Towards unbiased Dried blood spot analysis using temperature-enhanced flow-through desorption coupled online to solid-phase extraction and mass spectrometry

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"The hematocrit can have an effect on the size of the blood spot, on spot homogeneity and on extraction recovery in a compound-dependent manner" (*)

(*) The effect of hematocrit on bioanalysis of DBS: Results from the EBF DBS-microsampling consortium
Ronald de Vries, Matthew Barfield, Nico van de Merbel, Bernhard Schmid, Christoph Siethoff, Jordi Ortiz, Elwin Verheij, Ben van Baar, Zoe Cobb, Steve White & Philip Timmerman; *Bioanalysis* (2013) 5 (17)

"Going forward, the EBF considers DBS as a developing technology, and awaits further innovations and improvements to better balance the advantages of the technique versus its current limitations" (*)

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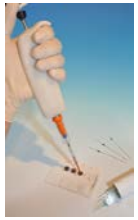
Outline

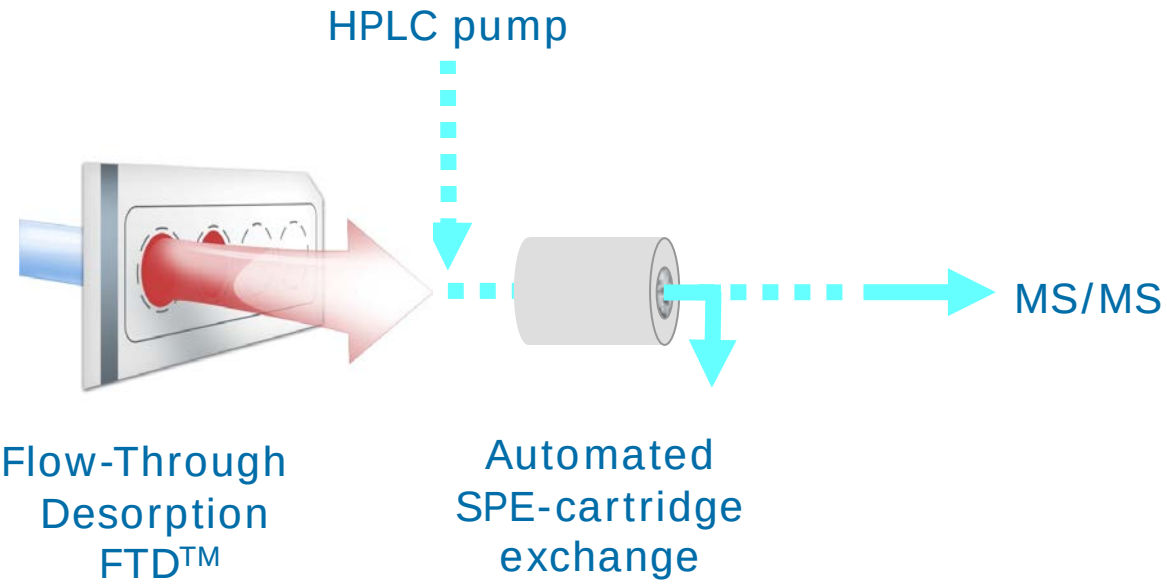
- Flow-Through Desorption (FTD) of DBS

- Evaluating full-spot DBS analysis



- Maximizing recovery to reduce the Ht effect

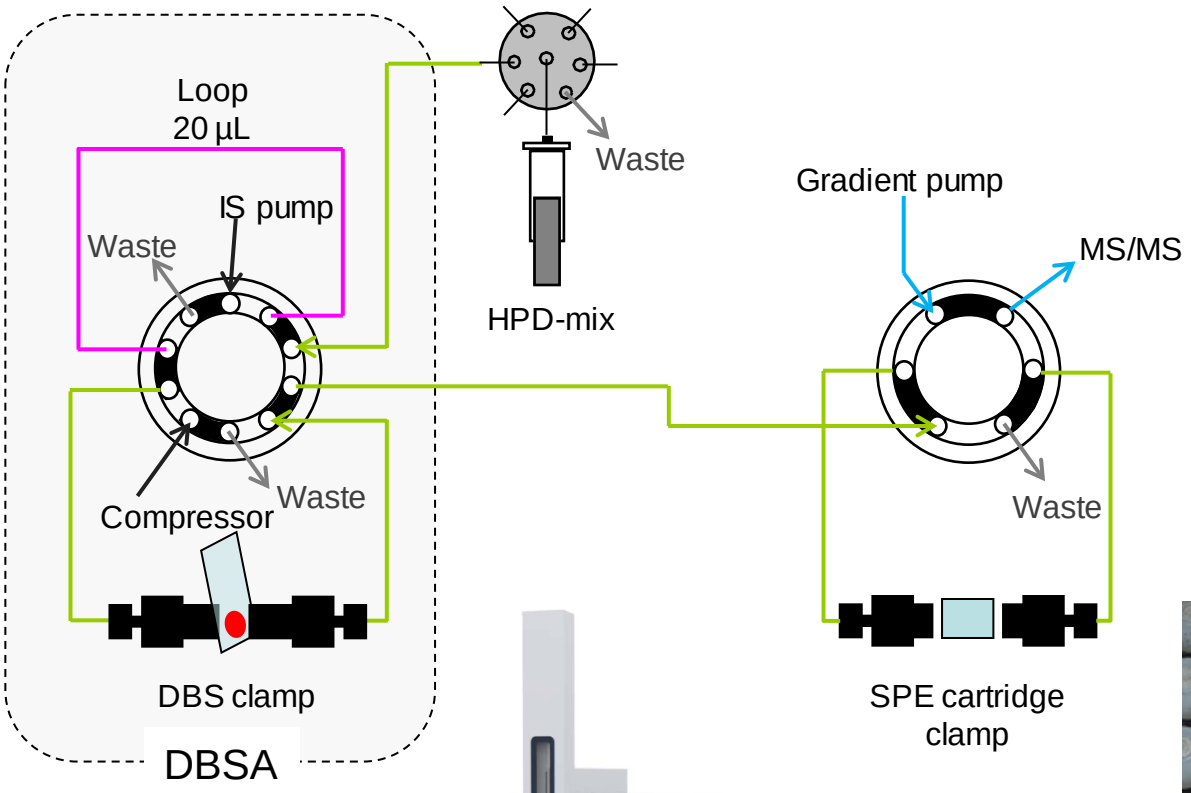
Online FTD-SPE-MS/MS – concept (*)



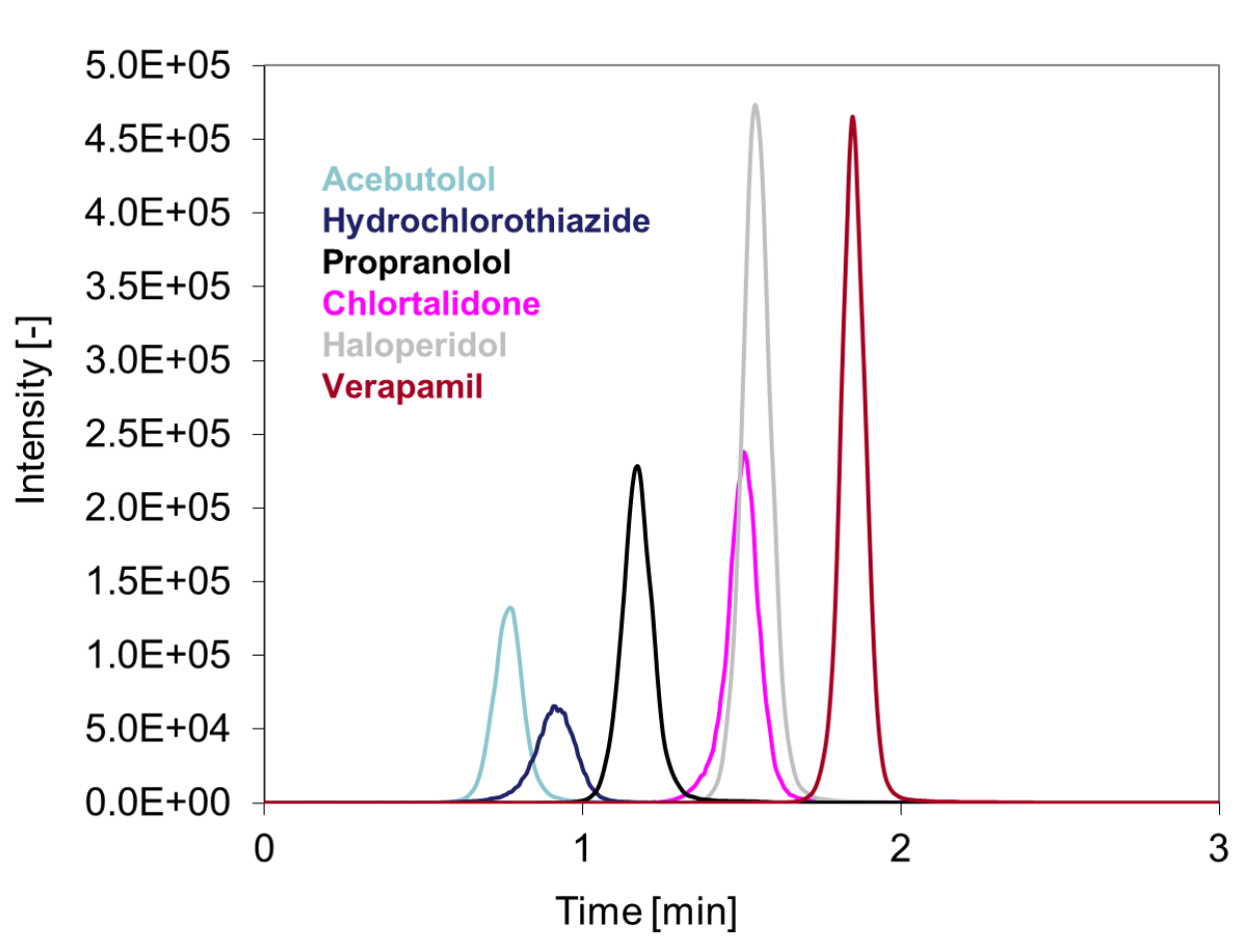
Clean-up and LC separation on a single high performance SPE cartridge

