

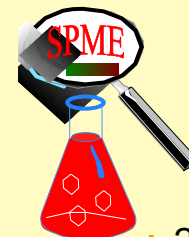
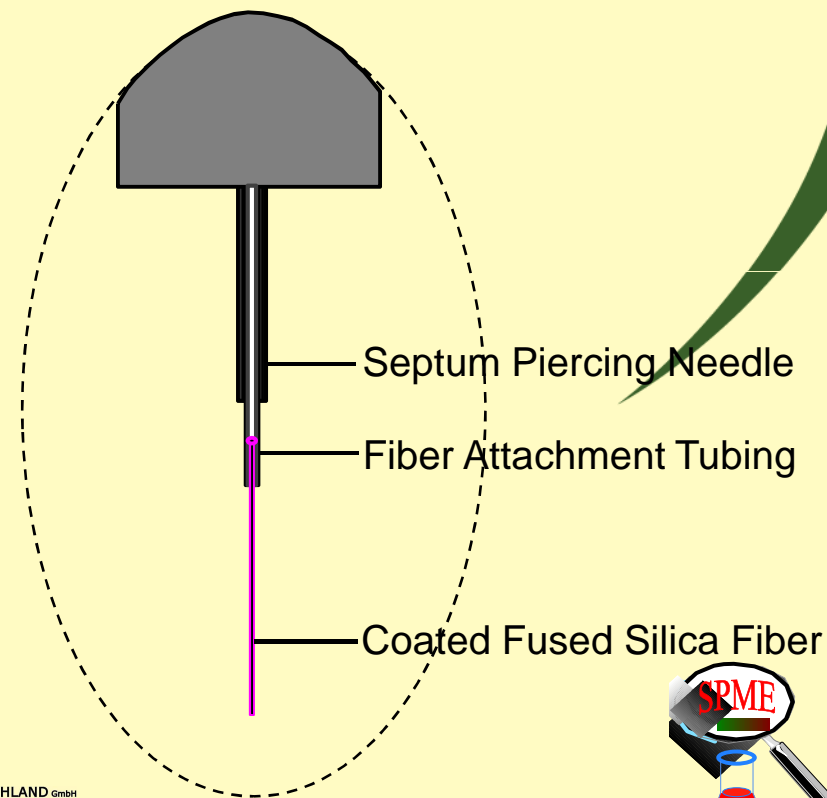
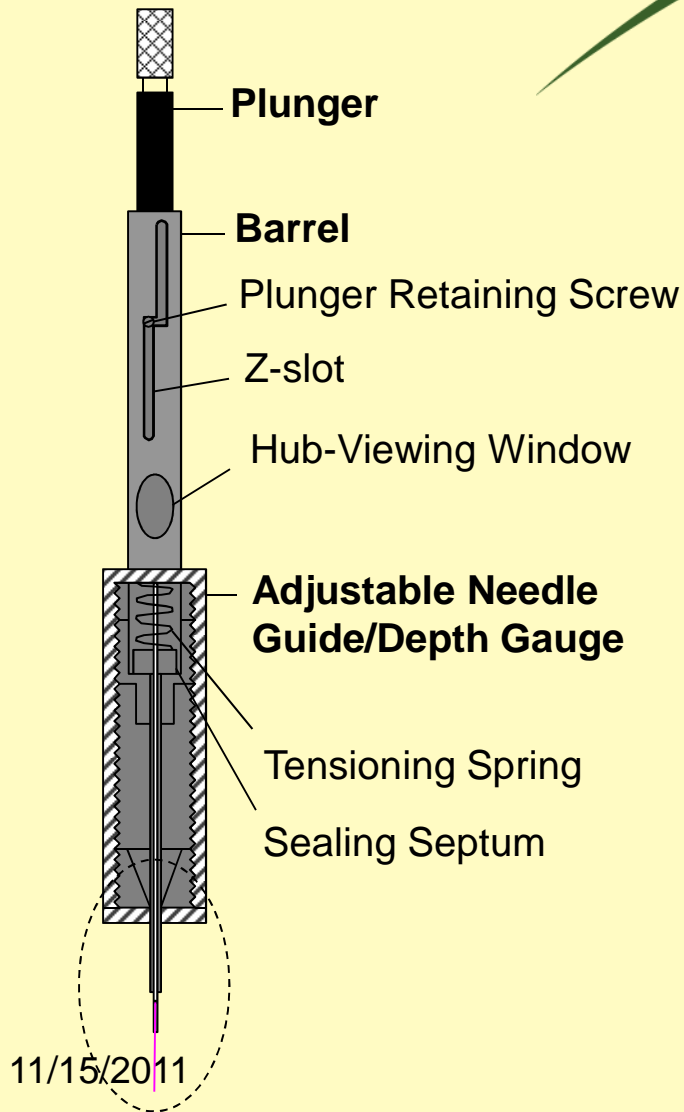


