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UNIVERSITÀ DI BOLOGNA

MICROSAMPLING FOR THE THERAPEUTIC DRUG MONITORING OF EATING DISORDER PATIENTS UNDER ANTIDEPRESSANT TREATMENT

Michele Protti

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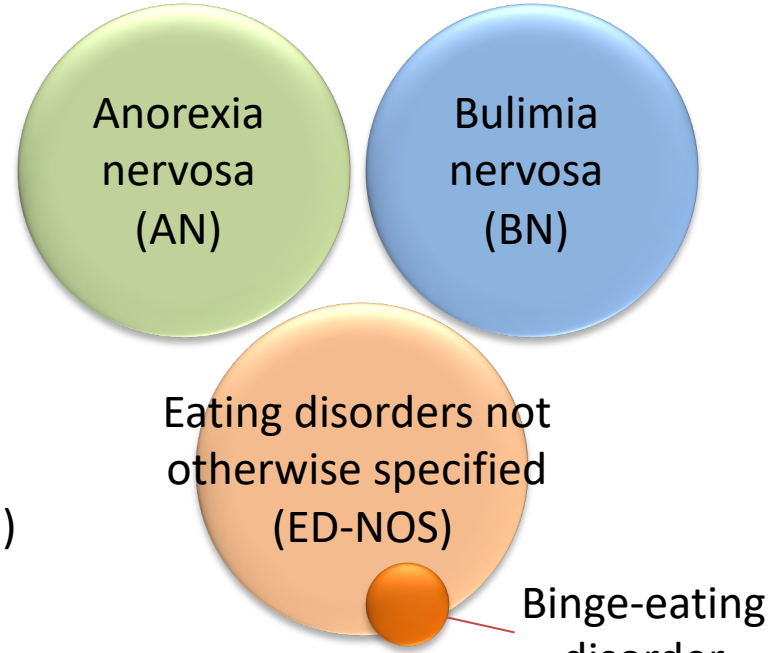
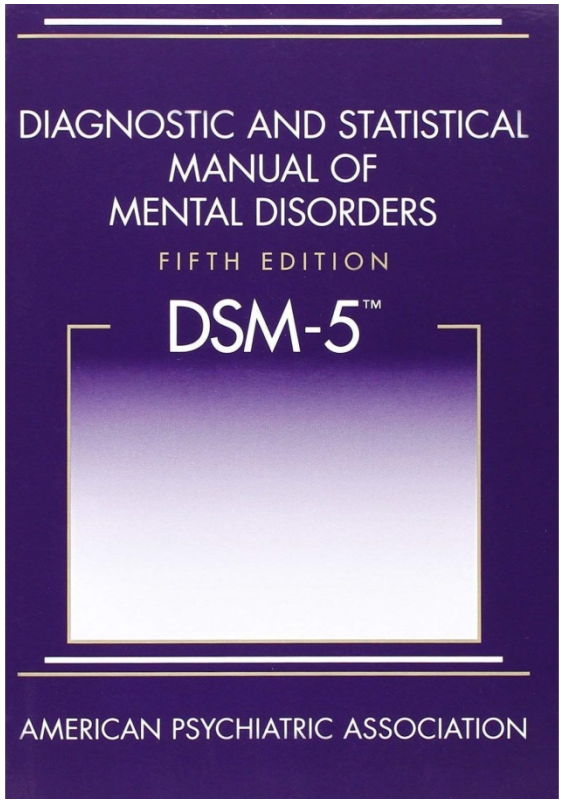
PTA Lab
Pharmaco Toxicological Analysis Laboratory

EATING DISORDERS

- Complex and multifactorial psychiatric diseases
- High mortality rates among psychiatric disorders (undernutrition, suicide)
- Prevalence 0.3-0.7% men, 2-3% women (higher among adolescent girls and young women)
- Complex counselling/psychotherapy + pharmacological treatment (comorbidity of affective, anxious and obsessive symptoms) Mainly antidepressants (e.g. SSRIs)
- Extreme BMIs need careful drug(s) dosage regimens → **TDM**





S.B. Murray, Eating Disorders 27 (2019) 6-17.

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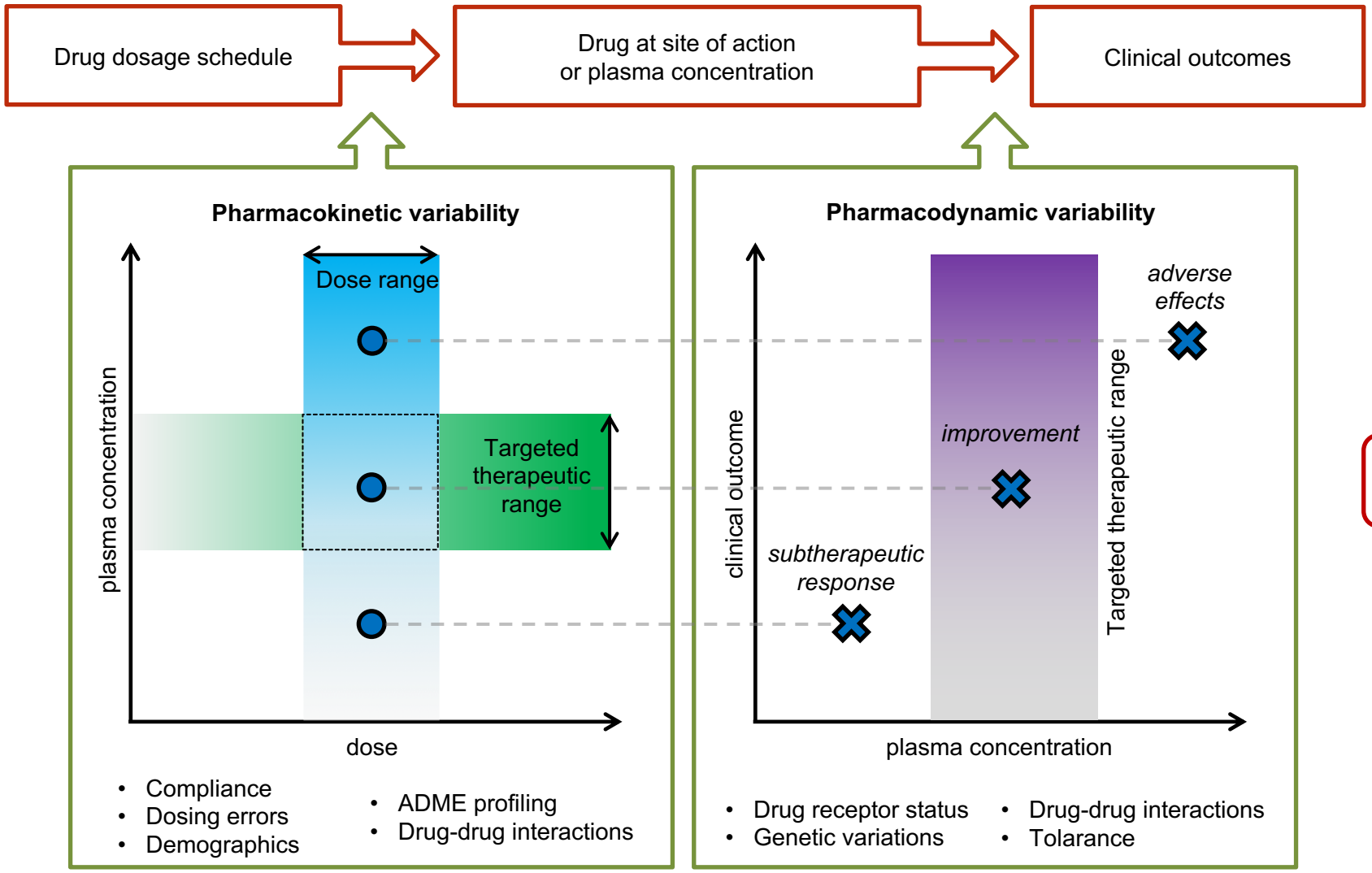
CURRENT THERAPEUTIC DRUG MONITORING