[*] Ooms JA, Knegt L, Koster EHM, Bioanalysis, October 2011,

Online FTD-SPE-MS/MS – System configuration



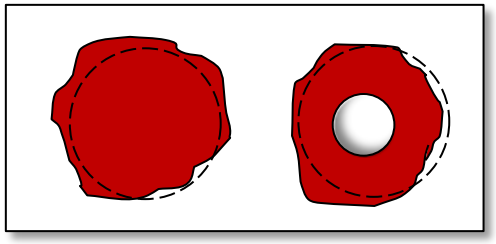
Online FTD-SPE-MS/MS - example



Overlay of pos and neg ESI chromatograms (ABSciex API 4000)

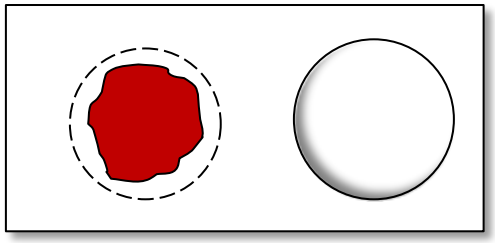
Ht effect in partial-spot versus full-spot analysis

Partial-spot analysis



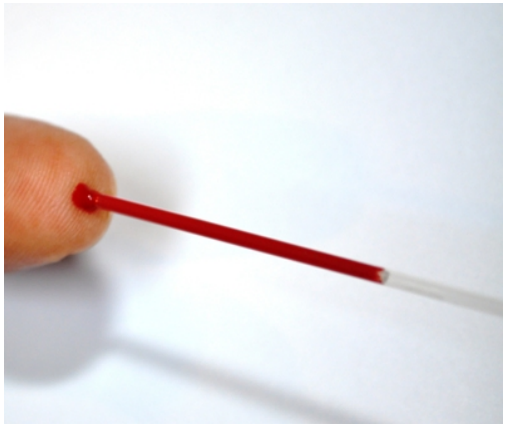
- Homogeneity issue
- Ht effect on punched blood volume
+ Simple blood application

Full-spot analysis



+ No homogeneity issue
+ No Ht effect on punched volume
- Accurate sample application required

Prototype multi-dispenser

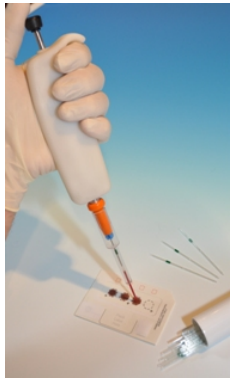


Acknowledgement:
Joe Siple and Jim Kenney,
Drummond Scientific, Broomall, PA

5 µL full-spot online FTD-SPE-MS/MS

5 capillaries were used to spot 4x5 µL blood (spiked with verapamil) on DBS cards (i.e. 4 spots from one capillary) using the Prototype Drummond Multi-dispenser. Spots were analyzed by FTD-SPE-MS/MS

Capillary	Precision %RSD 4 spots per capillary	Overall Precision %RSD 20 spots, 4 spots per capillary
1	3.3	3.4
2	2.5	
3	1.4	
4	5.2	
5	3.7	



"In case of punching the entire blood spot it still needs to be assured that the absolute recovery is the same at different hematocrits in both fresh and aged DBS" (*)

