



# On-line DBS-SPE-MS/MS

**Feasibility of a concept for rapid  
DBS analysis without punching**

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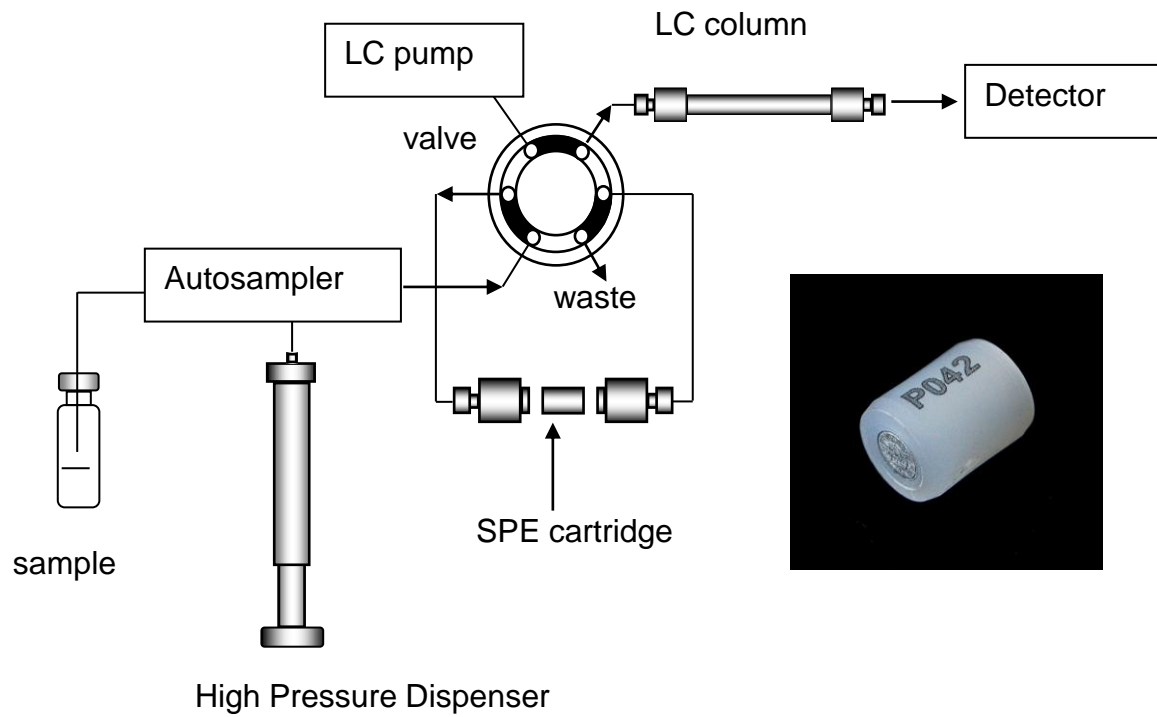
EBF workshop connecting strategies on Dried Blood Spots  
Brussels, June 17-18, 2010

## Outline



- Spark concepts for on-line Dried Blood Analysis
- Sorbent sampling
- On-line DBS extraction with loop interface
- on-line DBS extraction with SPE interface

# Spark Holland: Systems for on-line SPE-LC-MS



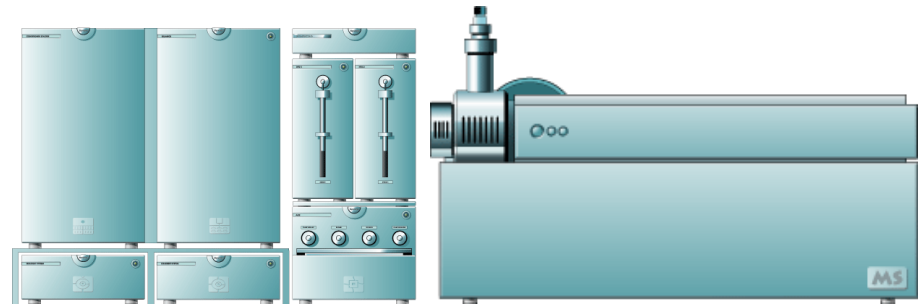
# Our mission



## The basis of our concepts for on-line Dried Blood Analysis



tem

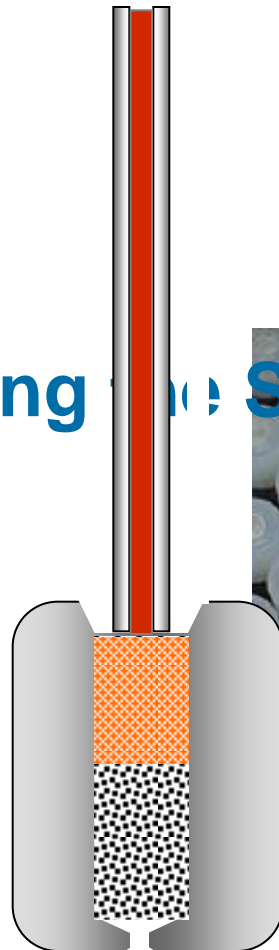
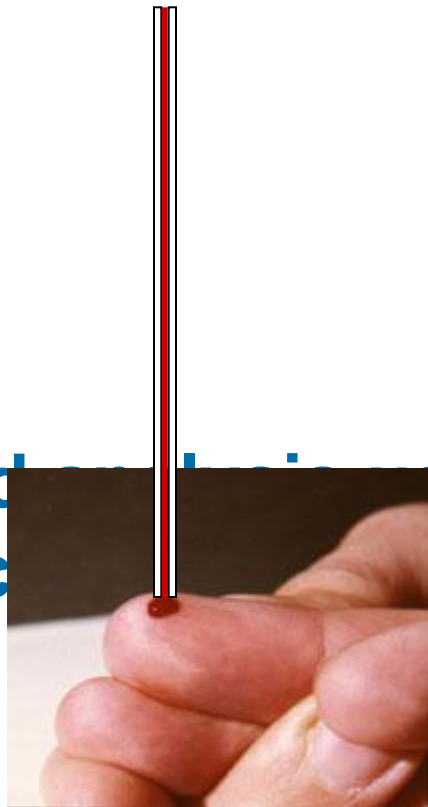
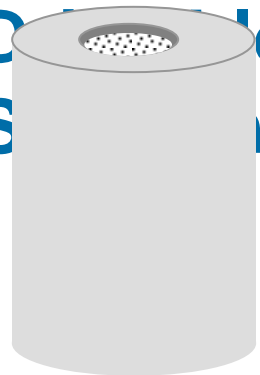