More frequent time points/
More feasible assessments

Accurate therapy personalisation

Precision medicine

R. Mandrioli, L. Mercolini, M.A. Saracino, M.A. Raggi, Current Medicinal Chemistry 19 (2012) 1846-1863.

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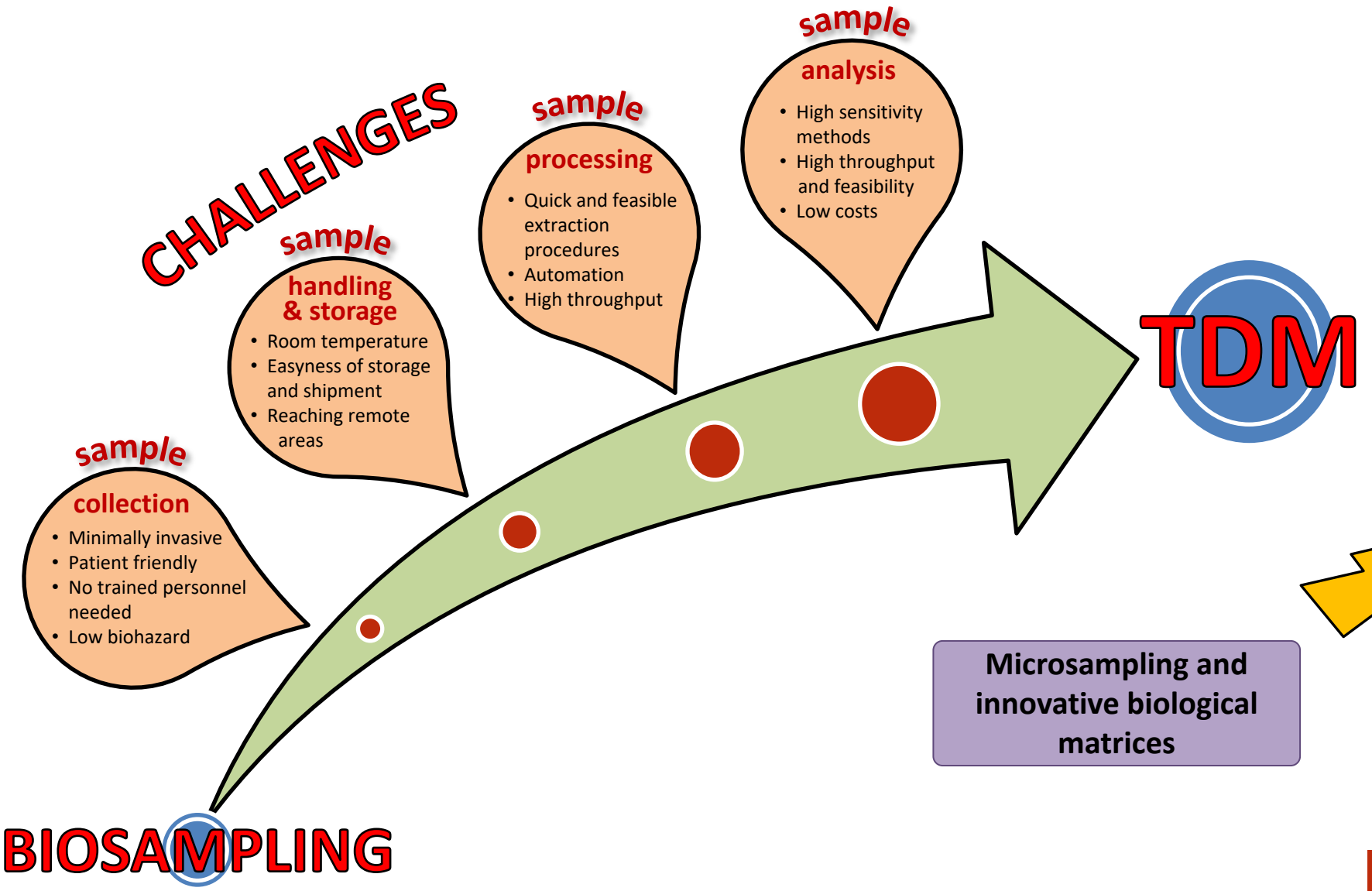
KEY POINTS FOR EFFECTIVE TDM



Therapy personalisation

- Dose-related efficacy
- Complinace
- Drug-drug interactions
- Toxicity
- Interindividual variability

Microsampling and innovative biological matrices

Overall health costs reduction and patient life quality improvement

R. Mandrioli, M. Protti, L. Mercolini. Current Medicinal Chemistry 25 (2018) 772-792.

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OUTLINE OF THE RESEARCH WORK

Aim

To design, develop and apply novel microsampling approaches coupled to LC-MS/MS for the TDM of eating disorder patients undergoing AD treatment

Agenda

- LC-MS/MS system and target analytes
- Microsampling approaches
  - HemaPen®
  - VAMS® Mitra®
  - HemaXis®
- Method development & pre-validation studies:
  - Selectivity
  - Extraction yield
  - Matrix effect
  - Carryover
  - Calibration
  - Precision
  - Accuracy
  - HCT assays
  - Storage conditions
- Application to patient samples and clinical implications
- Evaluation of oral fluid for TDM
- Conclusion