AUTOMATION OF LC/SPME-COUPPLING IN 96-WELLPLATE FORMAT

**Dietmar Hein
PAS Technology Deutschland GmbH**

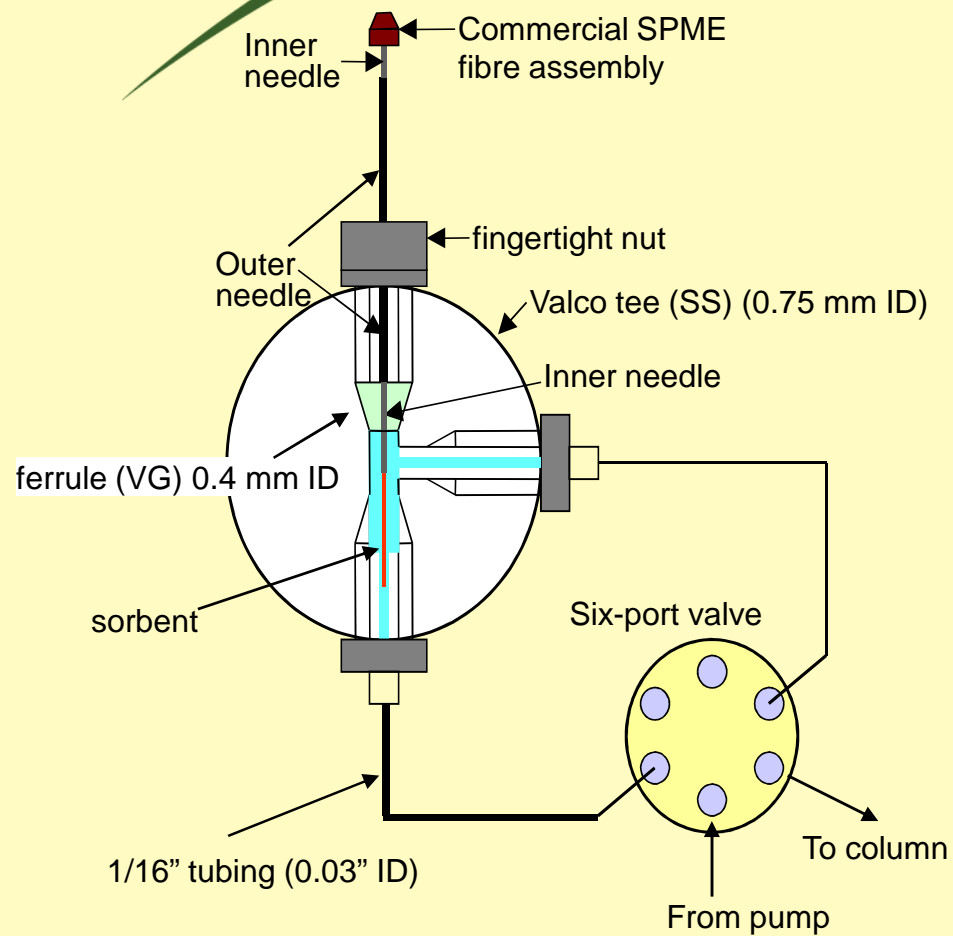
Professional Analytical Systems

Commercial Design of the SPME Device by Supelco



One step sample prep!

Schematic of Manual Interface



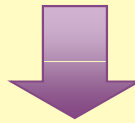
LC-SPME coupling

SPME-LC INTERFACES AND AUTOMATION IN THE PAST

SPME-LC INTERFACE	AUTOMATION	SAMPLE THROUGHPUT
MANUAL INTERFACE	LOW	LOW
IN-TUBE SPME	HIGH	LOW
OFF-LINE DESORPTION	LOW	HIGH