- Automated exchange of SPE cartridges with high pressure sealing
- High Pressure Solvent Dispenser
- Parallel SPE (dual cartridge clamps)



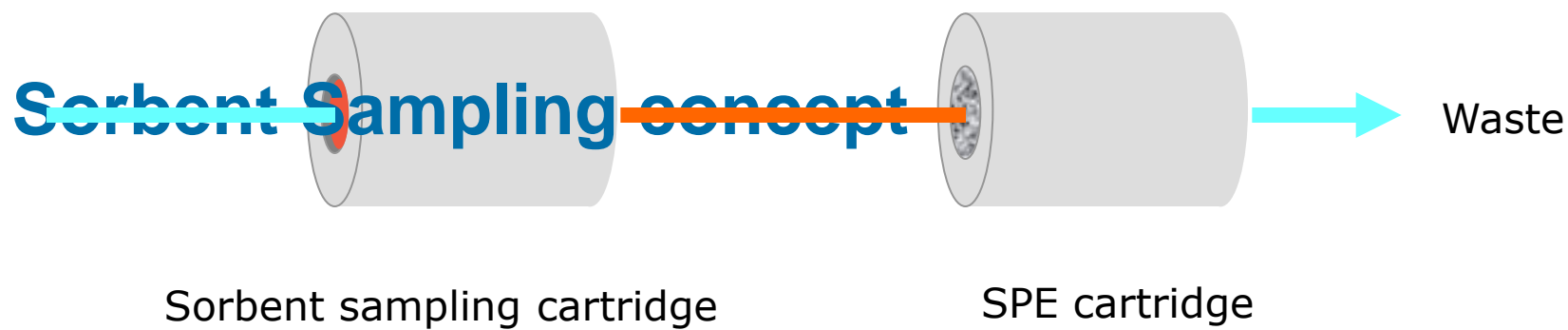


# Detecting blood alcohol using the Sorbent





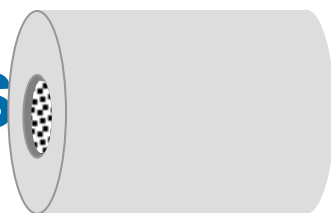
## Transfer to SPE



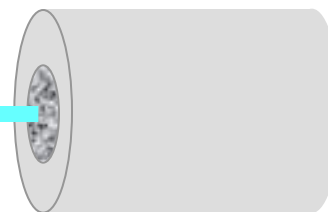


**Wash**

**Sorbent S** **concept**



Sorbent sampling cartridge



SPE cartridge



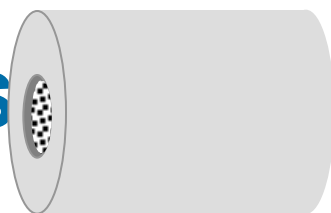
Waste





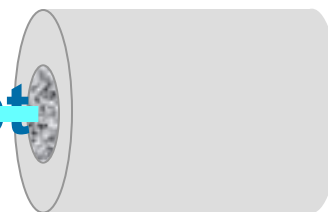
## Elute/analyse

Sorbent S



Sorbent sampling cartridge

concept

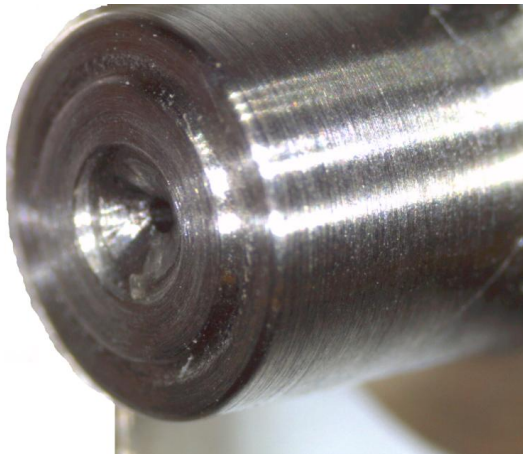
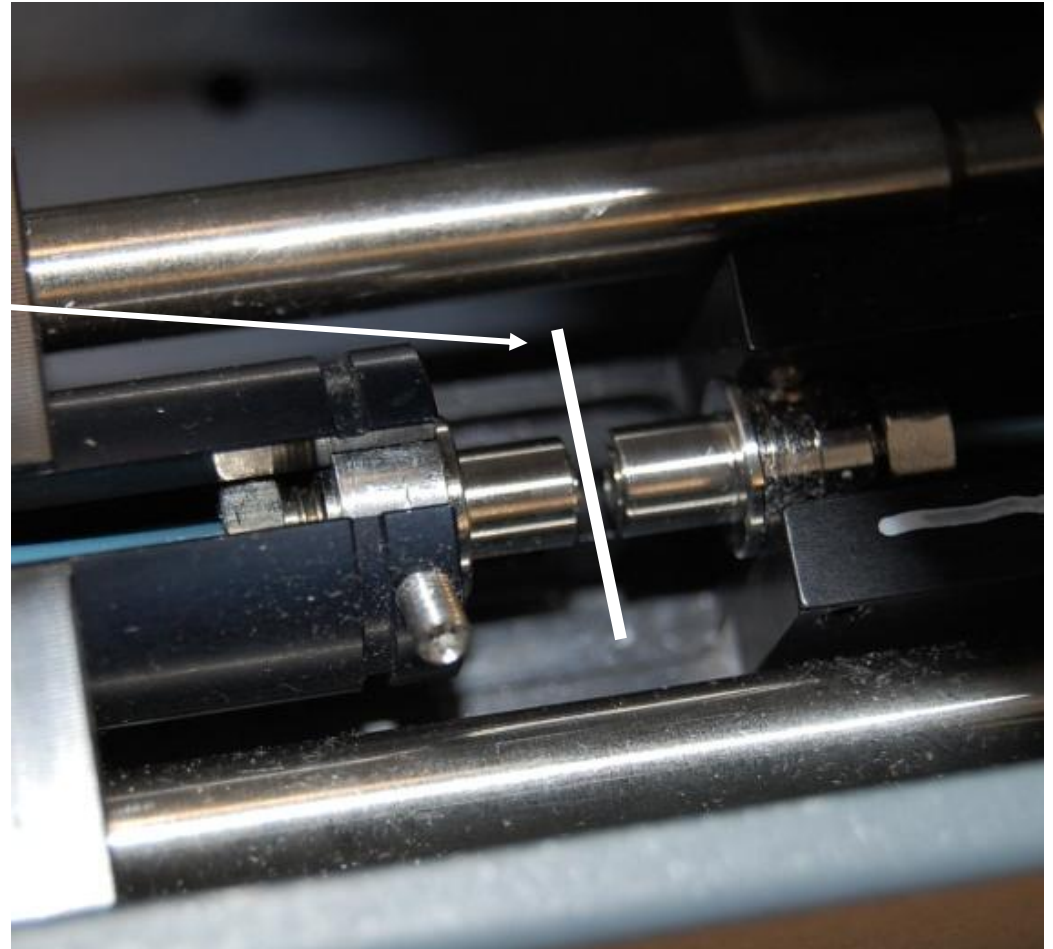


