A new method for fast and efficient analysis of POPs in low volume samples of plasma and serum

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MAN TECHNOLOGY ENVIRONMENT RESEARCH CENTRE

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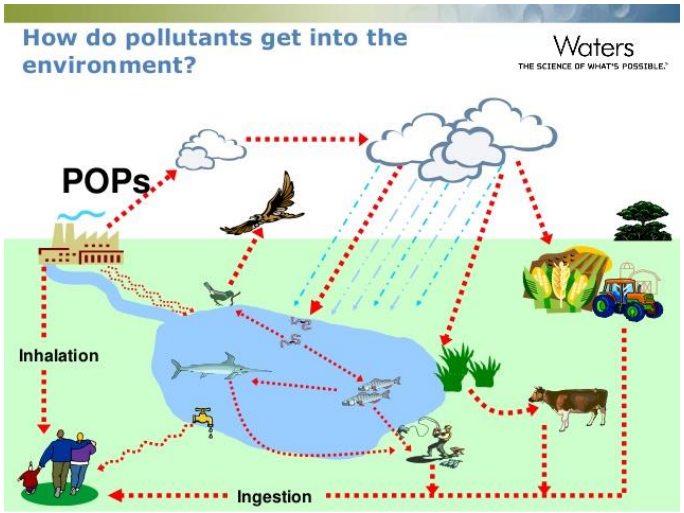
Stockholm Convention POPs

Addition to Stockholm Convention:

- 2001: PCBs, Pesticides, Dioxins
- 2009: PBDEs



Reasons for restriction:

- Persistent in Environment
- Bioaccumulate
- Endocrine disruption, diabetes, cardiovascular disease

Risk Assessement:

- Population-based biomonitoring → **Large sample count**
  Low sample volume

Current Sample Preparation

Typically

- Solid phase or liquid-liquid extraction
- 12 to 24 samples processed at one time
- Sample volumes of 0.5mL and up

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Volume 1216, Issue 6, 6 February 2009, Pages 897-901

A simple and fast liquid–liqui
determination of 2,2′,4,4′,5,5
1,1-dichloro-2,2-bis(*p*-chloro
human serum for epidemiolc

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Determination of selected perfluorinated alkyl acids and persistent organic pollutants from a small volume human serum sample relevant for epidemiological studies

Jani Koponen✉, Panu Rantakokko, Riikka Airaksinen, Hannu Kiviranta

Objective

- To increase the efficiency and cost-effectiveness of sample preparation procedures
  - extraction of Stockholm Convention POPs
  - low volumes of serum and plasma samples
- Develop and validate a miniaturized 96-well plate SPE procedure for high-throughput extraction of Stockholm Convention POPs from low volumes of plasma and serum
- Apply to large population-based biomonitoring and epidemiological studies
  - PIVUS Study

23 Target Cl/Br- POPs

PCBs (Polychlorobiphenyls)
Tetra: 74, 99
Penta: 118, 105,126
Hexa: 153, 138, 156, 157, 169
Hepta: 180, 170, 189
Octa: 194
Nona: 206
Deca: 209

PCDD (Polychlorinated dibenzo-*p*-dioxin)

OCDD (Octachlorodibenzo-*p*-dioxin)

OC (Organochlorine) pesticides

HCB (Hexachlorobenzene)

Trans and *Cis*-chlordane

Trans-nonachlor

p, p'-DDE

PBDE (Polybrominated diphenyl ether)

Tetra: 47

96-well plate SPE method and analysis

Precondition
Methanol and Water

Load
Sample: 150µL serum/plasma pretreated with sulfuric acid in water and acetonitrile in water