L. Mercolini, M.A. Saracino, M. Protti, Bioanalysis. 7 (2015)1925-1942.

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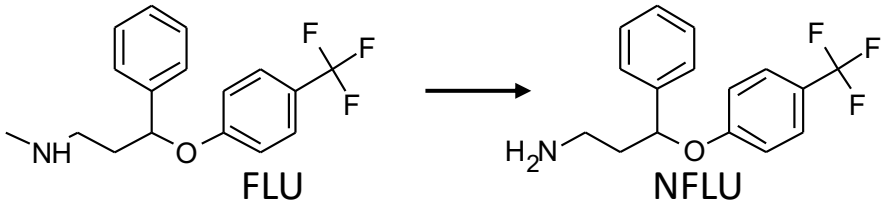
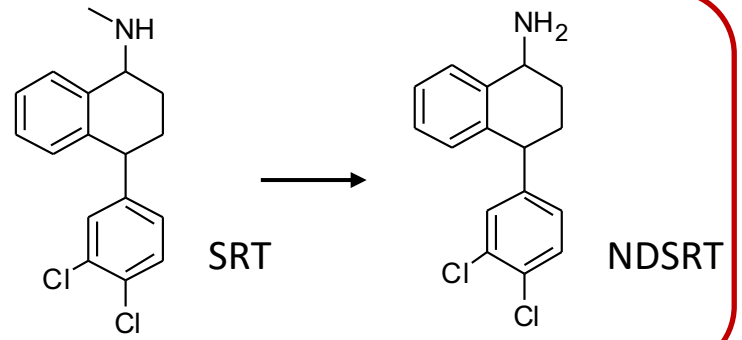
TARGET ANALYTES & LC-MS/MS SYSTEM

Fluoxetine



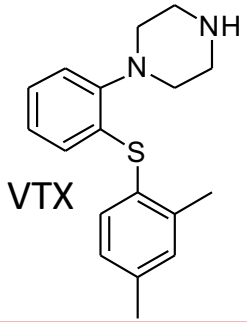
- selective serotonin reuptake inhibitor (SSRI)
- reduction of binge eating and vomiting in BN

Sertraline



- selective serotonin reuptake inhibitor (SSRI)
- Effective against BED

Vortioxetine



- serotonin modulator and stimulator (SMS)
- Effective against BED and AN

LC-MS/MS system

Stationary phase: RP C18 + guard column
Mobile phase: FA in H_2O / FA in ACN
gradient elution

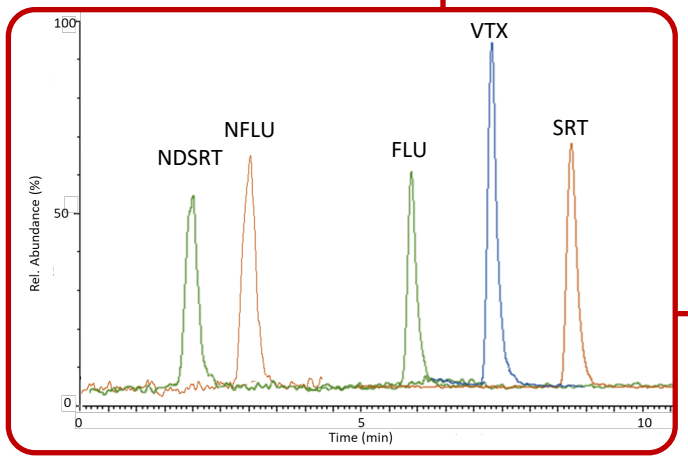
Flow rate: 0.1 mL/min

Injection volume: 10 µL

Analyser: Triple quadrupole (MS/MS), QTOF (HRMS)

Ionisation: ESI+

Acquisition: MRM



FLU	310.13	→	148.0
NFLU	296.11	→	134.9
SRT	306.34	→	275.2
NDSRT	292.17	→	159.0
VTX	299.40	→	150.1

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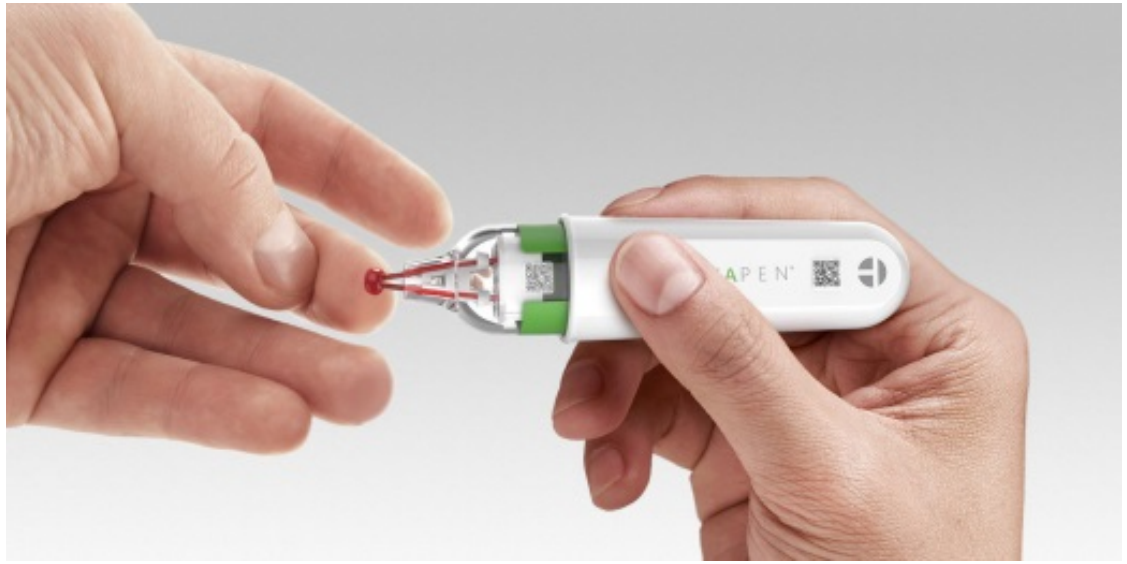
MICROSAMPLING APPROACHES/1

HemaPen®

- Simultaneous collection of 4x 2.74 µL replicates
- Generation of DBS on pre-punched disks
- Volumetric accuracy by glass capillaries
- Integrated storage
- Tamper-proof



TRAJAN

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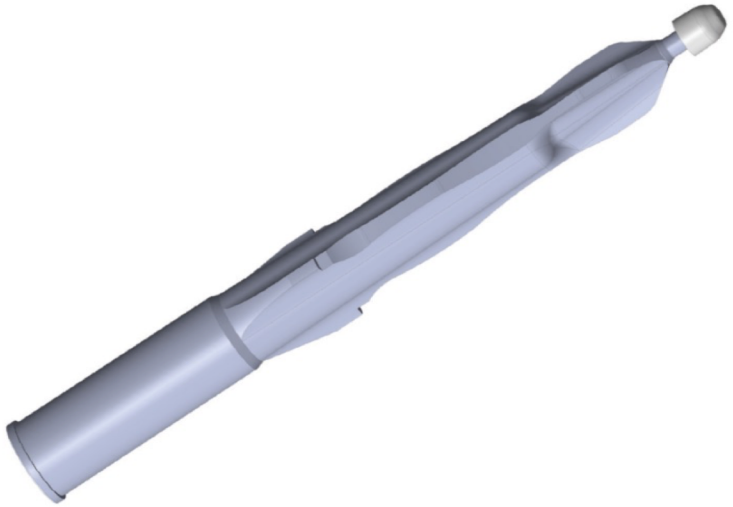
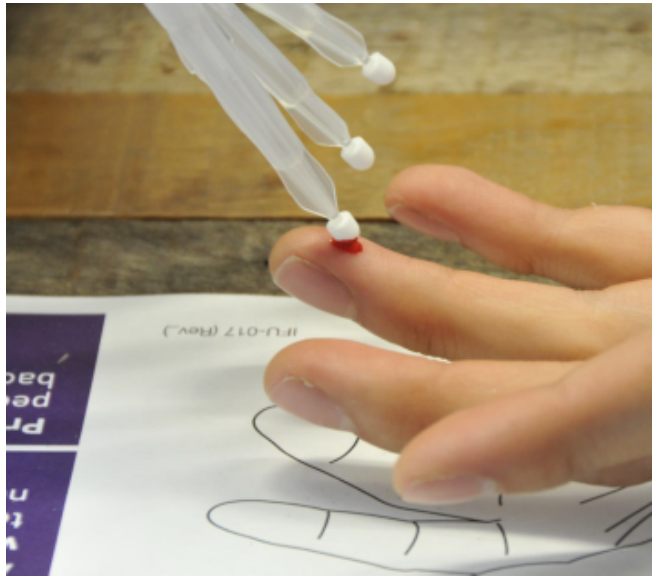
MICROSAMPLING APPROACHES/2