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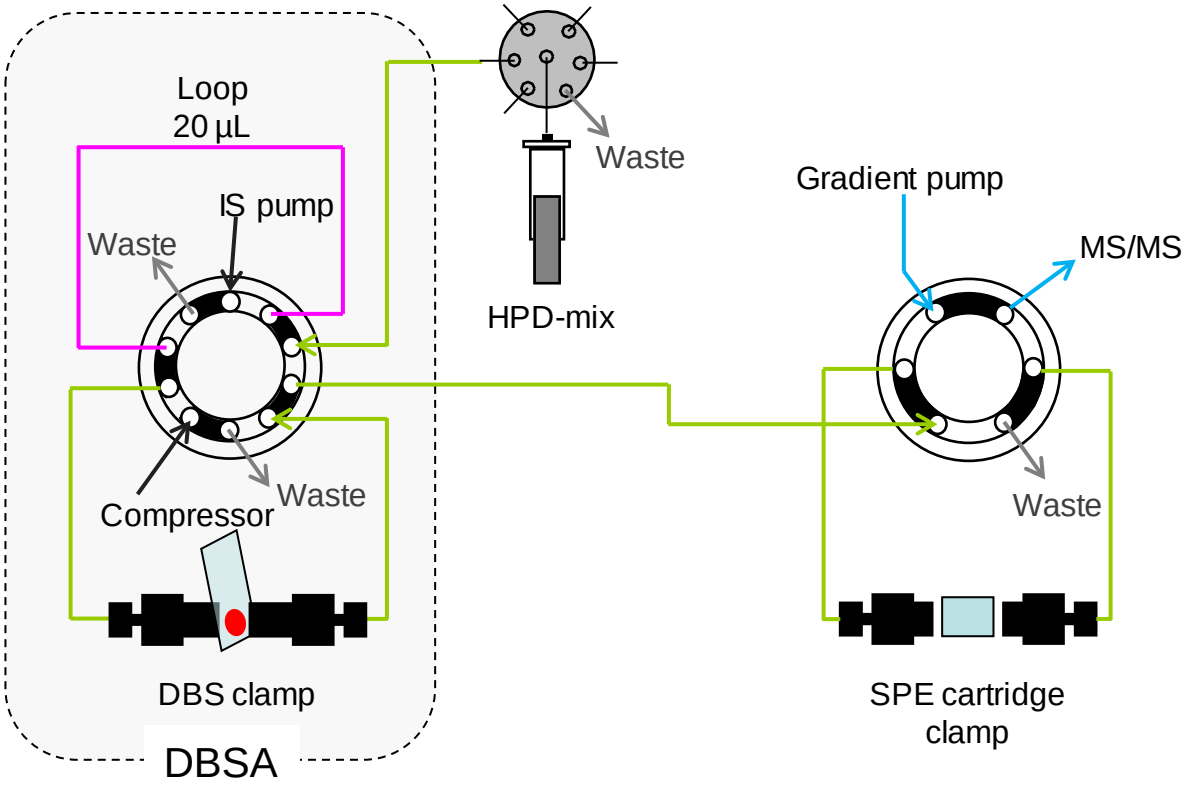
Measuring DBS desorption recovery

Compare results of 2 runs:

1. Analysis of 5-µL spiked DBS; loop filled with blank solution
2. Analysis of 5-µL blank DBS; calibrated loop filled with analyte standard mixture (as 100% recovery reference)