SPE cartridge



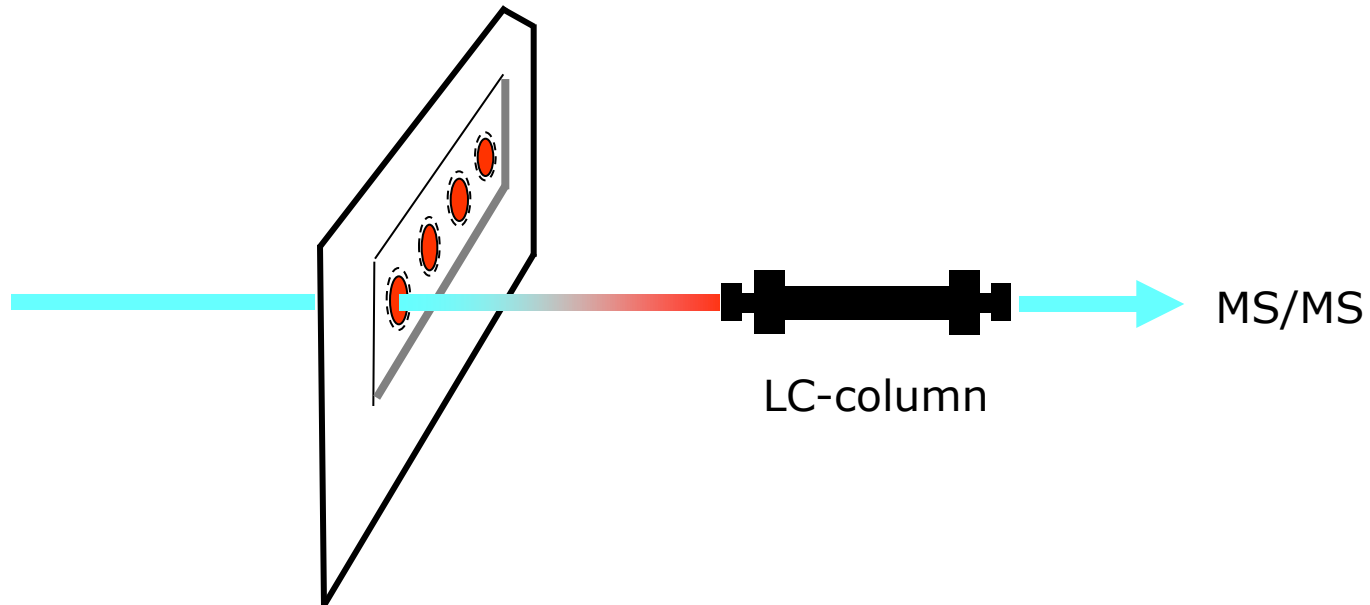
LC-column

# Dried Blood Spot analysis using on-line extraction of DBS cards

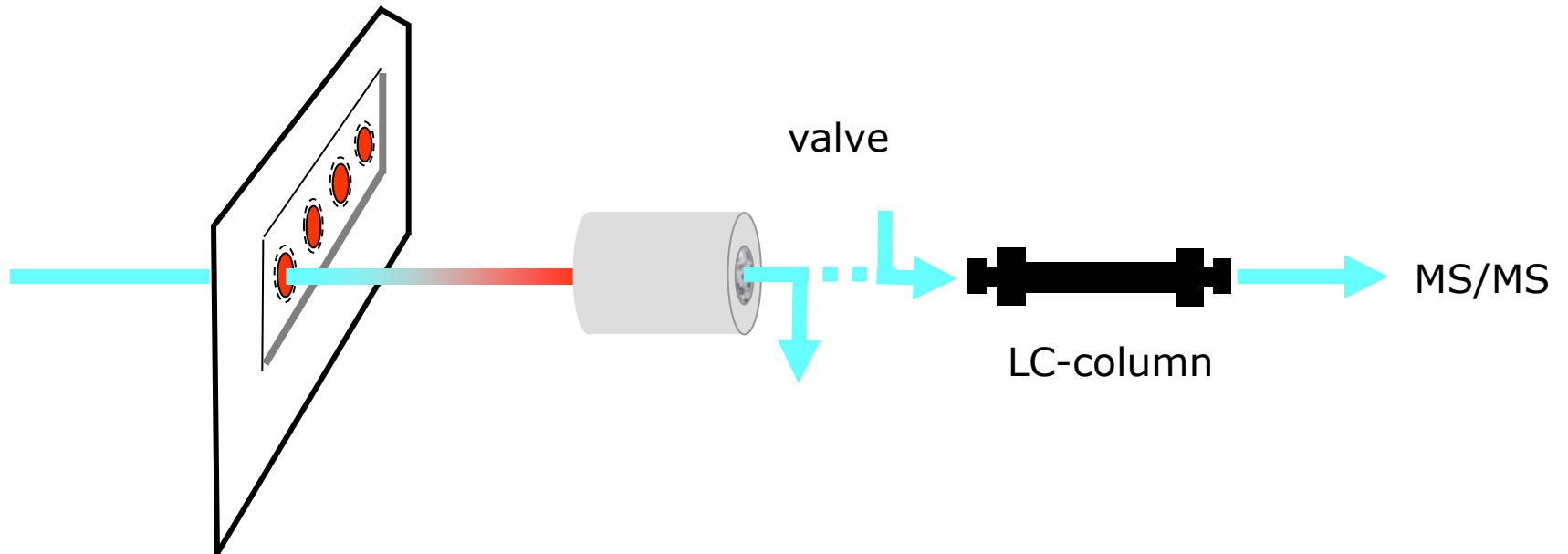


Patent pending

# On-line DBS extraction

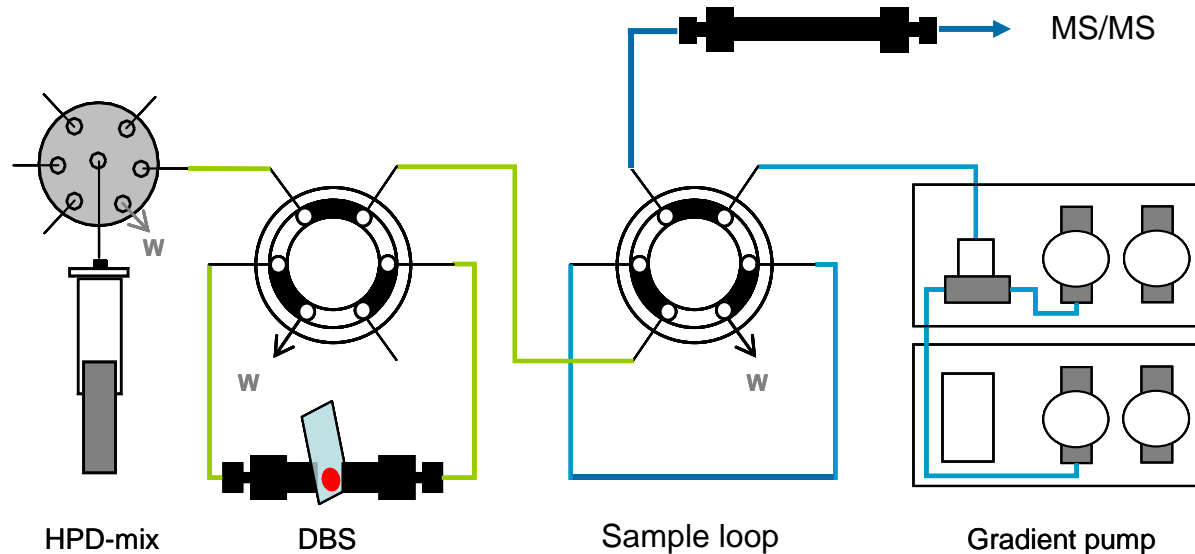


# Added SPE clean-up option



# On-line DBS extraction with loop interface

Feasibility test at GSK (\*)



## HPLC

Analyte: Sitamaquine  
Column: 50x2mm Varian  
Polaris C18A 5 $\mu$   
Eluent: 38% Acetonitrile  
62% 10mM  
Methyl ammonium  
acetate pH4.2  
Flow: 500  $\mu$ L/min  
Temp: 40° C