VAMS®

- Consecutive collection of 2/3/4/96x unique samples (depending on format)
- 10/20/30 µL
- Volumetric accuracy by absorption on porous polymeric tip
- Amenable to automation with standard equipment

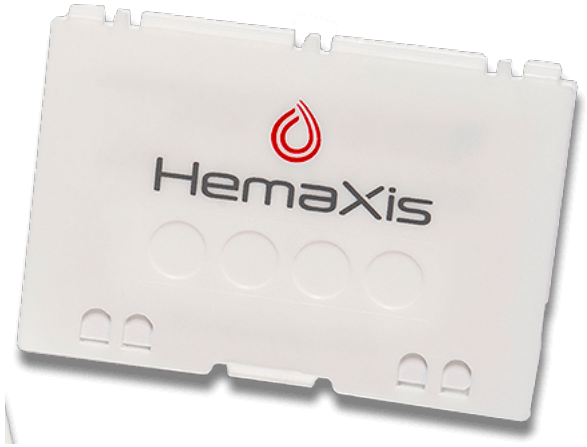


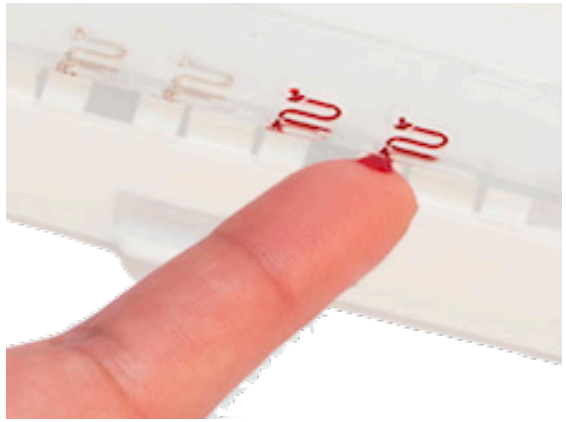
neoteryx™

MICROSAMPLING APPROACHES/3

HemaXis®

- Consecutive collection of 4 DBS samples on a single device
- Classic DBS card format
- 5/10 µL
- Volumetric accuracy by microfluidic channel chip
- Compatible with automated handling process





DBS System

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METHOD DEVELOPMENT & PRE-VALIDATION STUDIES: HEMAPEN®

Selectivity (n=6)	
Extraction yield (n=3)	> 81%
Matrix effect (n=3)	< 5.8%
Carryover (n=3)	
Calibration (5 conc., n=3)	5-5000 ng/mL
Precision (intraday, n=6)	RSD% < 7.3 (< 9.4 for LLOQ)
Precision (interday, n=6)	RSD% < 9.1 (< 12.0 for LLOQ)
Accuracy (n=3)	91-106%

Paper in preparation

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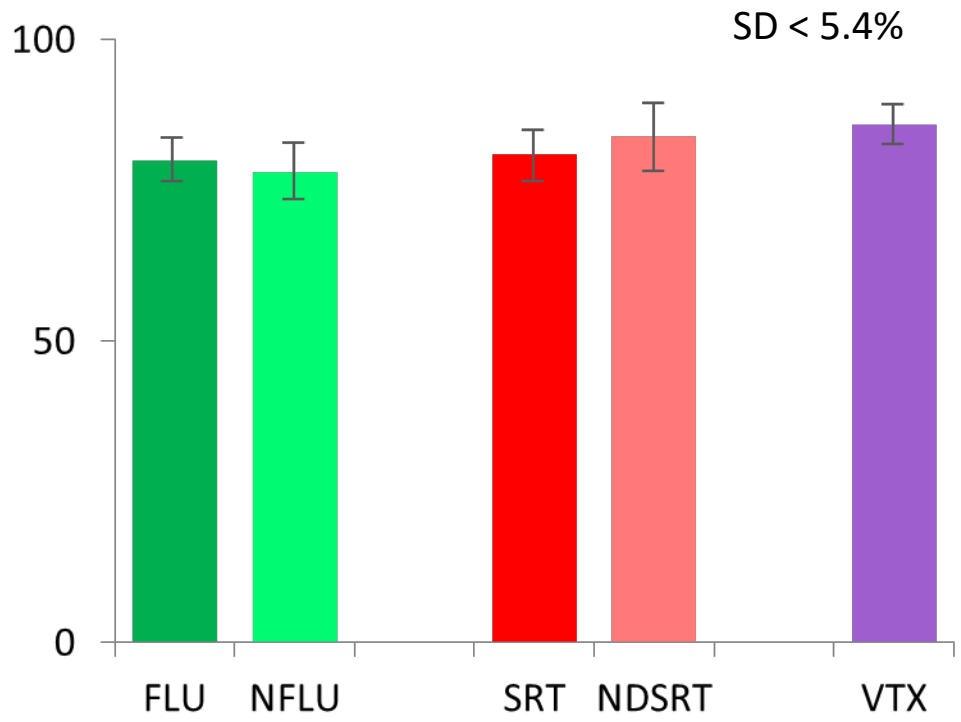
METHOD DEVELOPMENT & PRE-VALIDATION STUDIES: VAMS®

Extraction yields



extraction procedure

- 10 µL detached tip extracted with 500 µL of 0.1% FA in MeOH
- 30 min by ultrasonic-assisted extraction (UAE)
- Drying and re-dissolution

Matrix effect

< 4.7%

Accuracy

94-103%

Precision

RSD% < 7.6
(< 9.4 for LLOQ)