Note that both analyses have identical SPE breaktrough and matrix effects

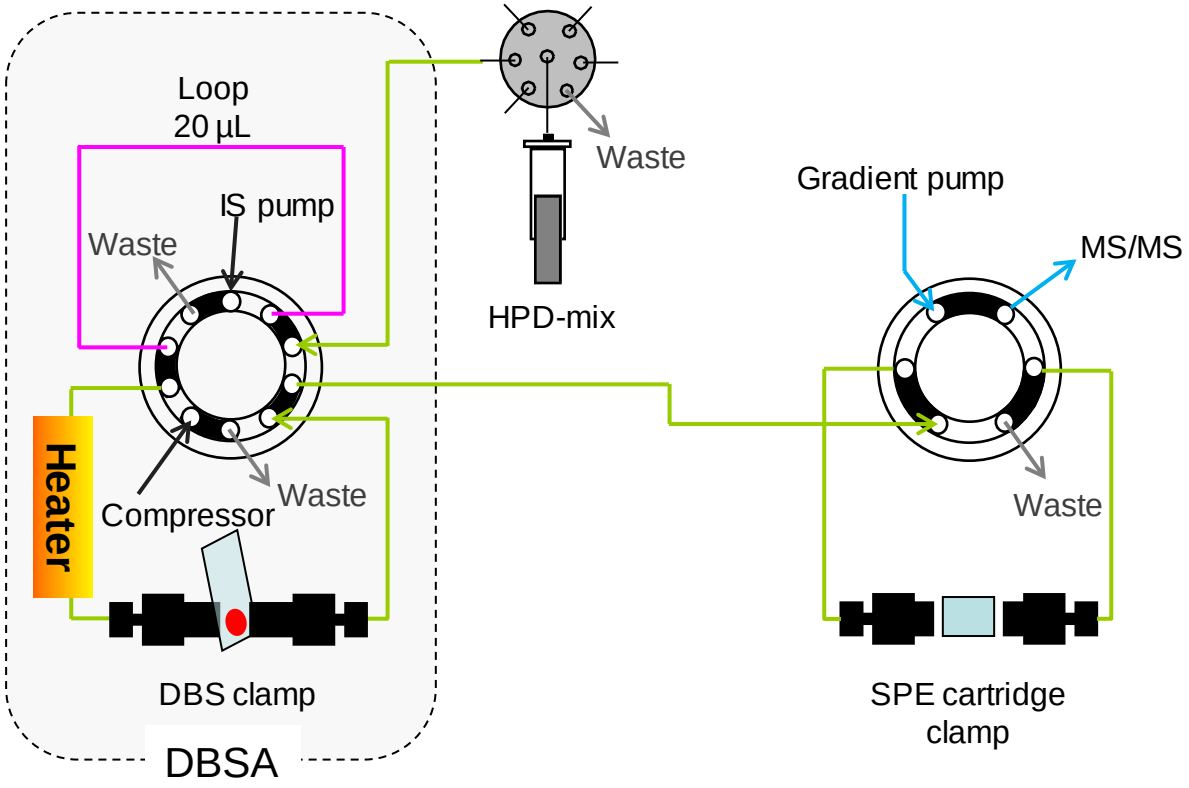


Effect of hematocrit on recovery

% Recovery difference Ht 0.7 vs Ht 0.3 (n = 3)	
Propranolol	-13
Haloperidol	- 22
Amitriptyline	-12
Verapamil	-14

2-stage desorption with 1 ml H2O 0.2% FA followed by 1 ml 5% ACN 0.2% FA

Online FTD-SPE-MS/MS with heated desorption



Temperature in DBS clamp: ~ 80^{o}C

Effect of Ht on recovery using heated desorption

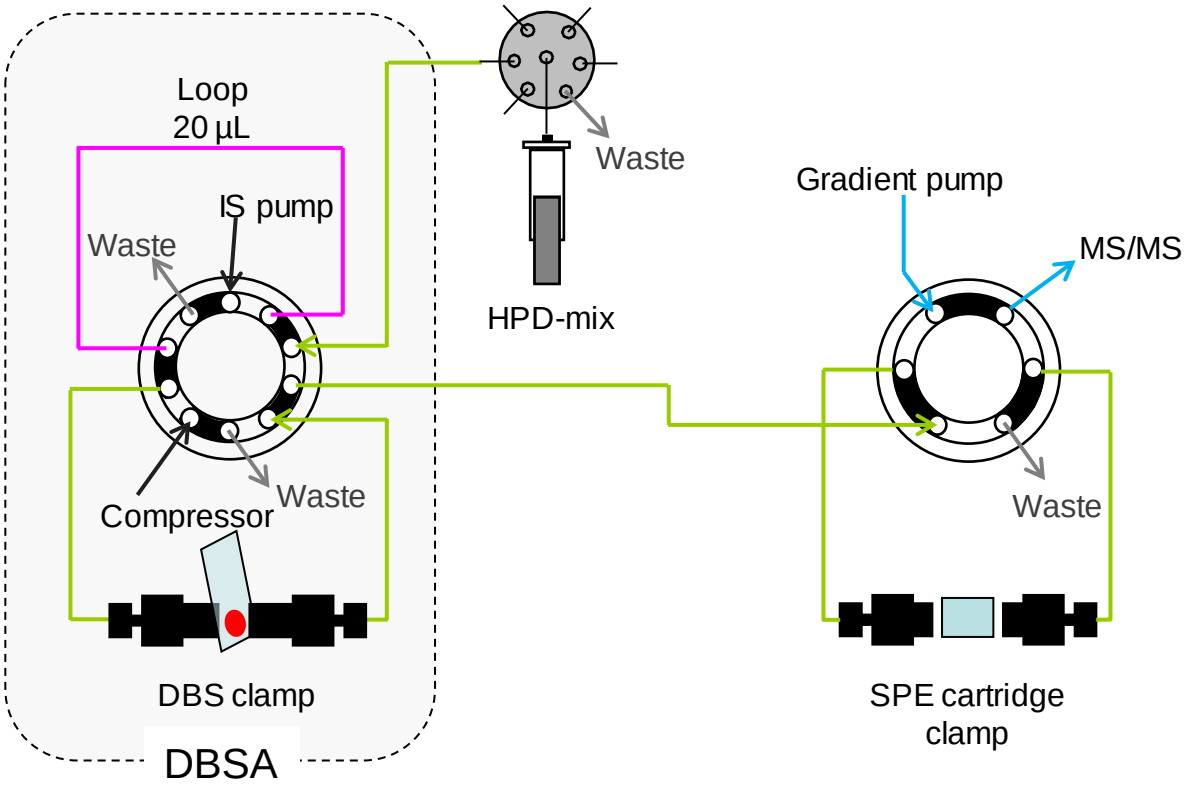
% Recovery (n = 3)			
Compound	**Ht 0.3**	**Ht 0.45**	**Ht 0.7**
Chlortalidone	96.8	96.0	97.2
Hydrochlorothiazide	95.6	103.6	92.6
Acebutolol	98.5	95.8	94.5
Haloperidol	101.1	96.9	88.9
Verapamil	106.7	101.3	99.7
Propranolol	91.0	104.3	107.9