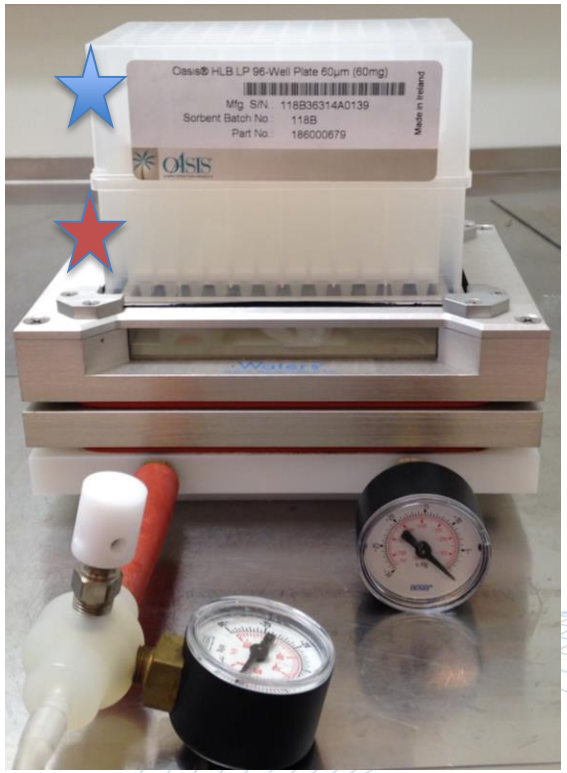
Wash
1.5mL methanol in water

Dry wells
15 minute centrifugation at 4,000 RPM
dried under vacuum with nitrogen stream for 3hrs (32 wells per hour)

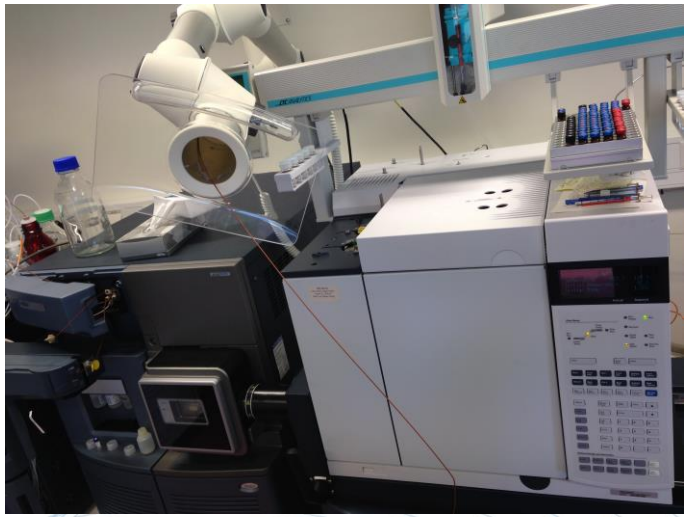
Elute
1.3mL (1:1) Dichloromethane:hexane collected in vials holding 20µL tetradecane

96-well SPE plate
- Oasis HLB 60mg sorbent/well (Waters Corporation)

96-well clean-up plate
- containing 40% H_2SO_4 modified silica and sodium sulfate



96-well plate SPE method and analysis



Instrumental Analysis

Agilent 6890 N GC
coupled to a Waters Micromass Autospec Ultima HRMS

Agilent 7890A GC coupled to a Waters APCI-MS/MS (Xevo TQ-S)

2µL injected onto a 30 m × 0.25mm i.d. × 0.25 µm DB-5MS capillary column

POPs quantified by using isotope dilution of ^{13}C-labeled standards.

Quality Assurance/Quality Control

Limits of Detection and Quantification:

- H_2O method blanks
- Newborn Bovine Serum (NBS) method blanks

Precision and Reproducibility:

- In-house reference plasma
- National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 1957

Accuracy:

- NIST SRM 1957

Quantification and Instrument Performance:

- Batch standards
- Instrument blanks
- Calibration curve

Difference in results between instruments (GC-HRMS vs. GC-APCI-MS/MS)

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Recoveries

C13-labeled POPs	Newborn bovine serum blank (RSD)	Reference plasma (RSD)
C13-PCB 70	**86** (20)	**75** (22)
C13-PCB 101	**76** (20)	**65** (24)
C13-PCB 118	**75** (19)	**64** (21)
C13-PCB 105	**76** (18)	**67** (21)
C13-PCB 153	**73** (21)	**60** (25)
C13-PCB 138	**76** (18)	**62** (21)
C13-PCB 156	**76** (21)	**60** (26)
C13-PCB 180	**76** (17)	**55** (31)
C13-PCB 170	**76** (17)	**52** (20)
C13-PCB 194	**74** (15)	**49** (31)
C13-PCB 206	**63** (14)	**37** (21)
C13-PCB 209	**56** (20)	**35** (31)
C13-HCB	**117** (10)	**107** (33)
C13-*p,p'*-DDE	**152** (23)	**126** (50)
C13-OCDD	**56** (15)	**40** (35)
C13-PBDE 47	**85** (52)	**78** (39)