Unpublished data

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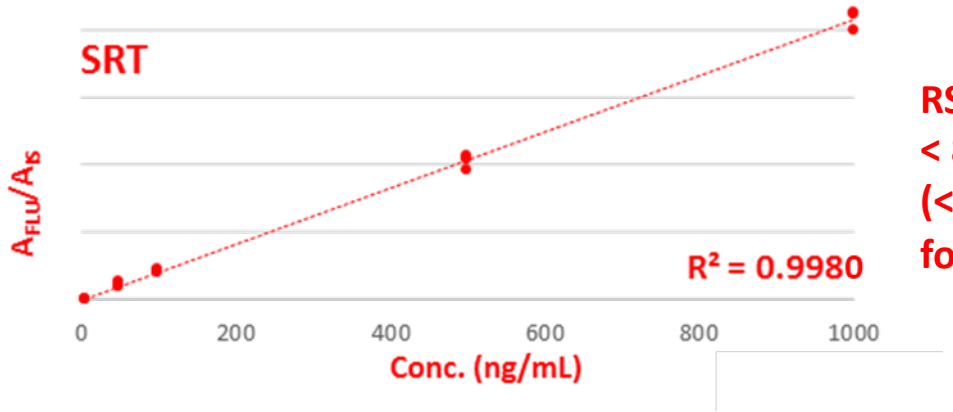
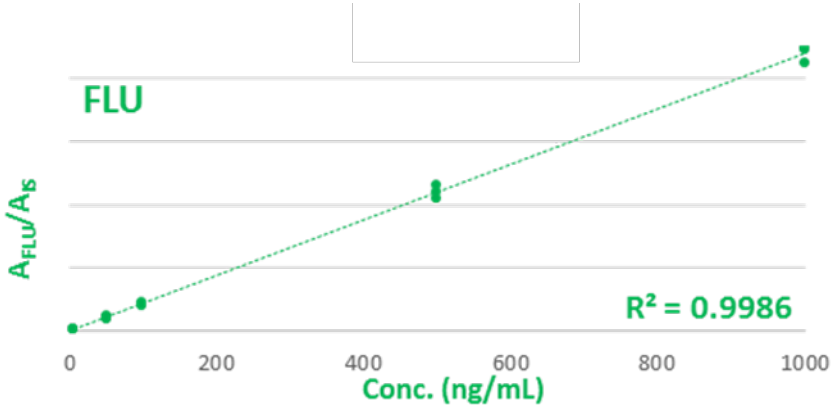
METHOD DEVELOPMENT & PRE-VALIDATION STUDIES

HEMAXIS®

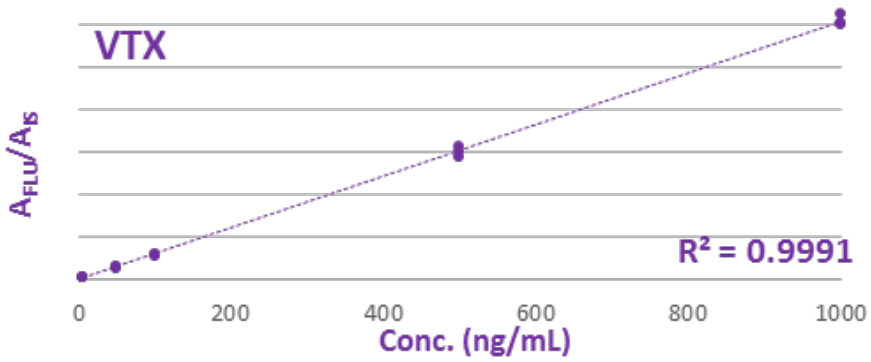
Calibration curves & precision



RSD%
< 8.4
(< 10.0
for LLOQ)



RSD%
< 7.3
(< 9.1
for LLOQ)



RSD%
< 6.8
(< 8.0
for LLOQ)

Unpublished data

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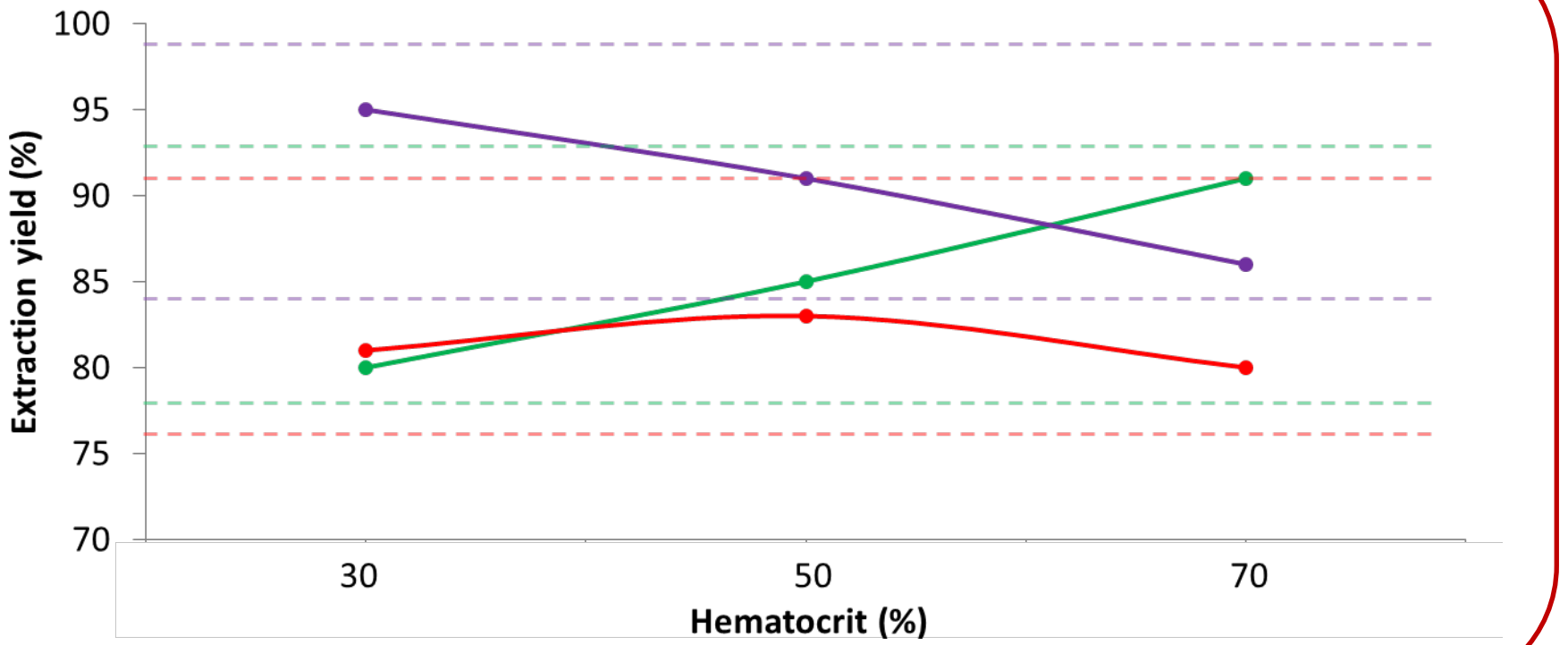
METHOD DEVELOPMENT & PRE-VALIDATION STUDIES

