Metabolomics by Solid Phase Microextraction – Liquid Chromatography-Mass Spectrometry (SPME-LC-MS)

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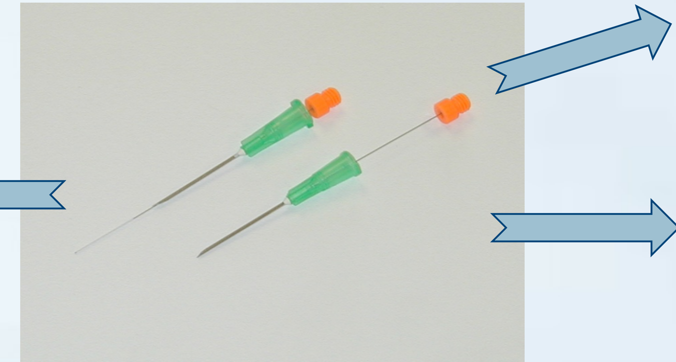
Introduction

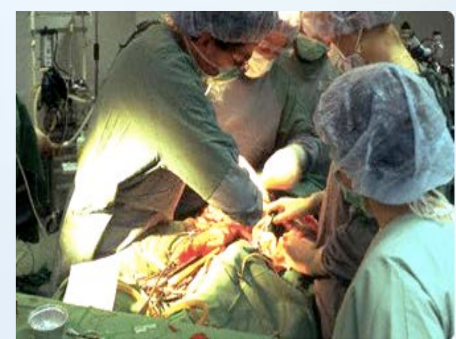
Metabolomics is powerful tool in the field of drug and biomarker discovery. It brings valuable information about response of the organism to external stimuli and allows defining changes induced by pathological factors. However, monitoring of hundreds or thousands of compounds requires high resolution and mass accuracy, which in turn force very efficient sample clean-up. This can be successfully accomplished by using solid phase microextraction (SPME), which applies no sample treatment or modification. This is particularly important in metabolomics, where obtaining true snapshot of metabolome is the main goal. We used SPME for in vivo and ex vivo metabolomics studies in animals and humans, respectively, showing application of the technique to preclinical and clinical studies.

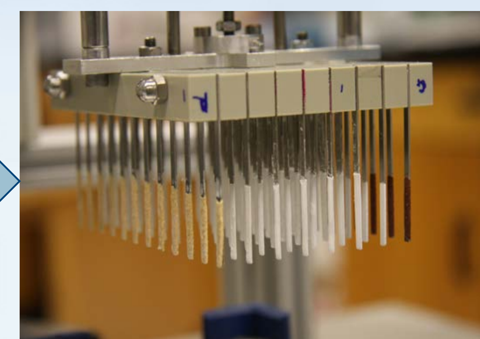
Methods



In vivo animal sampling: 45 µm thickness mix-mode (C18 with benzenesulfonic acid) SPME coating

- blood and perfusate: 15 mm coating length,
- lung and brain: 7mm coating length





Ex vivo extraction of human plasma:

- 15 mm coating length mix-mode fibres (manual extraction)
- thin film geometry divinylbenzene-polyacrylonitrile (DVB-PAN) coating probes ("blades") mounted to automated high throughput SPME system CONCEPT 96

RP method

Pentafluorophenyl column Discovery HS F5, 2.1 x 100 mm, 3 µm (Supelco); flow rate 300 µL/min;