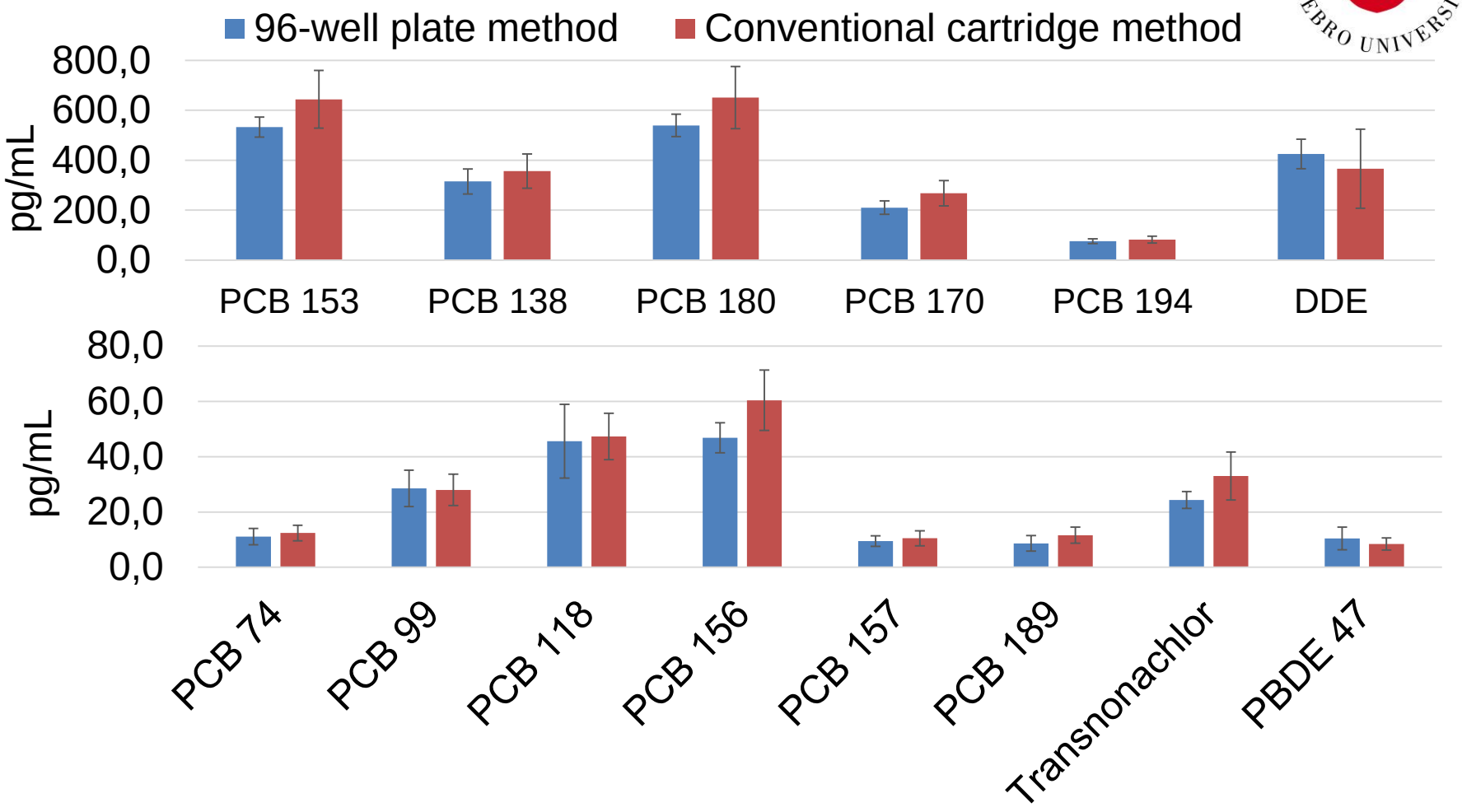
Limits of Detection

pg/mL

PCB 74	**4.7**	PCB 170	**2.0**
PCB 99	**19**	PCB 189	**1.2**
PCB 118	**37**	PCB 194	**2.0**
PCB 105	**12**	PCB 206	**1.4**
PCB 126	**2.1**	PCB 209	**1.4**
PCB 153	**38**	OCDD	**3.0**
PCB 138	**32**	HCB	**93**
PCB 156	**2.5**	*p,p'*-DDE	**12**
PCB 157	**1.4**	*Trans*-chlordane	**2.0**
PCB 169	**0.0**	*Cis*-chlordane	**2.0**
PCB 180	**6.2**	*Trans*-nonachlor	**3.6**
		PBDE 47	**4.8**