LC-SPME coupling

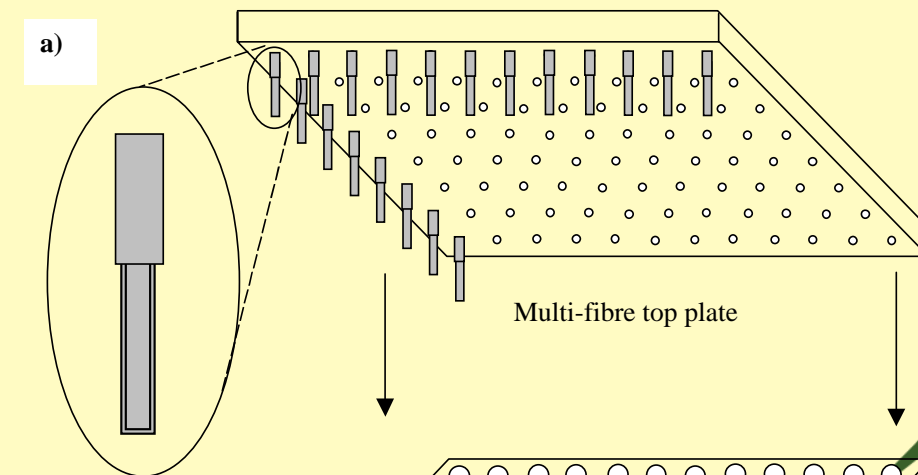
**ACHIEVE HIGH DEGREE OF AUTOMATION
AND HIGH SAMPLE-THROUGHPUT**



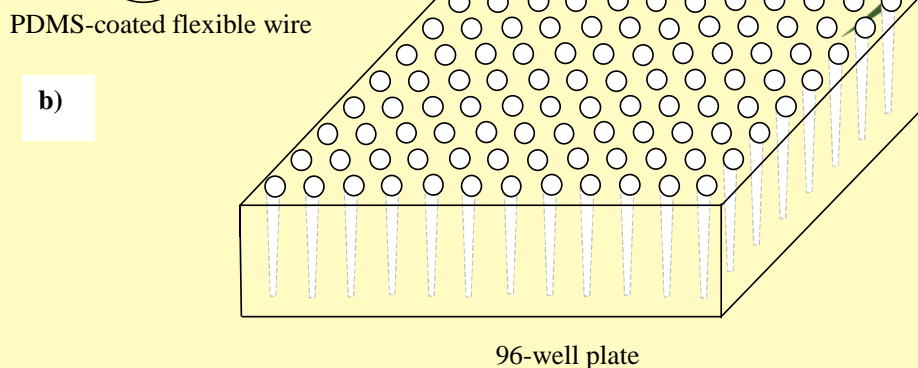
**PERFORM EXTRACTION AND
DESORPTION OF MANY SIMILAR
SAMPLES IN PARALLEL**

Fibre Multiwell System

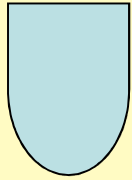
a) For simplification one row and one column of SPME fibres are shown to be inserted into the top plate.



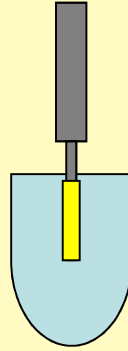
b) The multi-fibre top plate can then be placed into a commercial multi-well plates for extraction and desorption.



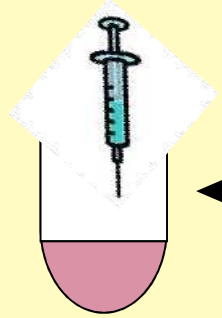
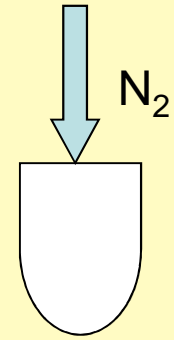
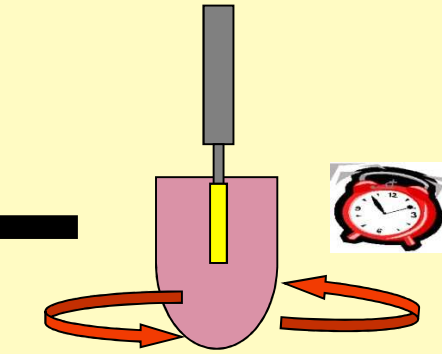
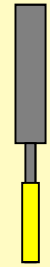
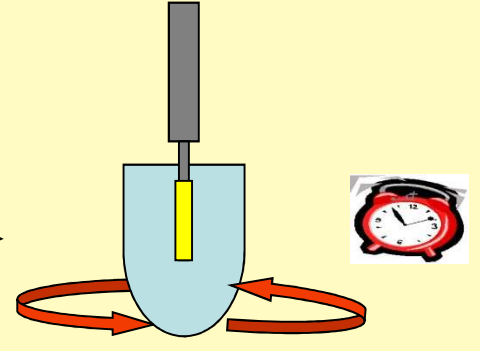
Well filled with sample



Insert SPME fibre for extraction



Agitate well until equilibrium is reached



Remove fibre from well

Desorb in well filled with solvent

Evaporate solvent

Reconstitute and inject into LC



Advantages of SPME

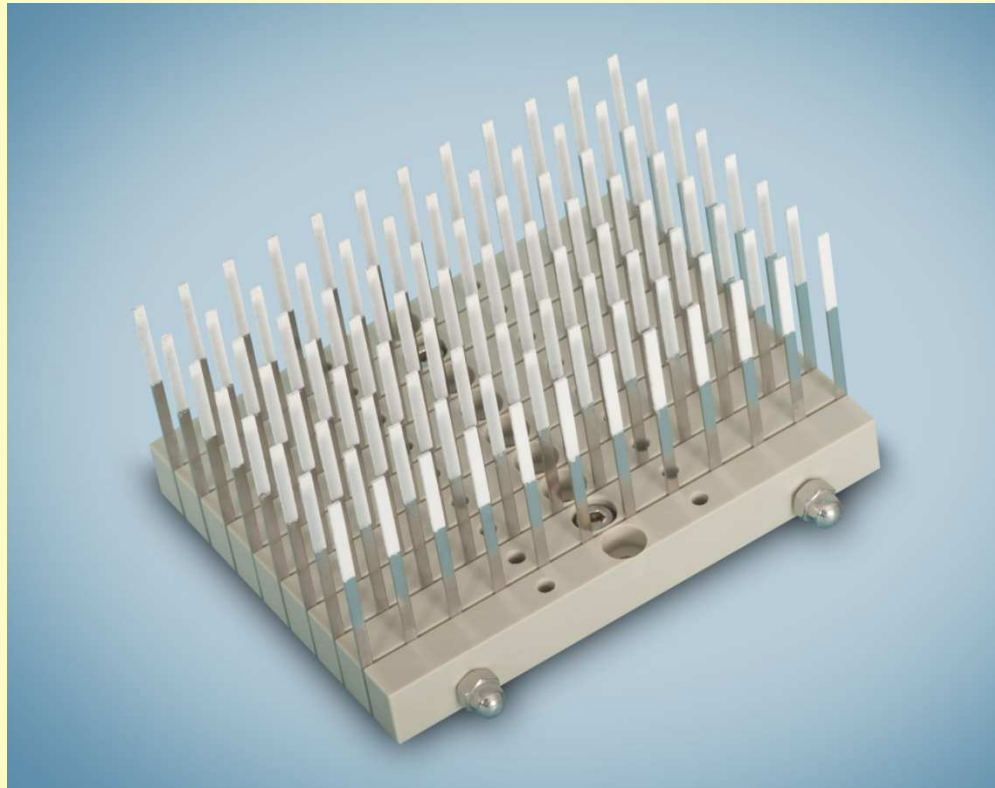
$$n = K_{fs} V_f C_0$$

- no further sample preparation!!!
- increasing sensitivity by increasing V_f !!!

$$V_f = d_f \times A_f$$

increased area improves sensitivity and extraction rate!!!