Validation samples eluted to loop with 35  $\mu$ L elution of 50/50 v/v methanol: water at 500  $\mu$ L/min

1. DBS containing analyte & IS; elute 50/50 MeOH: water.
2. DBS containing analyte; elute 50/50 MeOH: water containing IS

(\*) Philip Denniff, Paul Abu-rabie and Neil Spooner. GSK, Ware, UK (data presented at ASMS 2010)

# GSK results on-line DBS with loop interface



## 1. Analyte & IS Co-Spiked (\*)

Linearity

$$Y = 0.0313x + 0.0123$$

$$r = 0.9981$$

### Accuracy and precision

QC Conc (ng/mL)

	5	20	100	800	1000
	5.05	19.68	95.01	722.04	872.73
	5.09	19.76	91.65	732.96	883.89
	5.1	19.95	93.26	741	860.65
	5.23	19.79	95.7	739.64	841.69
	5.07	19.79	94.67	749.33	859.91
	4.85	19.98	94.18	757.92	846.72
Mean	5.07	19.83	94.08	740.48	860.93
CV	2.4%	0.6%	1.5%	1.7%	1.8%
Bias	1.3%	-0.9%	-5.9%	-7.4%	-13.9%

(\*) Philip Denniff, Paul Abu-rabie and Neil Spooner. GSK, Ware, UK (data presented at ASMS 2010)

# GSK results on-line DBS with loop interface



## 2. IS in Elution Eluent (\*)

Linearity

$$y = 0.00286x + 0.0355$$

$$r = 0.9971$$

### Accuracy and precision

QC conc (ng/mL)

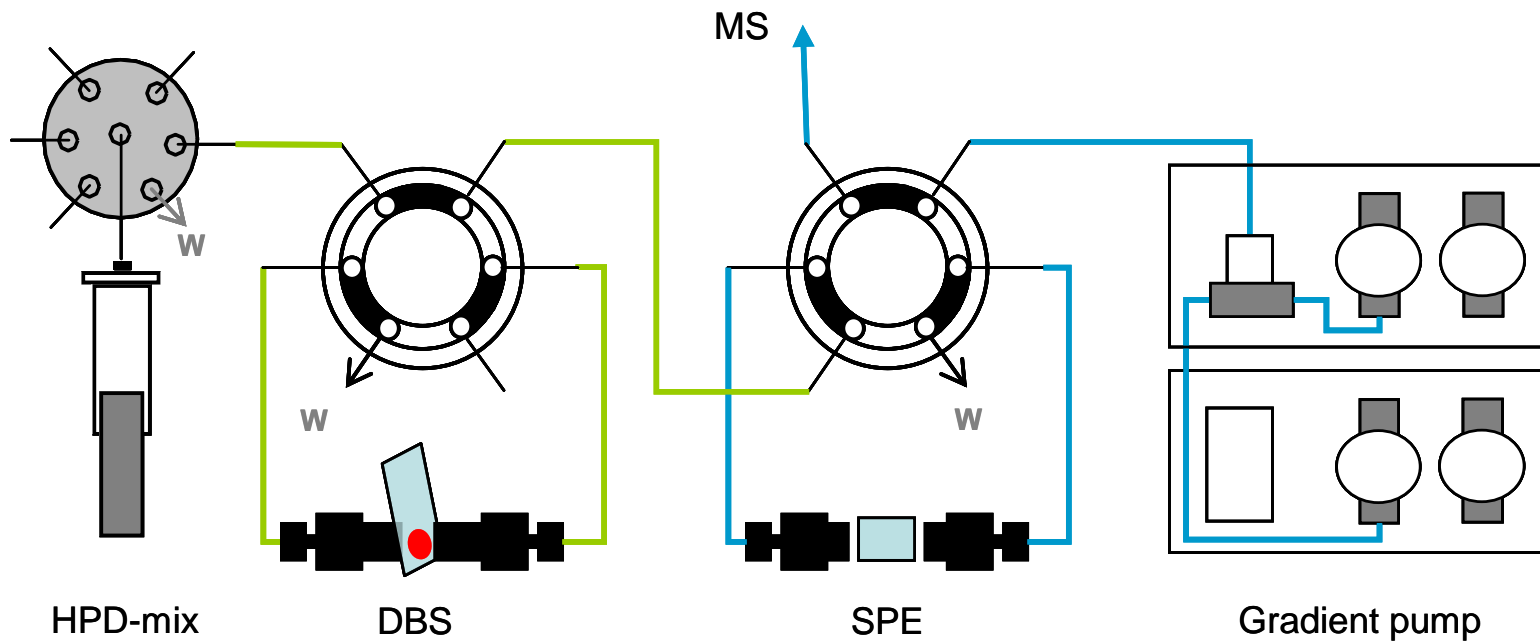
	5	20	100	800	1000
	4.9	17.17	90.6	750.73	[1745.38]
	4.49	18.85	87.02	757.62	966.45
	5.01	19.45	101	771.12	968.12
	4.2	17.88	87.34	735.91	893.94
	4.53	19.37	94.95	735.7	855.04
	4.36	18.64	101.62	704.71	866.91
Mean	4.58	18.56	93.76	742.63	910.09
CV	6.8%	4.8%	6.9%	3.1%	5.9%
Bias	-8.4%	-7.2%	-6.2%	-7.2%	-9.0%