HEMAPEN®

HCT study



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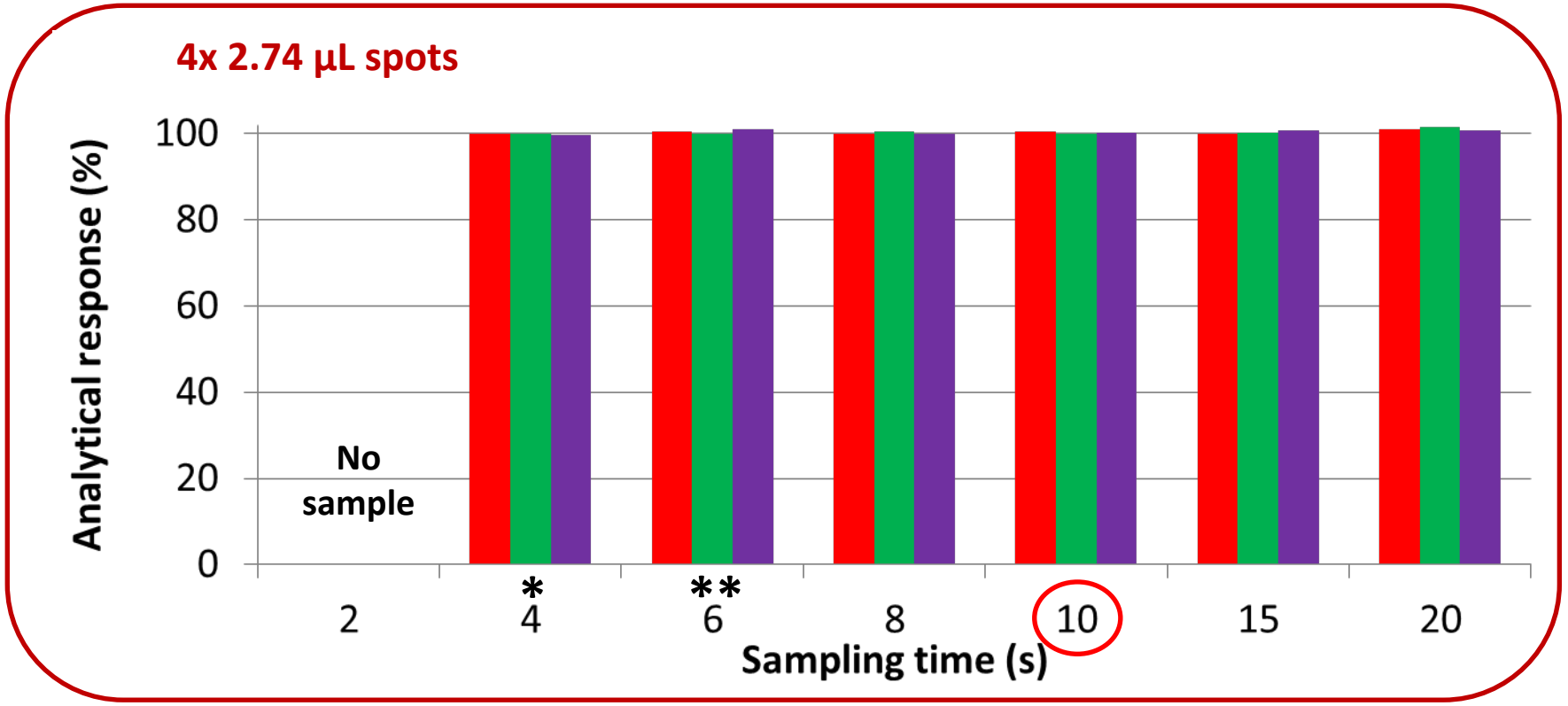
METHOD DEVELOPMENT & PRE-VALIDATION STUDIES

HEMAPEN®

Misuse assessment (under- & oversampling)



* 1 out of 4 samples

** 2 out of 4 samples

Sampling time reported by the manufacturer

FLU/NFLU

SRT/NDSRT

VTX

MICROSAMPLING APPLICATION TO ED PATIENTS TDM

16 patients diagnosed with ED were recruited for this study:

- 13 females, 3 males
- Age 18-52 years
- 6 BED, 7 AN, 3 BN
- BMI 14.1 - 47.3 kg/m^2
- Treated with 1 or more AD (fluoxetine, sertraline, vortioxetine)
- Other factors evaluated under the clinical point of view:
  - time from the beginning of therapy
  - time since the last drug intake
  - previous and current posology
  - consumption of alcohol and cigarettes

Concurrent collection of fluid blood, microsamples by fingerprick and oral fluid

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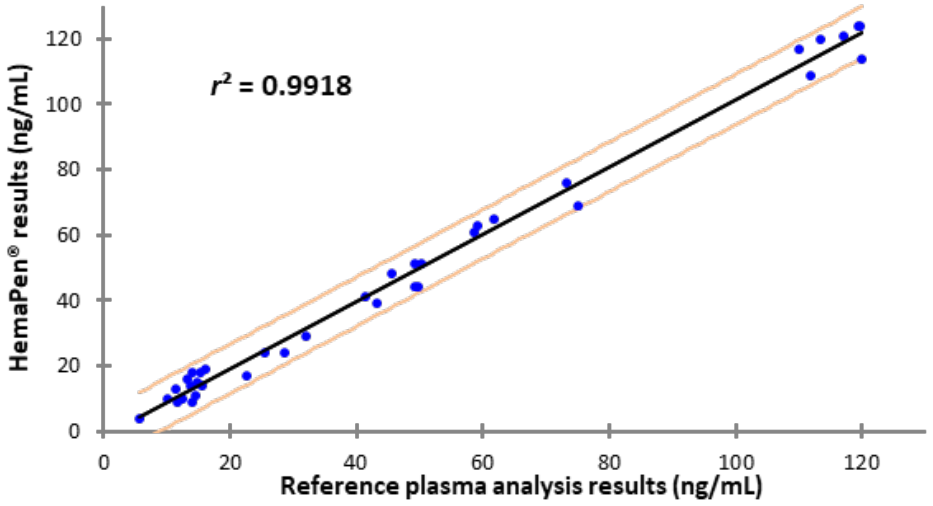
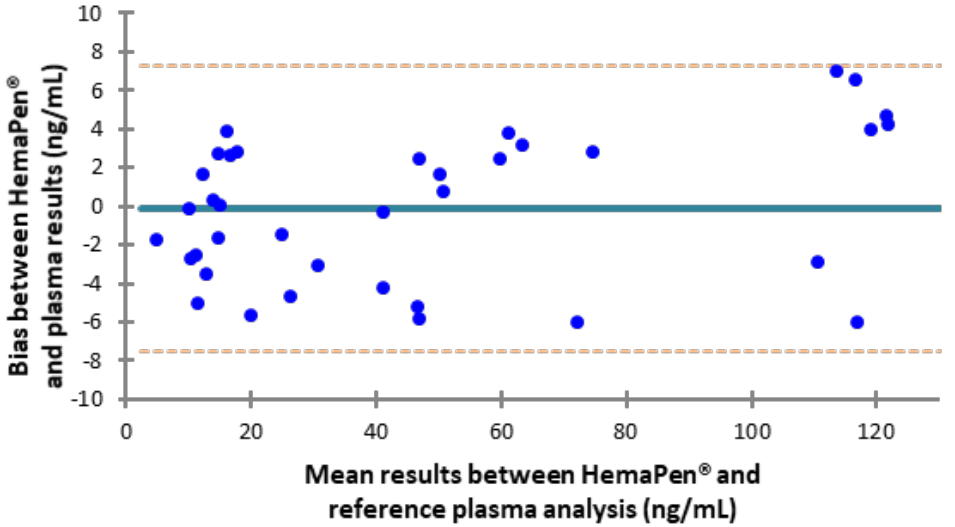
RESULTS FROM MICROSAMPLING APPLICATION TO TDM AND CLINICAL IMPLICATIONS