- “100%” recoveries
- Independent on Ht

Measuring matrix effects

Inject a standard mixture of analytes with the 20 µl loop and compare results for:

1. 5-µL blank DBS
2. a blank card (as ref for 100% signal i.e. zero suppression)



Matrix ionization effects

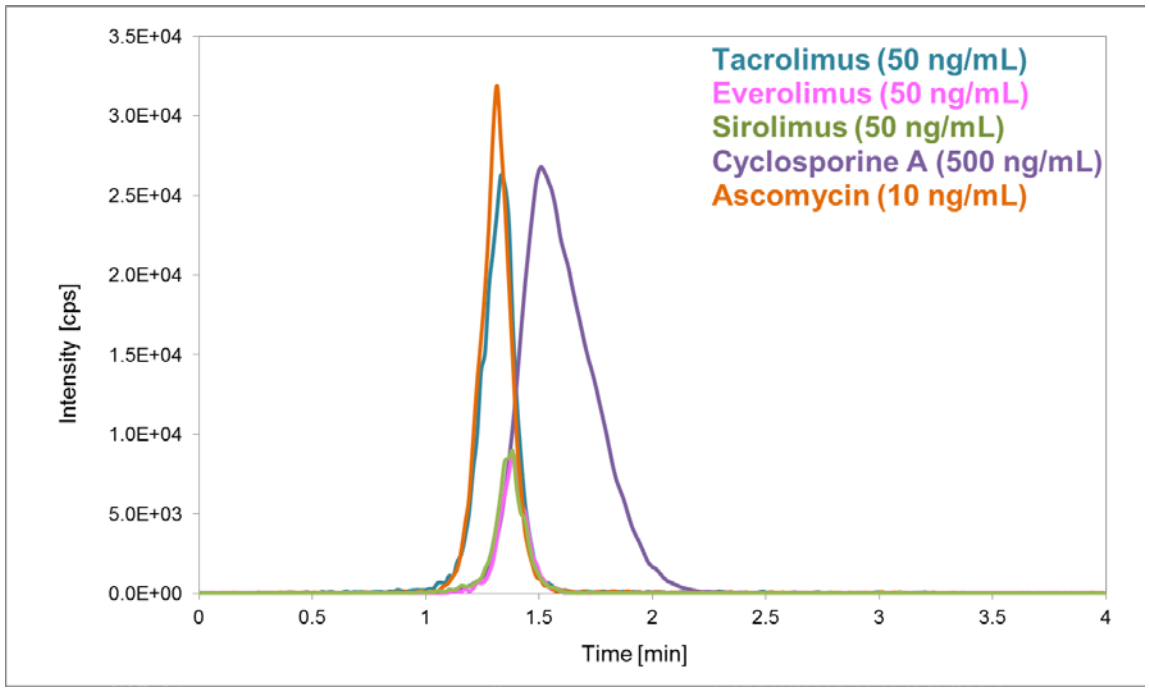
(Using heated desorption solvent)

Compound	Matrix Effect % signal enhancement/suppression n = 3
Chlortalidone	- 16.7
Hydrochlorothiazide	+ 2.7
Acebutolol	+ 9.9
Haloperidol	+ 5.4
Verapamil	- 11.6
Propranolol	- 9.6

Single I.S. would probably be OK

DBS analysis for Immunosuppressant drugs

- 5-µL DBS (Perkin Elmer 226 Bioanalysis Card)
- clamp head ∅ 6 mm → full-spot desorption
- FTD: 1 mL water 0.2% formic acid at 2 mL/min
  1mL 40% methanol 0.2% formic acid at 2 mL/min
  ⇒ Heater ON