IS half expected size

(\*) Philip Denniff, Paul Abu-rabie and Neil Spooner. GSK, Ware, UK (data presented at ASMS 2010)

# On-line DBS with SPE interface

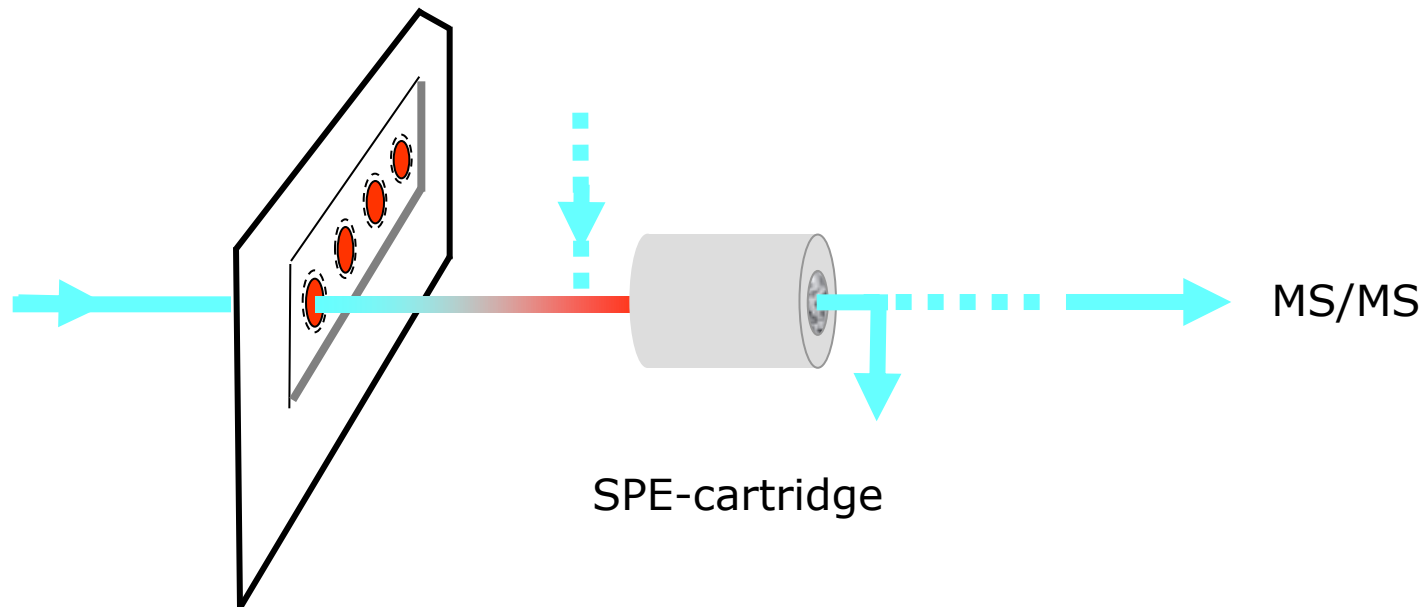
Feasibility testing at Spark Holland





# On-line DBS with SPE interface

Added clean-up on disposable “mini LC column”



# On-line DBS with SPE interface

## Experimental conditions

### DBS

Filter card: Whatman Protein Saver™ 903® Card  
Sample volume: 15 µL  
Sample matrix: Blood (Na<sub>2</sub>-EDTA) spiked with propranolol, haloperidol, amitriptyline, verapamil  
Desorption solvent: 1 mL water 0.2% FA at 2 mL/min (= sample transfer SPE)  
Clamp flush: 1 mL 80/20 acetonitrile/water 0.2% FA at 5 mL/min  
1 mL water 0.2% FA at 5 mL/min