HemaPen®





- Allows **simultaneous** collection of 4 identical samples
- **Integrated** silica desiccant: ready to store/ship without drying precautions
- Does not need additional packaging
- **Tamper proof** (needs a tool to be opened)
- Slightly longer training time needed
- Lower sample volumes

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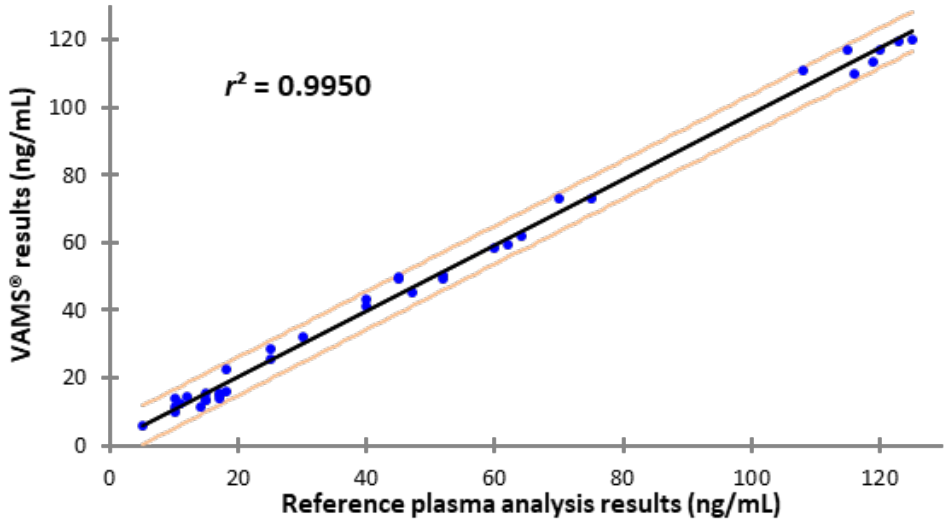
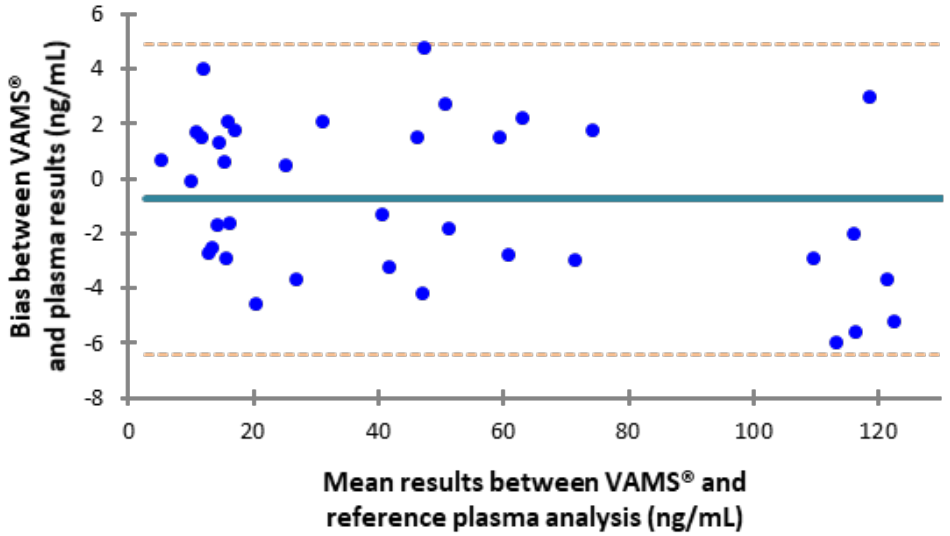
RESULTS FROM MICROSAMPLING APPLICATION TO TDM AND CLINICAL IMPLICATIONS

VAMS®





- Handy and **intuitive** use
- Amenable to **automation** with most liquid handling robots
- **Polymeric** material offers alternative selectivity for the retention of interferents
- Needs additional packaging/desiccant for long term storage

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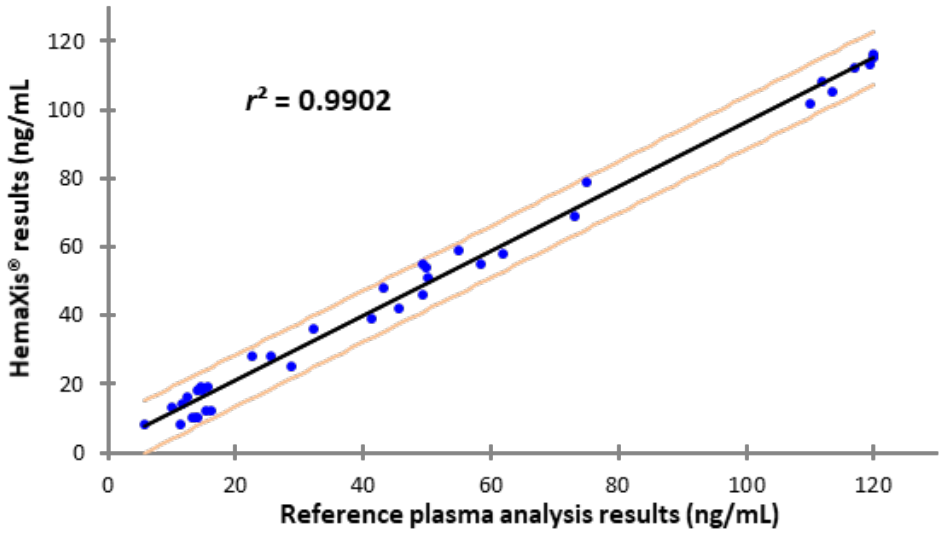
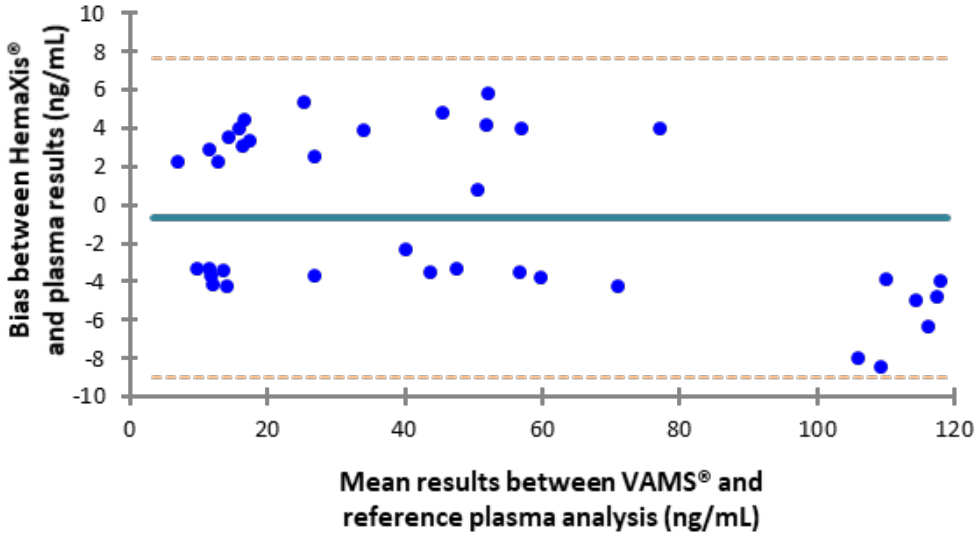
RESULTS FROM MICROSAMPLING APPLICATION TO TDM AND CLINICAL IMPLICATIONS