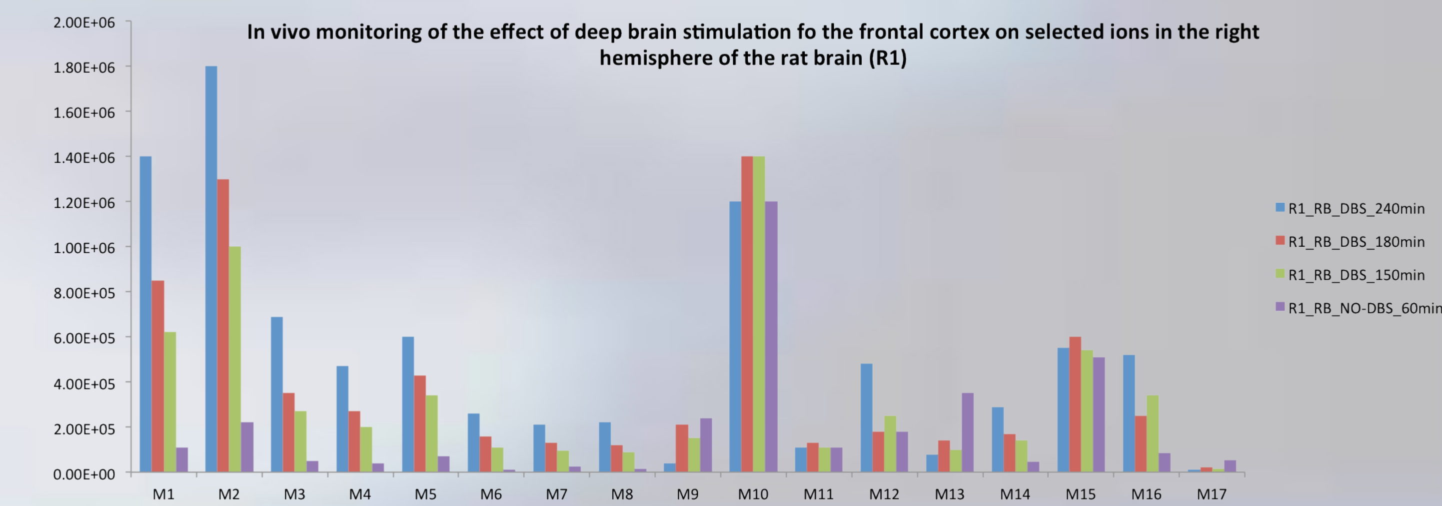
mobile phase A: water/formic acid (99.9/0.1, v/v); mobile phase B: acetonitrile/formic acid (99.9/0.1, v/v)

HILIC method

Luna HILIC column 2.0 x 100 mm, 3µm, 200A (Phenomenex); flow rate 400 µL/min; mobile phase A: acetonitrile/ammonium acetate buffer 20 mM (9/1, v/v): mobile phase B:acetonitrile/ammonium acetate buffer 20 mM (1/1, v/v)