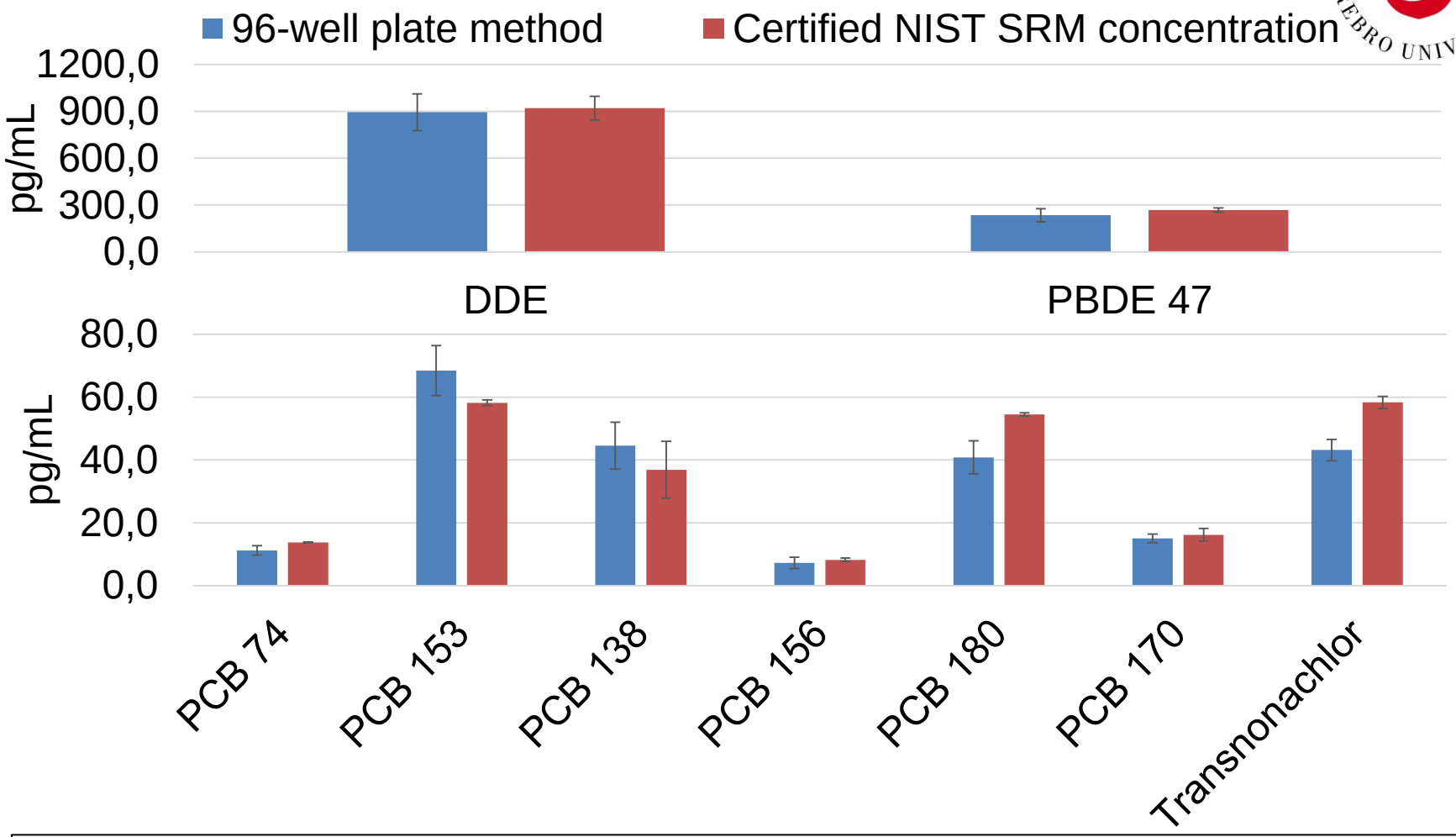
LOD (NBS blank)=average + 3x SD

Precision and Reproducibility



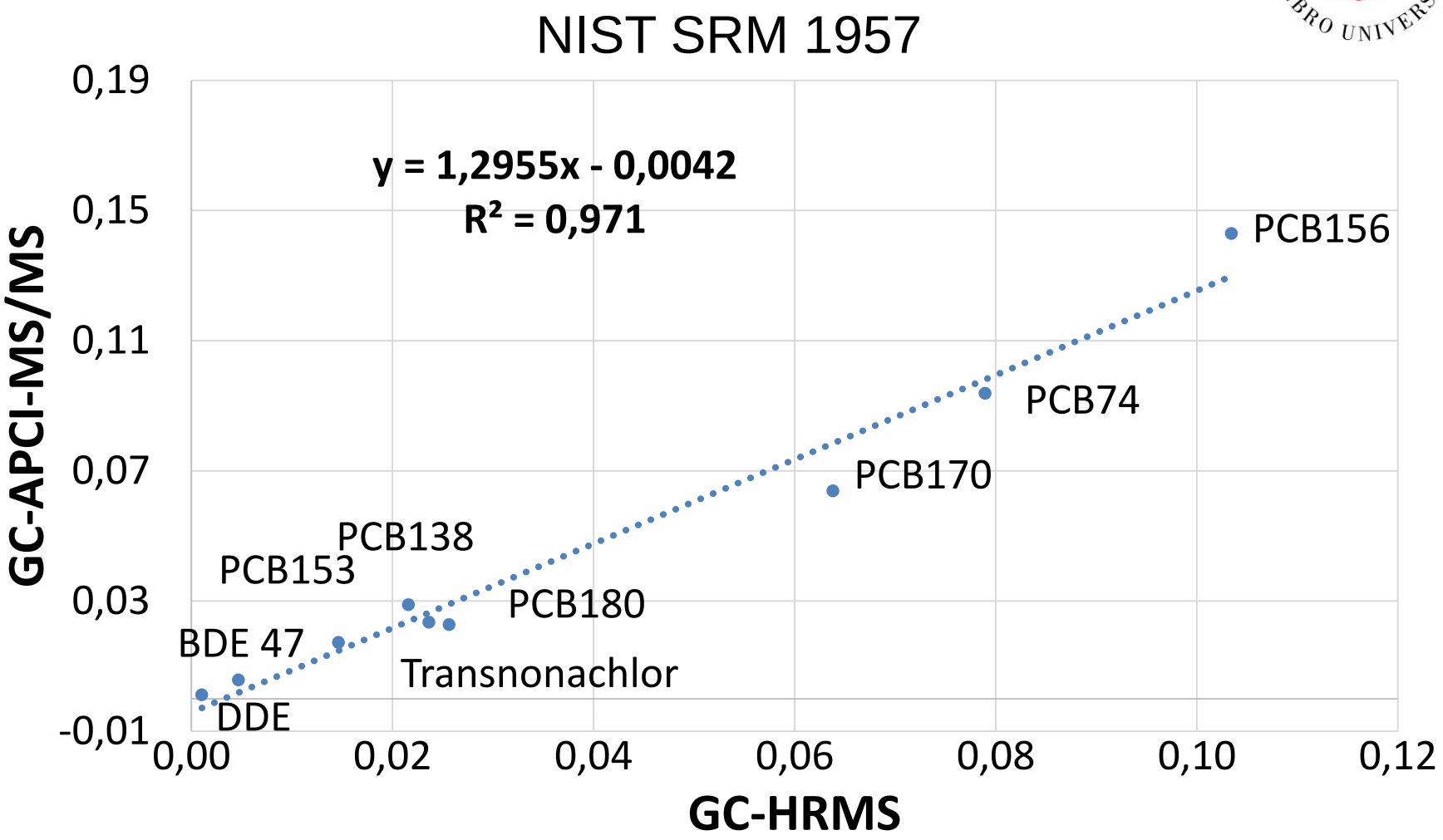
The average concentration and standard deviation of POPs in QC reference plasma between the 96-well plate method (N= 8;150µL plasma) and conventional SPE cartridge method (N=95; 500µL plasma) developed by Salihovic et al. 2012.

