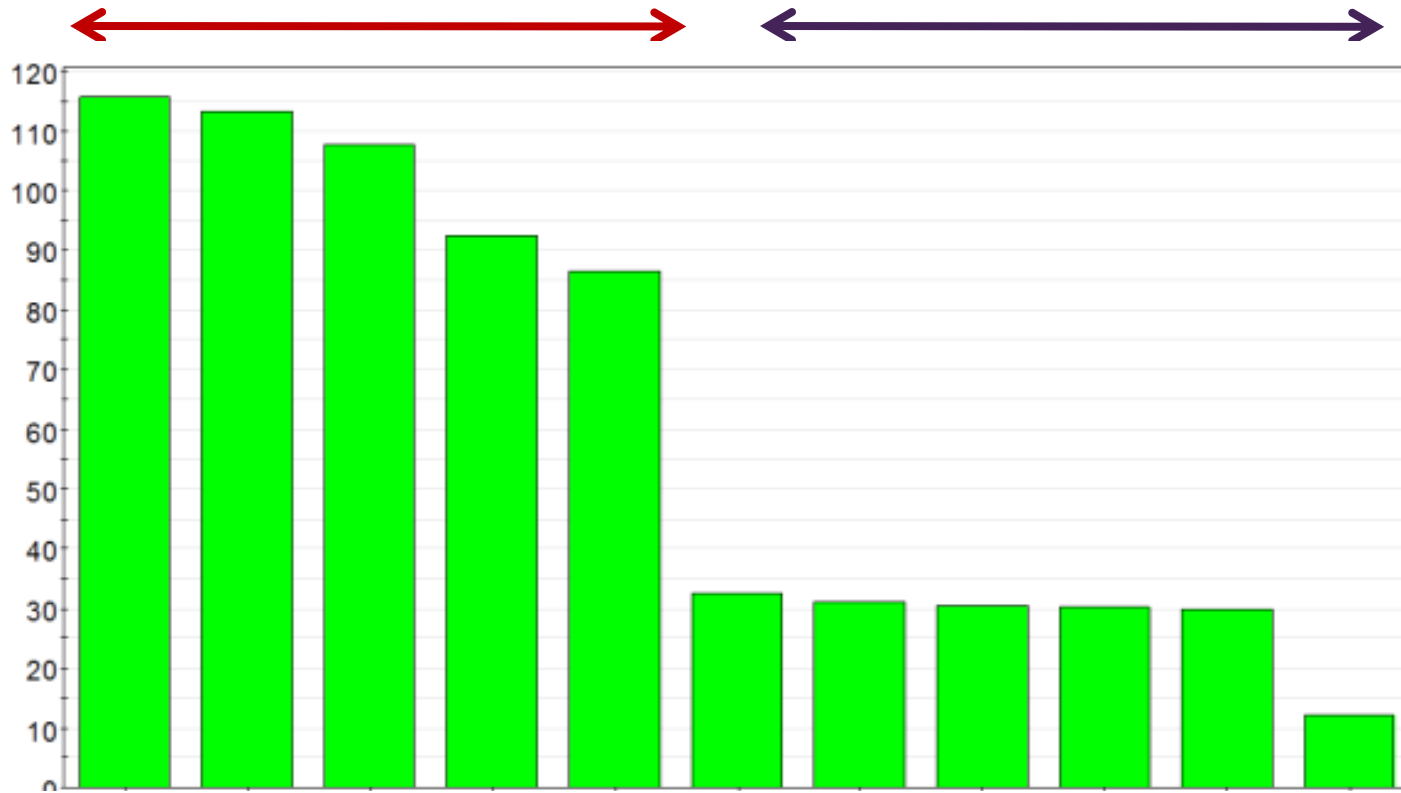


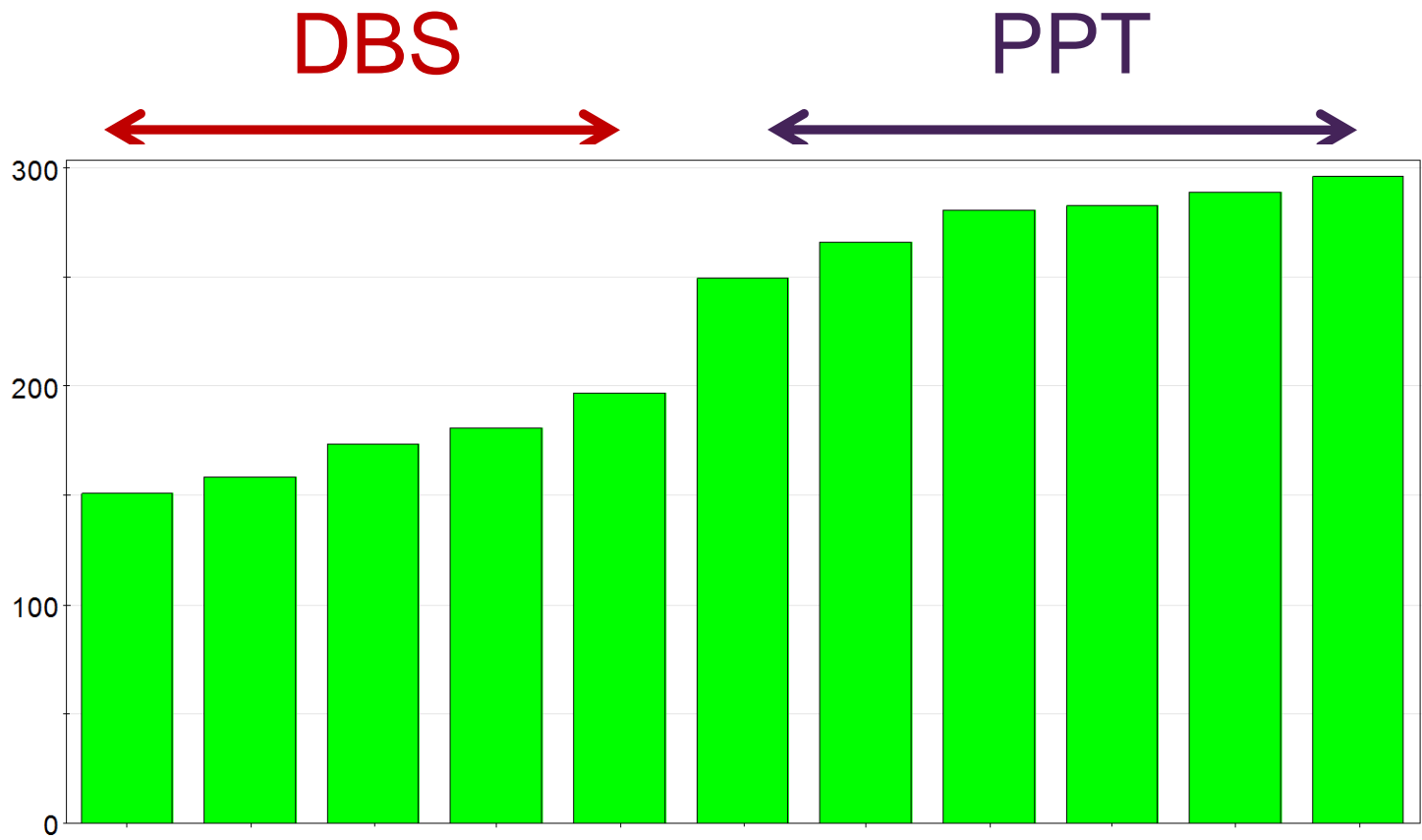
What is different?



DBS

PPT





Potential Differentiating Ions Between Blood Derived Samples

Positive Data

	389.25			365.13			568.35			544.34		
	RT	AVERAGE PEAK AREA	CV	RT	AVERAGE PEAK AREA	CV	RT	AVERAGE PEAK AREA	CV	RT	AVERAGE PEAK AREA	CV
PPT	4.93	196.19	5.93	6.57	3.27	38.95	8.30	185.19	3.89	8.33	919.31	6.86
DBS	4.93	435.37	3.13	6.57	1019.40	6.25	8.30	94.15	6.55	8.33	722.63	6.36
DPS	4.93	537.14	31.37	6.57	854.36	33.66	8.30	55.69	8.87	8.33	333.28	39.28

	520.34			518.30			522.35			538.40		
	RT	AVERAGE PEAK AREA	CV	RT	AVERAGE PEAK AREA	CV	RT	AVERAGE PEAK AREA	CV	RT	AVERAGE PEAK AREA	CV
PPT	8.35	1331.12	0.59	8.70	902.76	3.71	8.84	770.19	3.74	9.60	375.81	18.31
DBS	8.35	951.19	10.83	8.70	671.99	6.08	8.84	821.13	7.46	9.60	203.27	2.43
DPS	8.35	440.55	4.58	8.70	474.59	4.03	8.84	333.48	45.89	9.60	78.24	33.21