### SPE

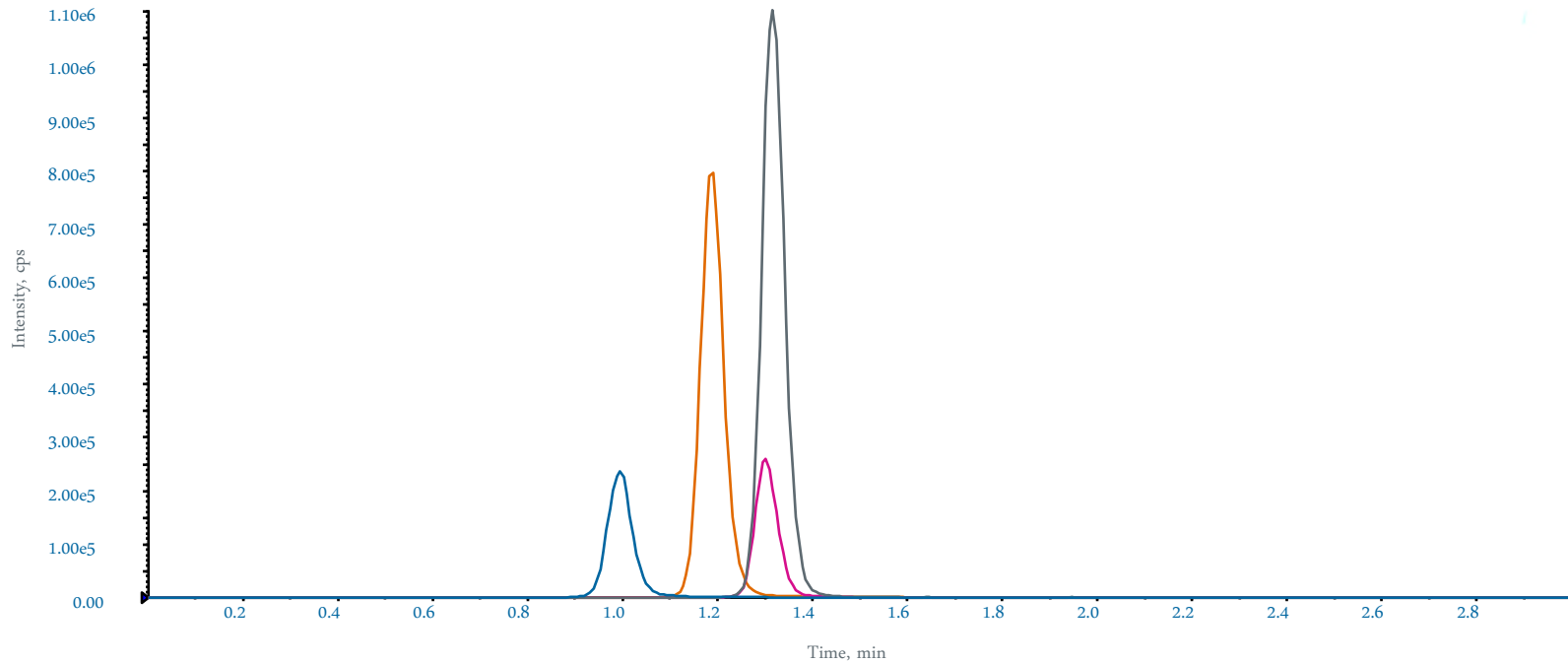
Cartridge: HySphere C18HD 10x2 mm  
Conditioning: 1 mL acetonitrile at 5 mL/min  
Equilibration: 1 mL water 0.2% FA at 5 mL/min  
Sample transfer: 1 mL water 0.2% FA at 2 mL/min  
Cartridge wash: 1 mL 5/95 acetonitrile/water 0.2% FA at 5 mL/min  
Elution: 3 min gradient  
A) water 0.2% FA; B) acetonitrile 0.2% FA  