HemaXis®





- Very **fast sampling** (+ careful spot generation)
- Fits classic **DBS cards**
- Amenable to already existing **DBS pretreatment instruments**
- Needs additional packaging/desiccant for long term storage
- Spots cannot be generated simultaneously (possible need for additional finger pricking)

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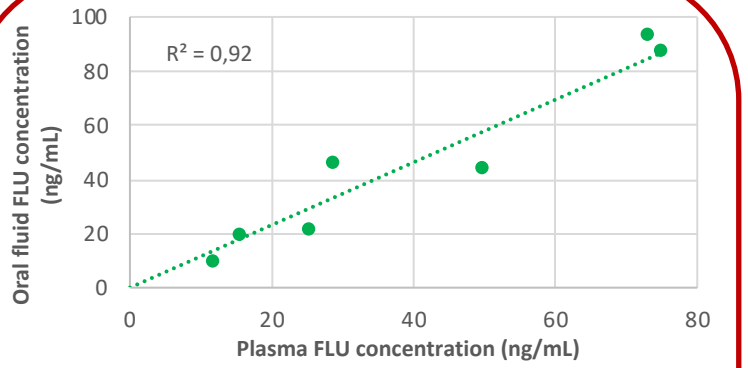
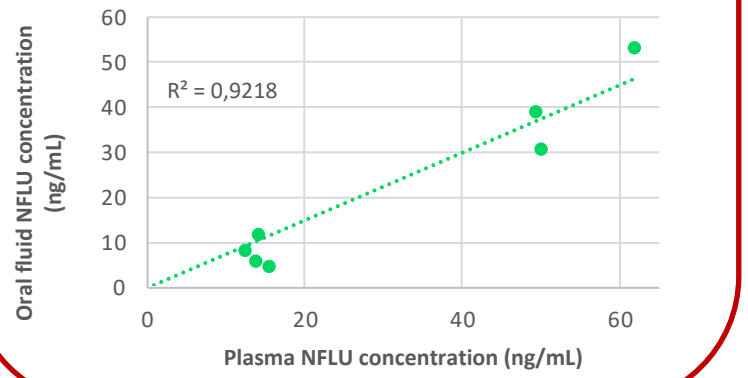
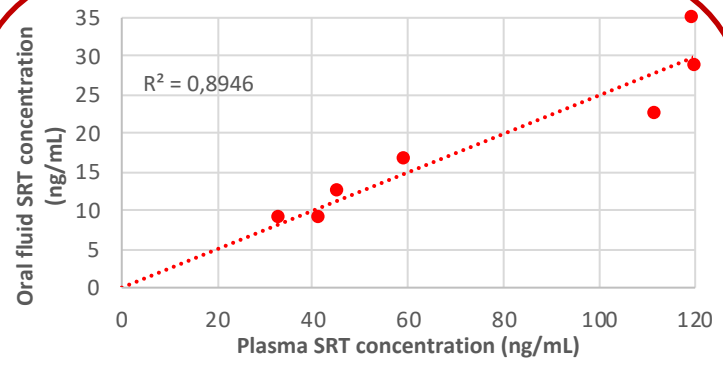
EVALUATION OF ORAL FLUID AS ALTERNATIVE MATRIX FOR TDM OF AD DRUGS

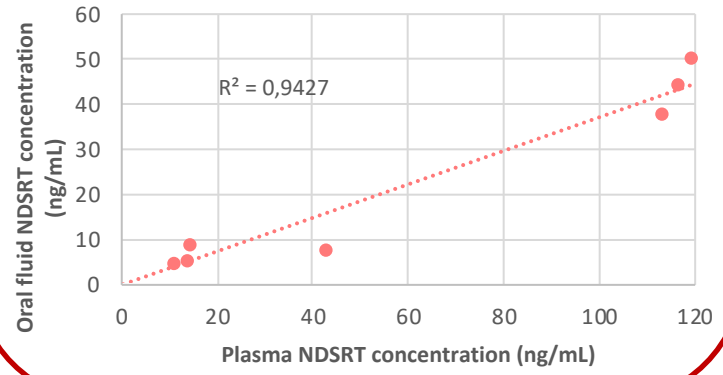
FLU-NFLU



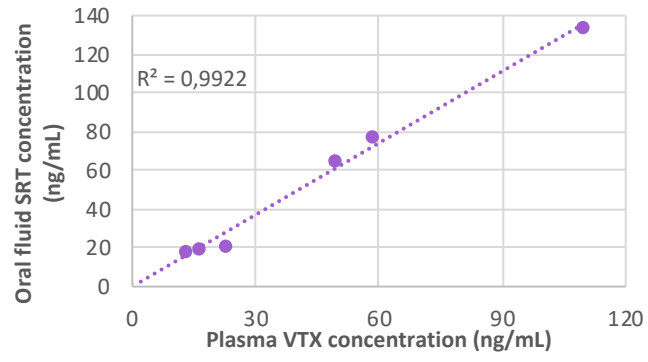


SRT-NDSRT





VTX



OF concentration of AD drugs and metabolites could potentially reflect plasma unbound fraction

Correlation studies need stronger evidence:

- Wider patient population
- Careful evaluation of factors involved in OF alteration/stimulation

CONCLUSION

- **Microsampling** represents a great opportunity and a potentially big improvement in **TDM practice**

Therapy personalisation → **Precision medicine**

- The bioanalytical landscape currently offers **different microsampling solutions** to answer many needs and applications
- HemaPen®, VAMS® and HemaXis® proved to be **suitable and promising strategies** for the **TDM of antidepressant drugs** and their metabolites in whole blood with comparable analytical performances
- The 3 approaches shows **unique features** that could best fit to different applications and requirements
- In addition, **oral fluid** could represent an **alternative/complementary matrix** to obtain additional information for clinicians in a totally non-invasive way

L. Mercolini, M.A. Saracino, M. Protti, Bioanalysis. 7 (2015)1925-1942.

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Laura Loste Cardona - International Erasmus+ student @ PTA Lab

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