The “Brush”



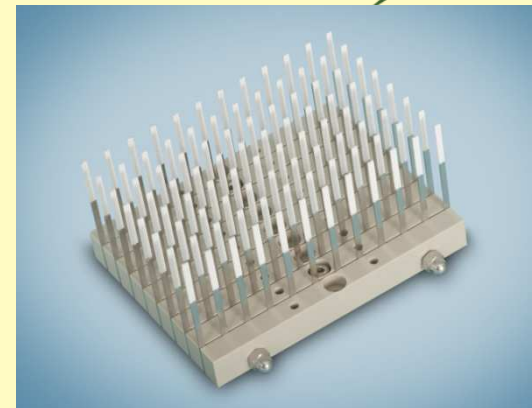
11/15/2011



CONCEPT 96

- Pre-washing/conditioning of fiber/membrane in well-plate
- Extraction (variable time setting) of analytes using a PAN/C18 coated blade device
- Washing of fiber/membrane prior to desorption
- Desorption (variable time setting) of analytes from coated blade device into different solvent system
- Evaporation of solvent – pre-concentration/re-constitution step (depends on type of solvent been used for desorption)
- (Injection)

CONCEPT 96



CONCEPT 96

- **4 stepper-motor driven axis (X, Y, Z, Z1)**

Axis	max. range	step width
Main axis Y	560 mm	0.250 mm
Arm axis X	170 mm	0.030 mm
Injector axis Z	160 mm	0.150 mm
Syringe piston Z1	70 mm	0.047 mm

- **2 pneumatically driven axis**

EXTRACTION-Tool (96 fiber „Brush“)
DRYING-Tool („Shower-Head“)

- **Up to 4 agitators (Conditioning, Extraction, Washing, Desorption/Evaporation), optionally heatable, (0 – 1.700 rpm)**

CONCEPT 96

Available coatings:

- C18-polyacrylonitrile (C18-PAN)
- Polystyryne-divynyl benzene- polyacrylonitrile (DVB-PAN)
- Phenylboronic acid-polyacrylonitrile (PBA-PAN)
- Methacryloyloxy ethyl phosphorylcholine (MPC)
- Chromabond Easy/PAN
- LC Diol/PAN
- C18SCX/PAN

CONCEPT 96

Method for determination of reusability and reproducibility

PRECONDITIONING
PAN-C18/ -PS-DVB

30 min

MeOH/water (1/1)

EXTRACTION

60 min, 1000 rpm

Wash

15 seconds

Purified water

Sample Prep. Time:
130 minutes!!