Clamp flush: 1 mL 80/20 acetonitrile/water 0.2% FA at 5 mL/min

#### Gradient program:

time (m:s)	flow (mL/min)	A %	B %
00:01	1.0	95	5
00:05	1.0	95	5
01:35	1.0	60	40
01:45	1.0	60	40
02:00	1.0	95	5
03:00	1.0	95	5

# On-line DBS with SPE interface

## Results

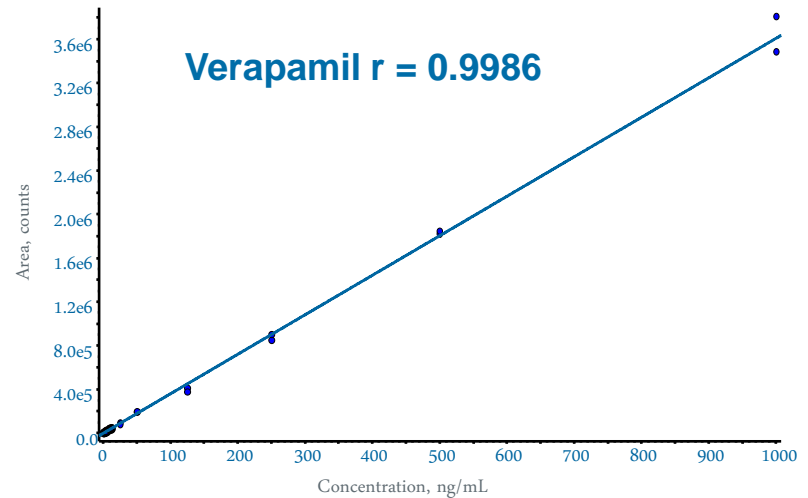
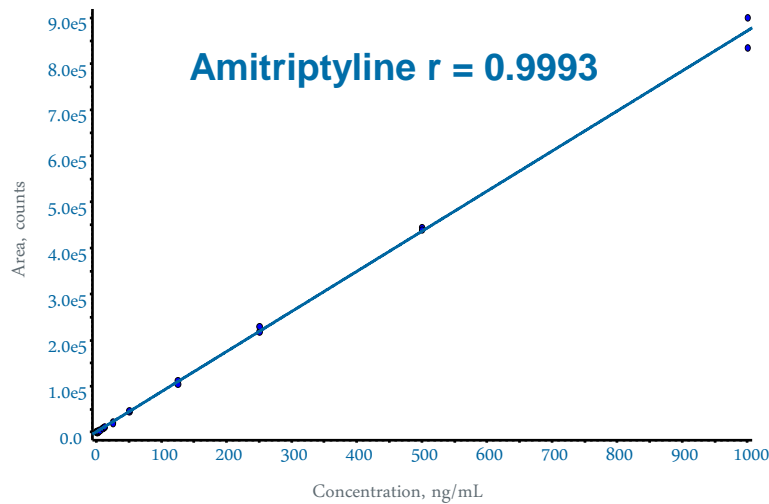
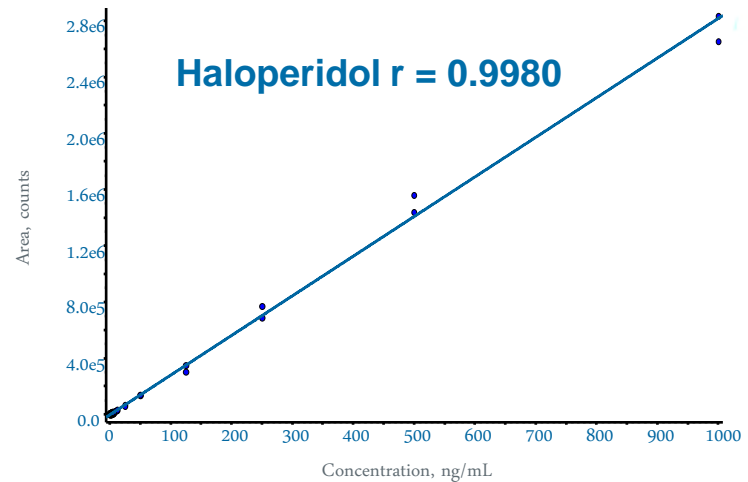
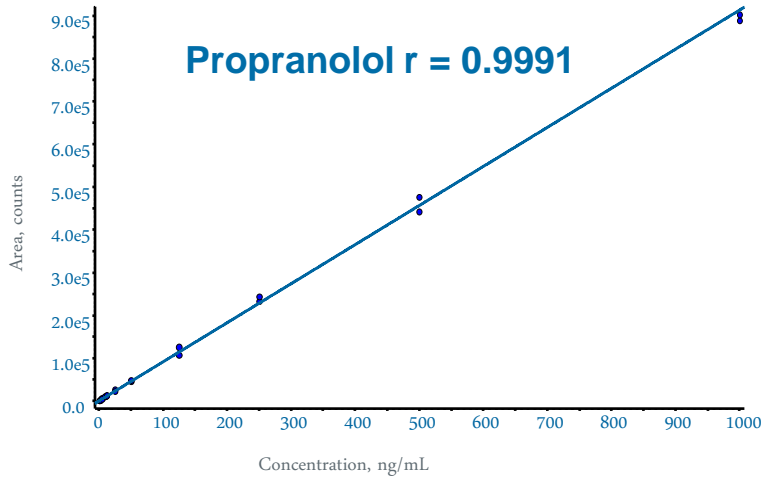