Accuracy



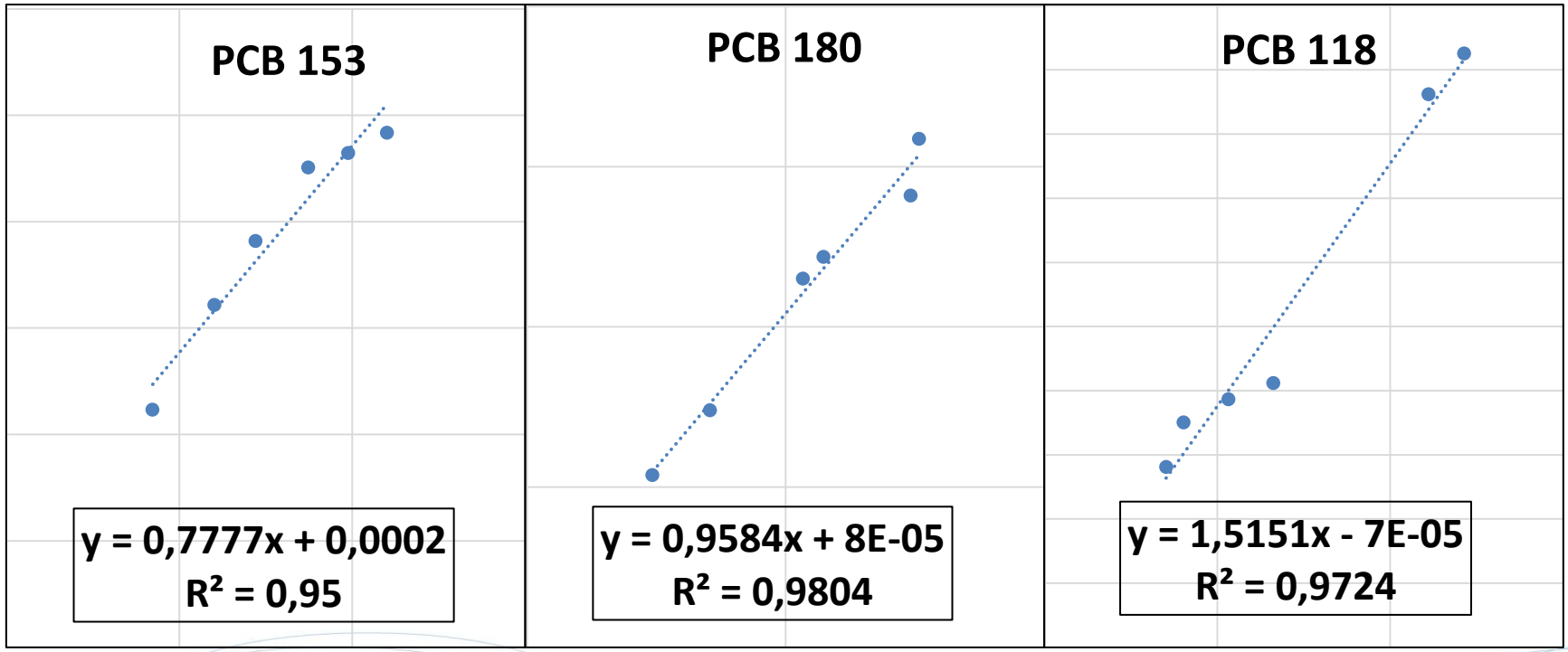
The average concentration and standard deviation of POPs in NIST SRM 1957 between the 96-well plate method (N= 8;150µL plasma) and certified reference values reported by NIST

GC-HRMS vs. GC-APCI-MS/MS



Application: Comparison of results between cartridge SPE and 96-well plate method

- POPs extracted from 6 epidemiological study samples using
  - 96-well plate method (y-axis)
  - Conventional cartridge method (Salihovic et al. 2012) (x-axis)



Benefits and Limitations

+ Increased sample throughput for large population based biomonitoring and epidemiological studies
+ Reliable quantification of Stockholm Convention POPs
+ Cost-effective and time-efficient

- For background level populations you may not be able to detect low-level analytes
  - OCDD will require larger volumes of plasma/serum (1pg/mL)
- Lowered recoveries can occur when
  - Wells are not thoroughly dried

Conclusions

- Miniaturized 96-well plate sample preparation developed for the extraction of Stockholm Convention POPs from low volume plasma and serum samples
- The sample throughput is increased by ~ 2 to 8-fold
  - One man/woman work week: process 73 to 146 samples vs. 10 to 40 samples
- Extraction and analysis provide reliable and accurate results
- Currently being applied to samples in epidemiological study

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Thank you!

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