Exactive orbitrap mass spectrometer operated in positive and negative mode

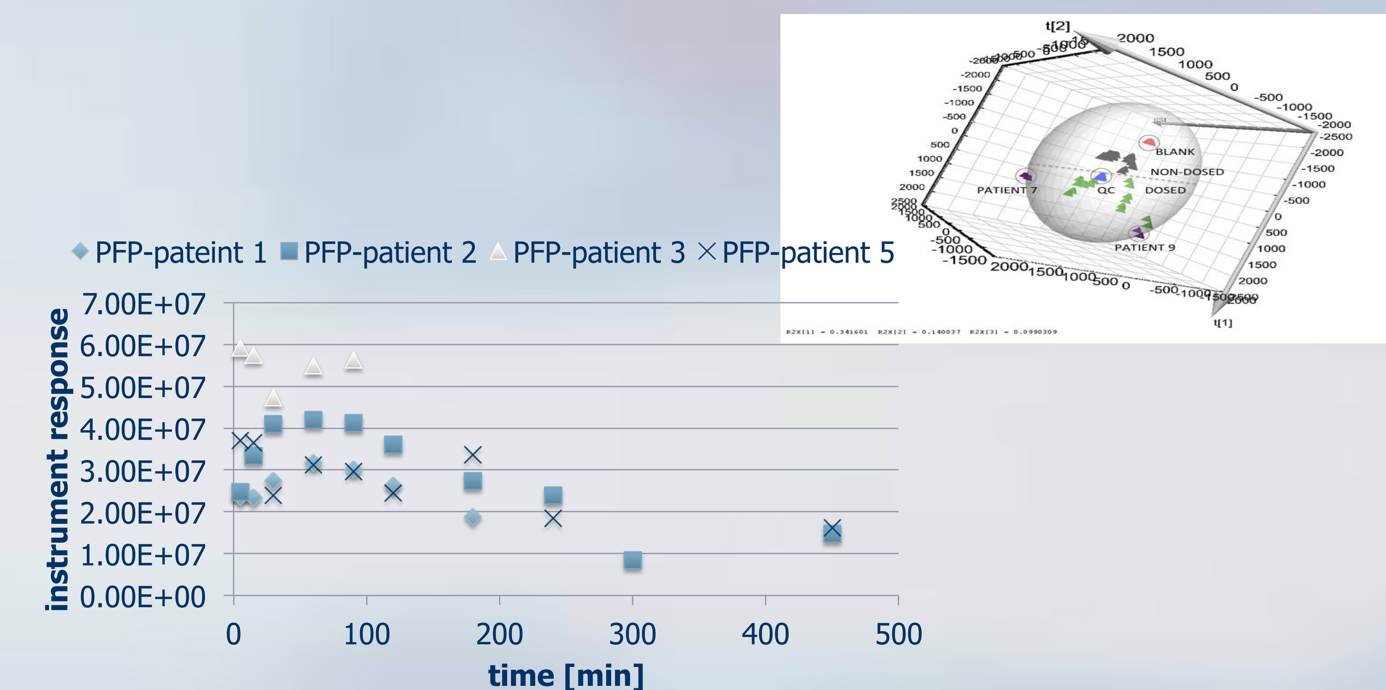
Results



	Qualitative identification	m/z	LogP	SPME		Microdialysis	
				No stimulation	Stimulation	No stimulation	Stimulation
M1	Triglyceride	529.4102	9.34	5e2 - 1.6e4	2.5e3-3.8e4	nd	nd
M2	Lysophosphatidylcholine	551.3923	23.75	9e3 - 7.6e4	1.6e3-1.5e4	nd	nd
M3	Arachinonyl carnitine	546.4367	3.47	2e4 - 7e4	6e3-1.2e4	nd	nd
M4	Ganglioside	530.4137	2.99	5.8e2 - 1.7e4	3e3-9e3	nd	nd
M5	Lysophosphatidylcholine	552.3958	3.14	5e2 - 1.8e4	1e3-4e3	5e0	1e1
M6	Eicosanoid acid	311.2947	8.4	1e4	1e3-1.5e4	nd	nd
M7	nonadeca-10(2)-enoic acid	329.3053	8.06	1.7e3 - 4e4	8e2-1e3	nd	nd
M8	diglyceride	547.44	9.25	3e3 - 6e3	1.6e3-3e3	nd	nd
M9	estradiol derivative	363.2536	3.9	1.4e4	1e4	1.5e2	1e2
M10	N-acetyl-D-mannosamine-6-phosphate or dimethyl heptanoyl carnitine	116.1437	-3.6	2.5e5	1.8e5	2e1	1.61
M*	N-acetyl-D-mannosamine-6-phosphate	116.1437	-3.6	8e4 - 2.3e5	6e4-9e4	nd	nd
M11	Unknown	153.139	1.85	8e3	7e3-1e4	7e1	9e1
M12	glutamine	147.0768	-3.6	6e4	2e4-3e4	3.5e4-1.9e5	1.8e5
M13	leukotriene/phytyl diphosphate	913.4786	4.8	2.6e3 - 5.4e3	4e3-5.8e3	nd	nd
M14	palmitaldehyde	132.077	5.[illegible]	3e4 - 5.7e4	7e3	7e3-1e4	6.5e3-1e4
M15	Phosphate	158.9643	-3.[illegible]	1.3e4 - 4.4e4	5e3-7.6e3	6e5-7.7e5	5.7e5-7.4e5
M16	leucine/amin[illegible]utyric acid	164.1286	-2.5	7e2 - 1e3	5e1-7.6e2	1e1-3.7e1	1.9e1-3.8e1
M17	methylglutaco[illegible] acid	223.0642	0.29	1.5e3	5e3-7e3	2.6e2	2.4e2-1.4e3

very polar salt, median concentration in SPME, very high concentration in MD (present in dialysate buffer)

hydrophobic compounds, tend to stick to dialysis membrane and do not pass to the dialysate



Conclusion

- uniform and simple protocol for different matrices enabling on-site sampling by medical personnel
- complementary information to standard sampling methods
- biomarker discovery
- personalized therapy
- simultaneous pattern recognition/ biomarker analysis and drug monitoring

Future Direction

In vivo SPME coupling with nanospray needle, Direct Analysis in Real Time (DART) or other ambient ionization technique

rapid diagnostic tool for intrasurgical monitoring of biomarkers and drug concentration

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