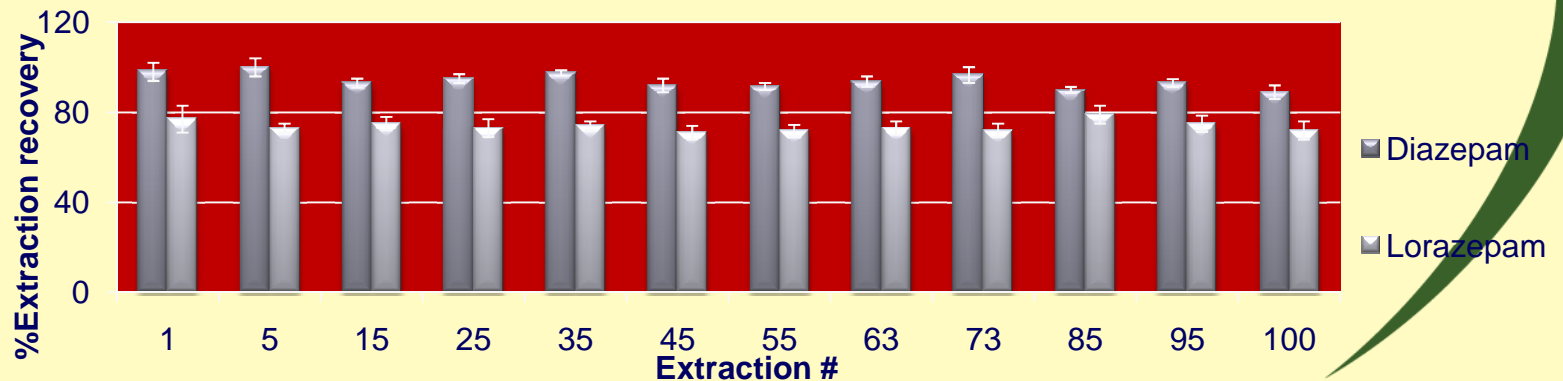
DESORPTION

40 min 120 rpm

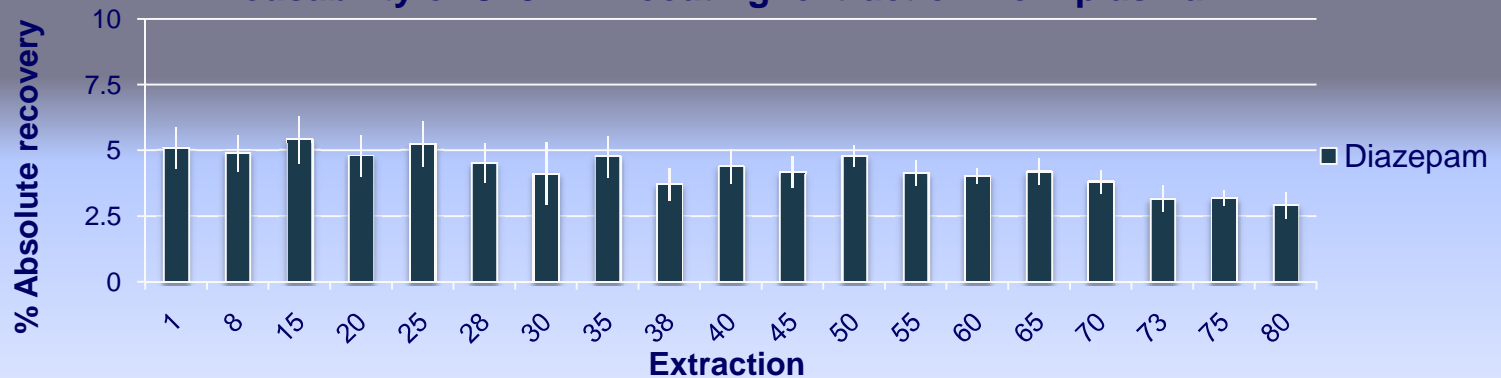
Acetonitrile/water (1/1)

Reusability and reproducibility of C18-PAN coating for extraction from PBS and plasma

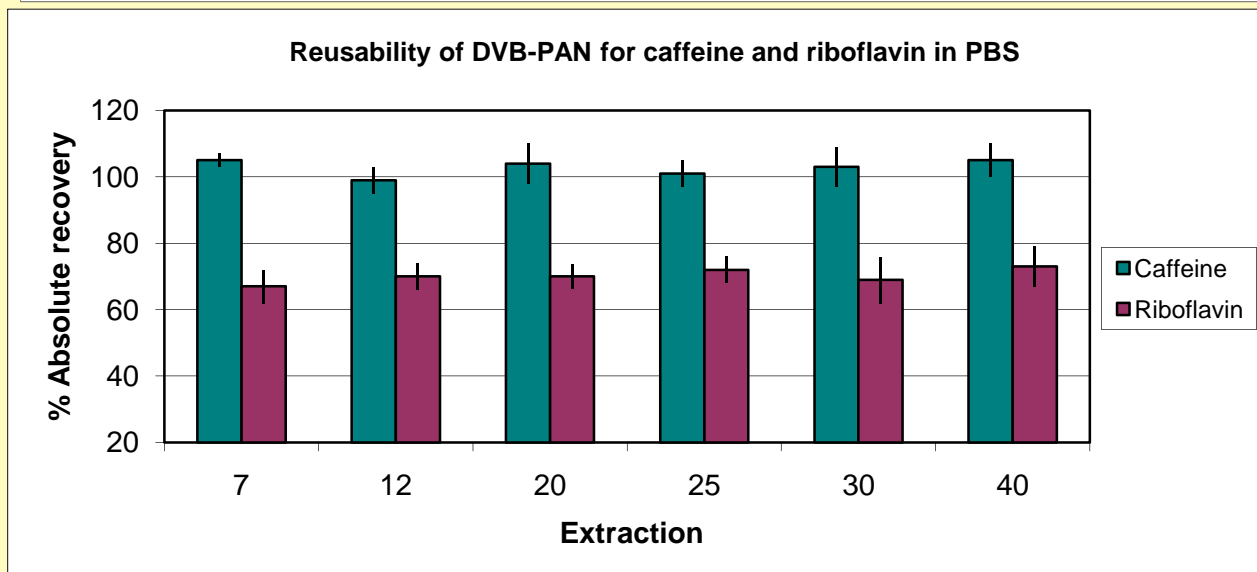
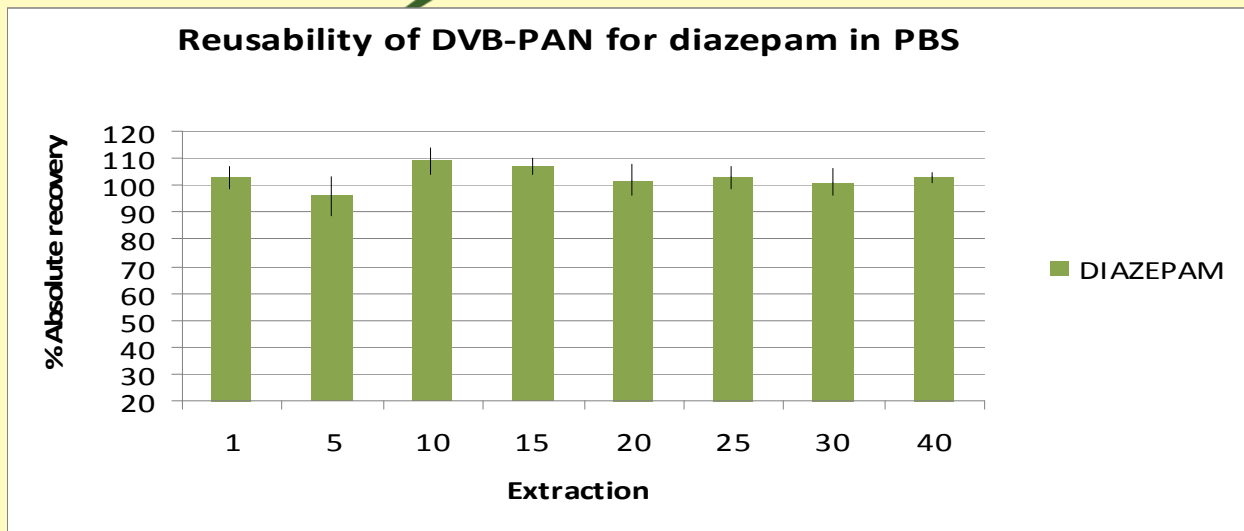
Reusability and reproducibility of C18-PAN: extraction from PBS