Online FTD-SPE-MS/MS example chromatogram

Acknowledgement:
Jac van der Heijden (DBS Lab, Geleen, Netherlands) for providing the immunosuppressants

Effect of Ht on recovery – heated desorption

Recovery (%)	heater (OFF) vs heater ON		
	Ht 0.25	Ht 0.45	Ht 0.65
Cyclosporine A	(88.6) 88.9	(84.9) 88.4	(53.7) 83.6
Everolimus	(26.1) 67.8	(22.1) 72.4	(24.4) 72.5
Sirolimus	(20.4) 59.5	(16.0) 62.5	(13.5) 67.0
Tacrolimus	(42.4) 78.5	(36.6) 79.9	(34.8) 76.2

- Higher recoveries
- Less dependent on Ht

Matrix Effects

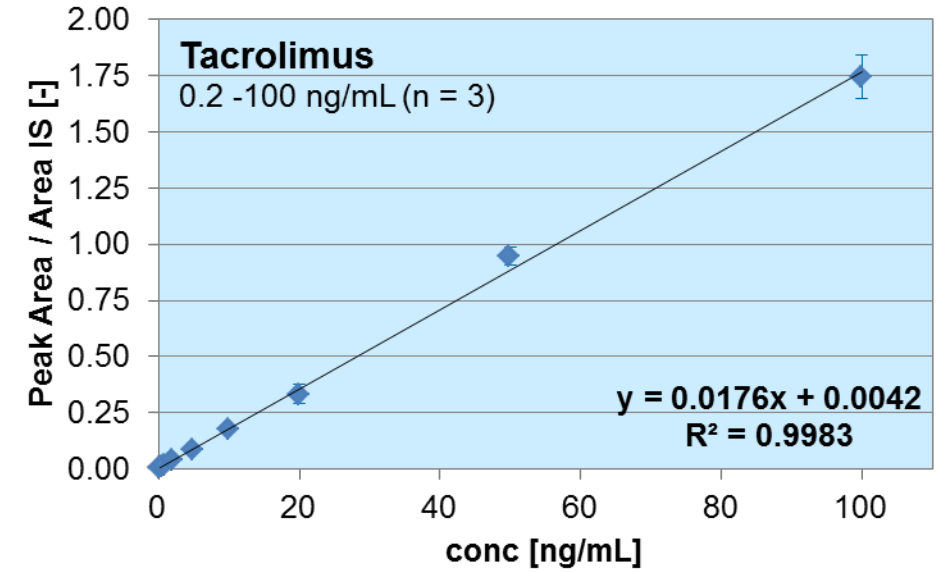
Matrix Effect as % signal enhancement / suppression (n = 4, heater ON)		
	Without I.S.	Using deuterated I.S.
Cyclosporine A	- 54.5	12.5
Everolimus	-27.7	3.5
Sirolimus	-26.6	-0.7
Tacrolimus	-42.3	1.3