Negative Data

	m/z 552.31			m/z 528.30			m/z 504.30			m/z 480.30		
	RT	AVERAGE PEAK AREA	CV	RT	AVERAGE PEAK AREA	CV	RT	AVERAGE PEAK AREA	CV	RT	AVERAGE PEAK AREA	CV
PPT	8.30	54.57	2.44	8.32	261.26	2.20	8.36	305.50	41.66	8.70	630.33	0.72
DBS	8.30	16.16	8.25	8.32	198.06	9.48	8.36	452.87	4.73	8.70	645.06	6.35
DPS	8.30	12.05	10.04	8.32	123.91	3.30	8.36	256.09	3.83	8.70	420.39	3.47

	m/z 566.34			m/z 568.36			m/z 355.31			m/z 281.24		
	RT	AVERAGE PEAK AREA	CV	RT	AVERAGE PEAK AREA	CV	RT	AVERAGE PEAK AREA	CV	RT	AVERAGE PEAK AREA	CV
PPT	8.86	112.57	2.11	9.40	149.03	2.22	9.58	1.61	58.26	9.71	96.48	9.15
DBS	8.86	80.45	8.37	9.40	130.43	8.53	9.58	733.87	10.98	9.71	67.05	12.85
DPS	8.86	58.33	4.83	9.40	70.17	6.08	9.58	177.42	19.42	9.71	46.55	8.43

Phospholipids Related Data from Blood Derived Samples

	184.07			496.33			524.37		
	RT	AVERAGE PEAK AREA	CV	RT	AVERAGE PEAK AREA	CV	RT	AVERAGE PEAK AREA	CV
PPT	8.30	1683.33	3.25	8.68	1853.15	3.76	9.39	1424.04	1.72
DBS	8.30	1934.01	3.44	8.68	1446.06	7.74	9.39	1507.64	2.02
DPS	8.30	773.16	19.61	8.68	878.11	6.61	9.39	656.30	46.44

	703.58			758.56			806.56		
	RT	AVERAGE PEAK AREA	CV	RT	AVERAGE PEAK AREA	CV	RT	AVERAGE PEAK AREA	CV
PPT	11.25	98.16	30.36	11.40	855.67	13.31	11.40	922.10	1.48
DBS	11.25	336.63	3.08	11.40	769.88	7.26	11.40	682.59	6.52
DPS	11.25	96.77	15.07	11.40	420.19	27.87	11.40	311.98	14.19

184.07: Phosphocholine

496.33: 1-palmitoyl-2-hydroxy-sn-glycero-3-phosphocholine

524.37: 1-stearoyl-2-hydroxy-sn-glycero-3-phosphocholine

703.58: glycerophosphocholine lipid

758.56: 1-hexadecanoyl-2-(9Z,12Z-octadecadienoyl)-sn-glycero-3-phosphocholine

806.56: 1-(9Z,12Z-octadecadienoyl)-2-(5Z,8Z,11Z,14Z-eicosatetraenoyl)-sn-glycero-3-phosphocholine



Potential Differentiating Ions from Urine

Derived data

Negative Data

	m/z 149.90			m/z 172.85			m/z 177.90			m/z 286.80		
	RT	AVERAGE PEAK AREA	CV	RT	AVERAGE PEAK AREA	CV	RT	AVERAGE PEAK AREA	CV	RT	AVERAGE PEAK AREA	CV
CONTROL URINE	2.39	250.40	12.39	3.00	134.00	18.21	3.66	670.83	8.89	4.21	20.07	22.65
DUS	2.39	47.46	5.78	3.03	19.10	3.69	3.66	174.49	4.98	4.26	2.99	9.97

	m/z 186.85			m/z 268.95			m/z 540.85			m/z 330.91		
	RT	AVERAGE PEAK AREA	CV	RT	AVERAGE PEAK AREA	CV	RT	AVERAGE PEAK AREA	CV	RT	AVERAGE PEAK AREA	CV
CONTROL URINE	4.81	2960.66	8.17	6.51	148.16	17.04	7.37	320.34	45.73	8.21	462.67	13.37
DUS	4.90	657.81	4.46	6.51	15.09	10.55	7.37	62.63	59.61	8.21	70.05	6.08



Conclusion

- Preliminary results show DBS, DPS and DUS represent an interesting alternative to liquid samples for generating metabolite profiles.
- The DBS represents a good procedure for when there is reduced sample.
- Other work has shown other biofluids such as bile can be used – DBileS
- Sensitivity issue, five cores used to achieve required response – Capillary or chip base technology could offer a solution to this.



Acknowledgements

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