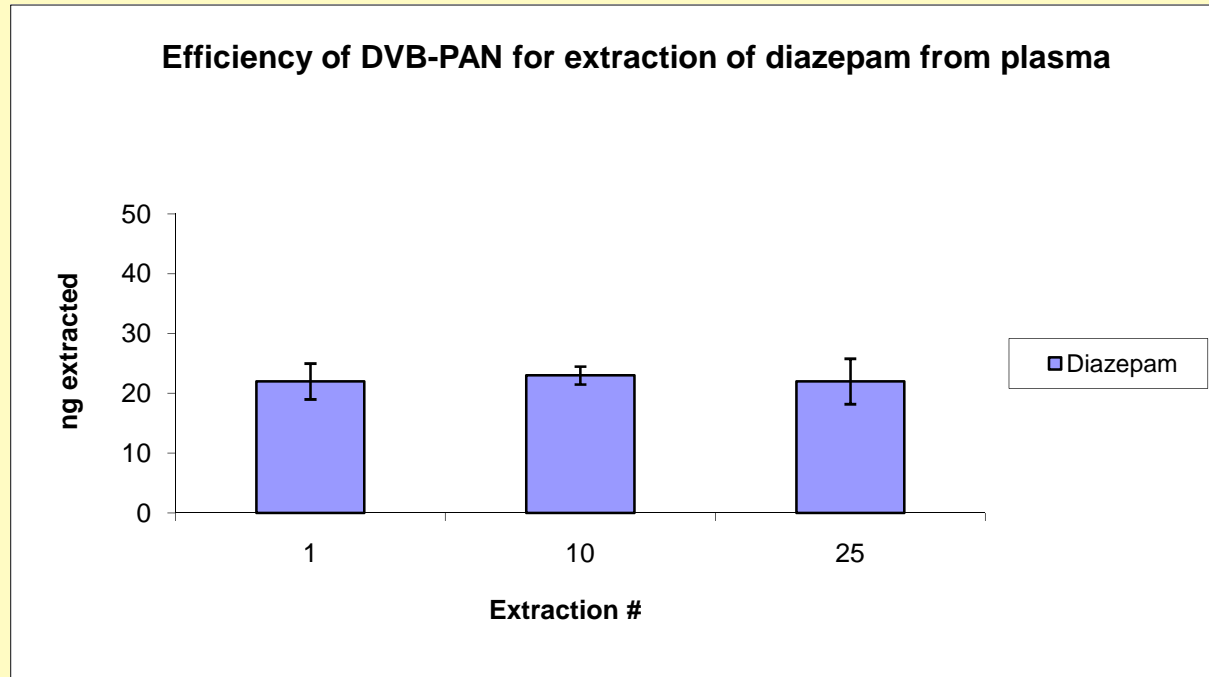
Reusability of C18-PAN coating: extraction from plasma



Reusability and reproducibility of DVB-PAN coating in PBS



Reusability and reproducibility of DVB-PAN coating in Plasma



Comparison of absolute recovery of the PAN-PS-DVB coating with C18-PAN

Comparison of absolute recovery of the PAN-PS-DVB coating with C18-PAN (extraction from PBS and n=12)

Analyte	Log P	Pka	%recovery for PAN-PS-DVB coating	%recovery for PAN-C18 coating
Diazepam	2.82	3.4	96 ±4	97±3
Oxazepam	2.24	12.4	97±3	80±4
Caffeine	-0.07	10.4	99±5	40±6
Riboflavin	-1.46	10.2	75±4	60±5
Sucrose	-3.7	12.6	3±0.2	-

Limits of detection and quantitation

LOD and LOQ for extraction of benzodiazepines from plasma

LOD for extraction from plasma (3×S/N)	LOQ for extraction from plasma (10×S/N)
0.1- 0.3 ng/mL	0.5-1 ng/mL

PAN-PS-DVB SPME-LC-MS/MS detection and quantitation

Analyte	LOD (extraction from plasma) ng/mL	LOQ (extraction from plasma) ng/mL
Oxazepam	0.1	0.5
Diazepam	0.3	1
Caffeine	0.3	1
Riboflavin	0.5	1.5
Sucrose	10	25

CONCEPT 96

Application

ANALYSIS OF BENZODIAZEPINES IN WHOLE BLOOD

PRECONDITIONING

RPA coating

30 min

Methanol/water (1/1)