DBS-SPE-MS/MS chromatogram of **Propranolol**, **Haloperidol**, **Amitriptyline** and Verapamil in blood at highest limit of quantitation (1000 ng/mL)

# On-line DBS with SPE interface

## Results

linearity 1-1000ng (no internal standard used)



# On-line DBS with SPE interface

## Results



Precision and carry-over (spiked blood samples, no internal standard used)

Relative standard deviation (%) of DBS-SPE-MS/MS at low, medium and high concentration (n=9)

Concentration level	Propranolol	Haloperidol	Amitriptyline	Verapamil
Low (10 ng/mL)	4.11	5.48	6.84	7.20
Medium (400 ng/mL)	5.55	5.75	4.32	3.24
High (800 ng/mL)	5.38	4.44	5.09	6.12

Carry-over of DBS-SPE-MS/MS after analysis of high concentration sample (n=2)

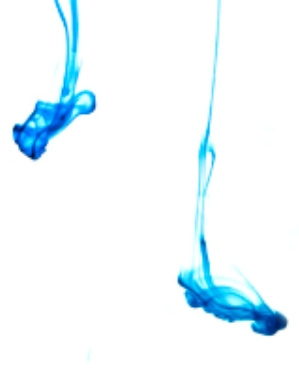
Sample	Propranolol	Haloperidol	Amitriptyline	Verapamil
Spiked blood (1000 ng/mL)	9.11E+05	2.95E+06	9.78E+05	3.80E+06
Blank blood	< LOD	1.08E+03	5.17E+02	< LOD
Carry-over (%)	n.d	0.04	0.05	n.d

LOD = limit of detection defined as 2 times peak to peak noise; n.d. = not detected

# On-line DBS with SPE interface

## Conclusion

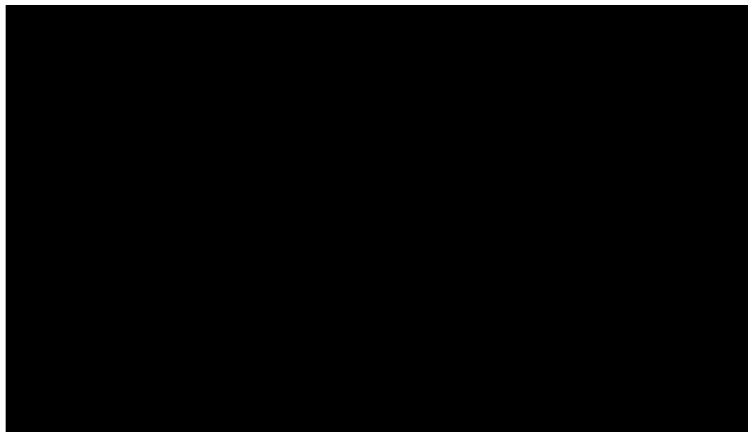
- Clean-up and LC separation combined in a single disposable SPE cartridge
- Good linearity over the 1-1000 ng/ml range ( $r > 0.998$ )
- RSD is well within the guidelines for bioanalysis (3.2 – 7.2%) without use of an internal standard
- and capable of analyzing at least 20 samples per hour
- Generic approach, easy to use



# What's next



Upgraded devices for easy testing the on-line DBS concept at various sites



Fully automated DBS card selection from stacker, identification, inspection and on-line extraction

# Acknowledgement

## GSK:

Neil Spooner

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## SparkHolland:

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