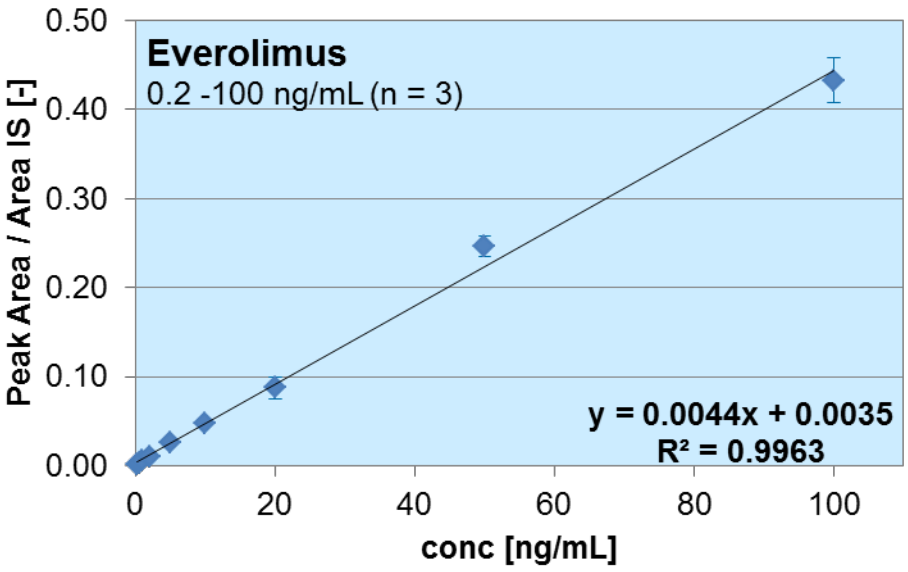
First validation results

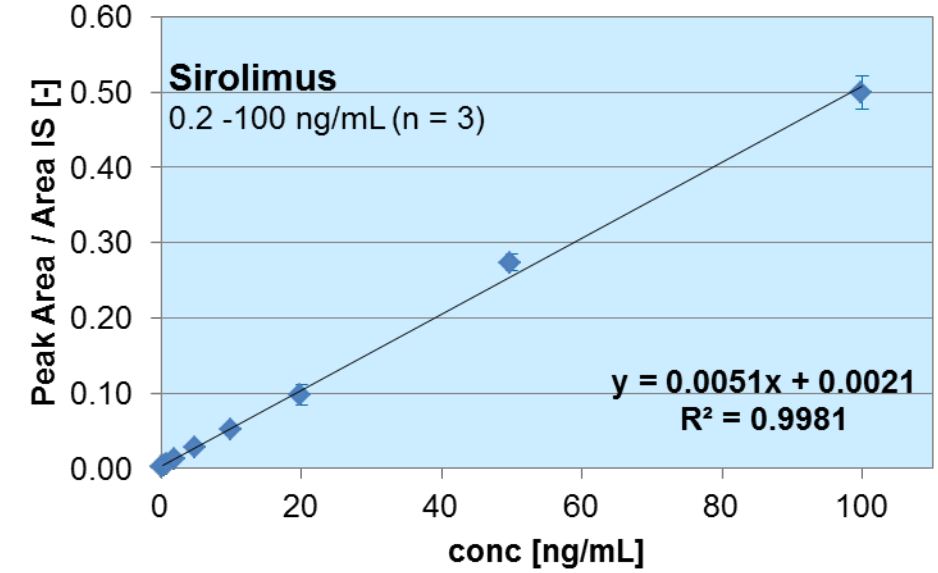
Precision and Accuracy (n = 6)			
Analyte	**Concentration ng/mL**	**Precision RSD %**	**Accuracy %**
Tacrolimus	1	4.0	85.3
	5	4.3	106.7
	50	3.4	98.3
Everolimus	1	6.3	85.4
	5	3.3	102.7
	50	2.6	100.8
Sirolimus	1	3.8	110.7
	5	4.0	100.5
	50	4.7	97.9
Cyclosporine A	2	10.3	108.8
	50	4.0	97.0
	500	5.6	99.6

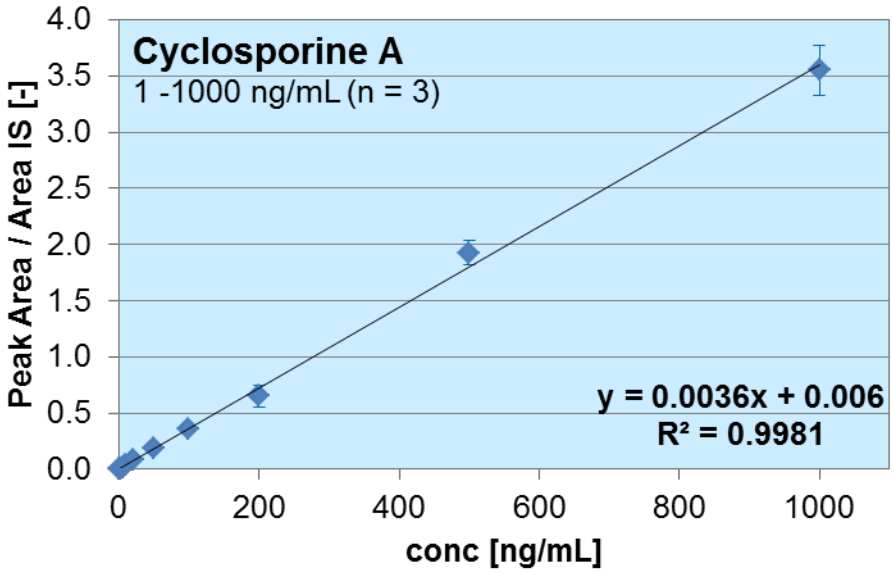
Using deuterated internal standards

Calibration curves









Conclusion

- Flow-through desorption of DBS coupled online to SPE-MS/MS enables simple automation of the entire DBS workflow.

- A prototype multi-dispenser for application of 5 µl blood spots enables reliable full-spot DBS analysis. Eliminating the homogeneity issue and Ht effect on punched blood volume.

- Maximizing recovery using heated desorption significantly reduces hematocrit effect on recovery.

- Going forward: further improve ease of use of the multi-dispenser and validation with patient samples