EXTRACTION

30 min, 850 rpm

Whole blood or plasma
(0.8 mL) + IS

RINSE

30 seconds
Purified water

**Sample Prep. Time:
90 minutes!!**

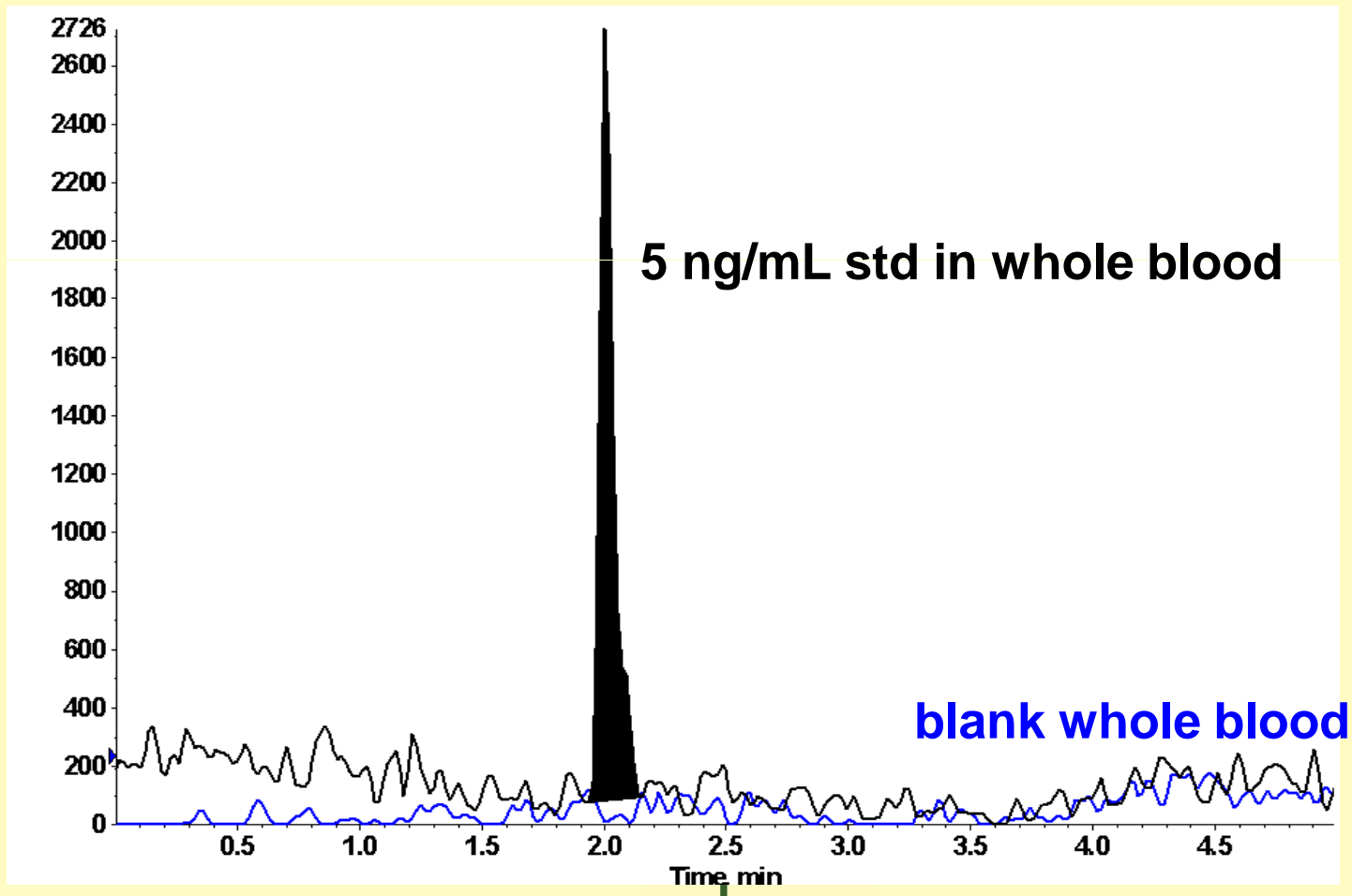
DESORPTION

30 min, 850 rpm
Acetonitrile/water (1/1)

CONCEPT 96

Application

ANALYSIS OF BENZODIAZEPINES IN WHOLE BLOOD



CONCEPT 96

Application

ANALYSIS OF BENZODIAZEPINES IN WHOLE BLOOD

Validation Parameter	Diazepam	Oxazepam	Nordiazepam	Lorazepam
LLOQ (ng/mL)	4	4	4	4
LLOQ Accuracy and Precision (%)	102 (11)	111 (17)	105 (9)	102 (14)
LLOQ S/N RATIO	5	19	16	16
Linear Range (ng/mL)	4-1000	4-500	4-1000	4-500
Accuracy (%)	94-103	91-98	98-106	97-106
Intra-batch Precision (%)	2-8	8-20	5-6	7-11
Inter-batch Precision (%)	3-6	7-12	2-4	7-12

CONCEPT 96

Application

AUTOMATED DRUG-PROTEIN BINDING STUDY

- Determine time required to reach equilibrium between ligand and receptor
- Obtain fibre constant (f_c) by calibration using standard solutions containing no receptor
- Prepare standard solutions containing different amounts of ligand and constant amount of receptor
- Perform SPME-LC to determine the amount of analyte extracted by the fibre (m)
- Calculate free concentration of ligand (C_f)

CONCEPT 96

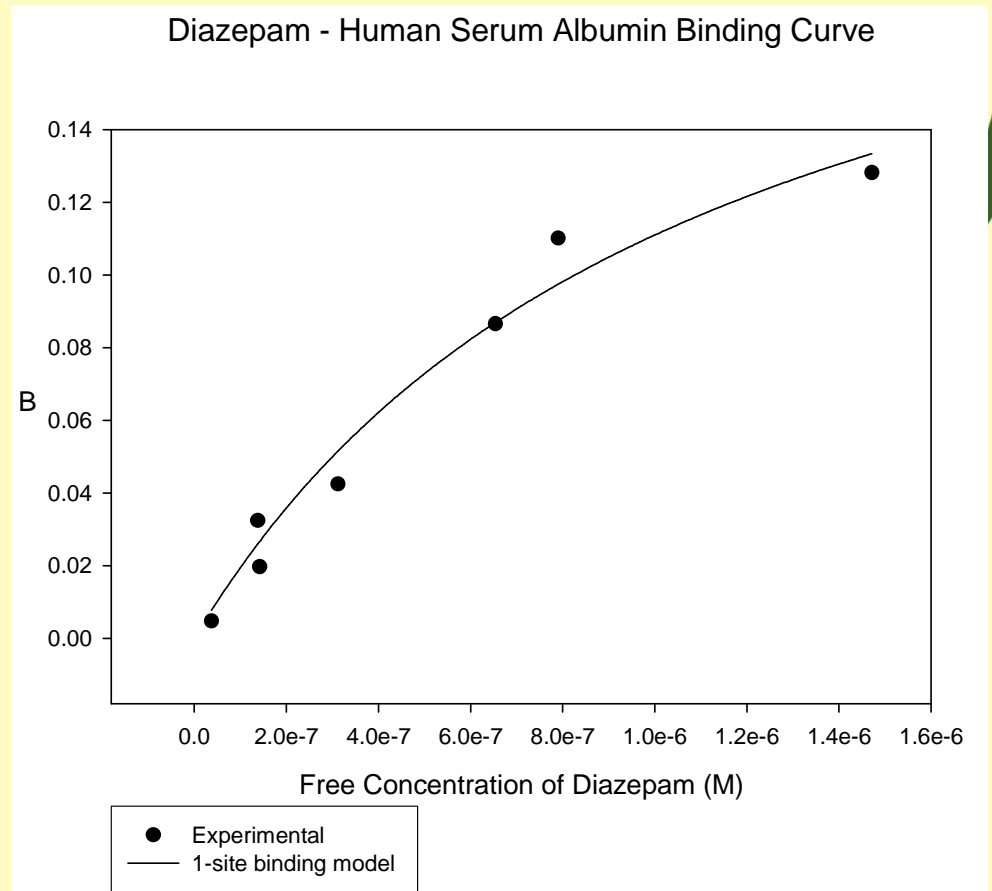
Application

AUTOMATED DRUG-PROTEIN BINDING STUDY

**BINDING OF DIAZEPAM
AND
HUMAN SERUM
ALBUMIN**

**1-SITE BINDING
MODEL**

**regression coefficient =
0.991**



CONCEPT 96

Application

AUTOMATED DRUG-PROTEIN BINDING STUDY

ADVANTAGES OF SPME TO STUDY LIGAND-RECEPTOR BINDING

- Ability to study binding under any conditions and for any concentrations of receptors and ligands
- Fast
- Works well for highly bound drugs
- Automation and increased sample throughput

CONCEPT 96

Application

Ochratoxin A in Human Urine

Ochratoxins:

- structurally related secondary metabolites, produced by *Penicillium verrucosum*
- a common storage fungus
- OTA has nephrotoxic, carcinogenic and immunosuppressive properties

CONCEPT 96

Application

Ochratoxin A in Human Urine

- Sample volume: 1 mL urine
- Extraction Time: 1 h @ 850 rpm
- Extraction Fiber: 12 mm of Carbon Tape on 0.061 SS-wire
- Desorption Solvent: 0.9 ml MeOH
- Desorption Time @ 15 min
- Injection Volume LC-MS/MS: 20 μ l

**Sample Prep. Time:
75 minutes!!**

CONCEPT 96

Application

Ochratoxin A in Human Urine

Validation Parameter	Ochratoxin A – Summary of Validation results		
	1 ng/mL	10 ng/mL	50 ng/mL
LOD (ng/mL)	0.3		
LLOQ (ng/mL)	0.7		
Linear Range (ng/mL)	0.7-50		
Accuracy and Intra-batch Precision (%)	106 (12)	114 (2)	93 (4)
Accuracy and Inter-batch Precision (%)	91 (14)	100 (5)	109 (4)

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- Fatemeh Mirnaghi, Janusz Pawliszyn, ASMS 2011 Poster „Modified PAN-PS-DVB 96-Thin-FilmSPME System, Capable of Extracting wide polarity range of Analytes from Biological Fluids“

Acknowledgements

- Prof. Pawliszyn and his group members, specifically Fatemeh Mirnaghi, Erasmus Cudjoe and Dajana Vuckovic, who supplied most of the data
<http://www.spme.uwaterloo.ca/>
- Supelco
http://www.sigmaaldrich.com/Brands/Supelco_Home/Spotlights/SPME_central.html