UNIMORE
UNIVERSITÀ DEGLI STUDI DI MODENA E REGGIO EMILIA

Centro Interdipartimentale Grandi Strumenti
CINQUANTA

MICROSAMPLING E SPETTROMETRIA DI MASSA PER UN MONITORAGGIO TERAPEUTICO PERSONALIZZATO

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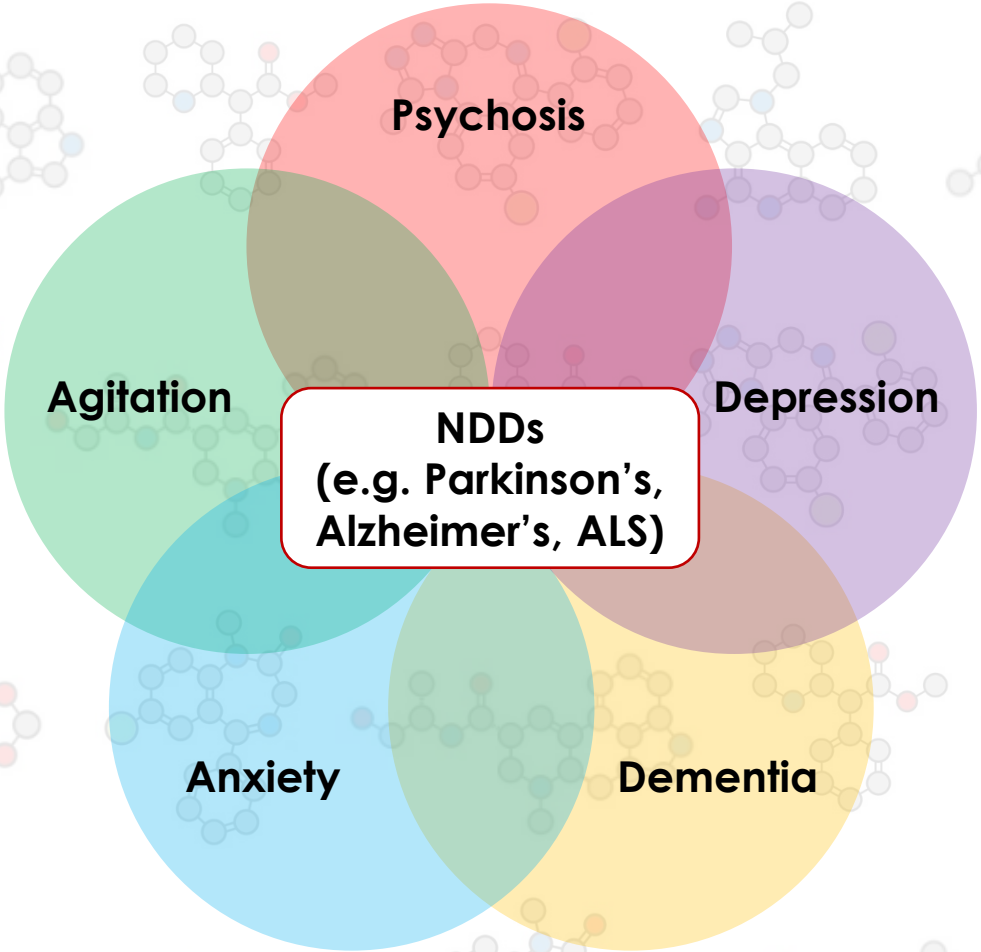
ALMA MATER STUDIORUM UNIVERSITÀ DI BOLOGNA
DIPARTIMENTO DI FARMACIA E BIOTECNOLOGIE

Behavioral and psychiatric symptoms in neurodegenerative disorders



High prevalence in the elderly

- High hospitalisation rates
- Delicate population
- Co-morbidities

Polypharmacy regimens

- Antidepressants
- Antipsychotics
- Mood stabilisers
- Sedative-hypnotics
- Opiates
- Anticholinergics
- Beta-blockers

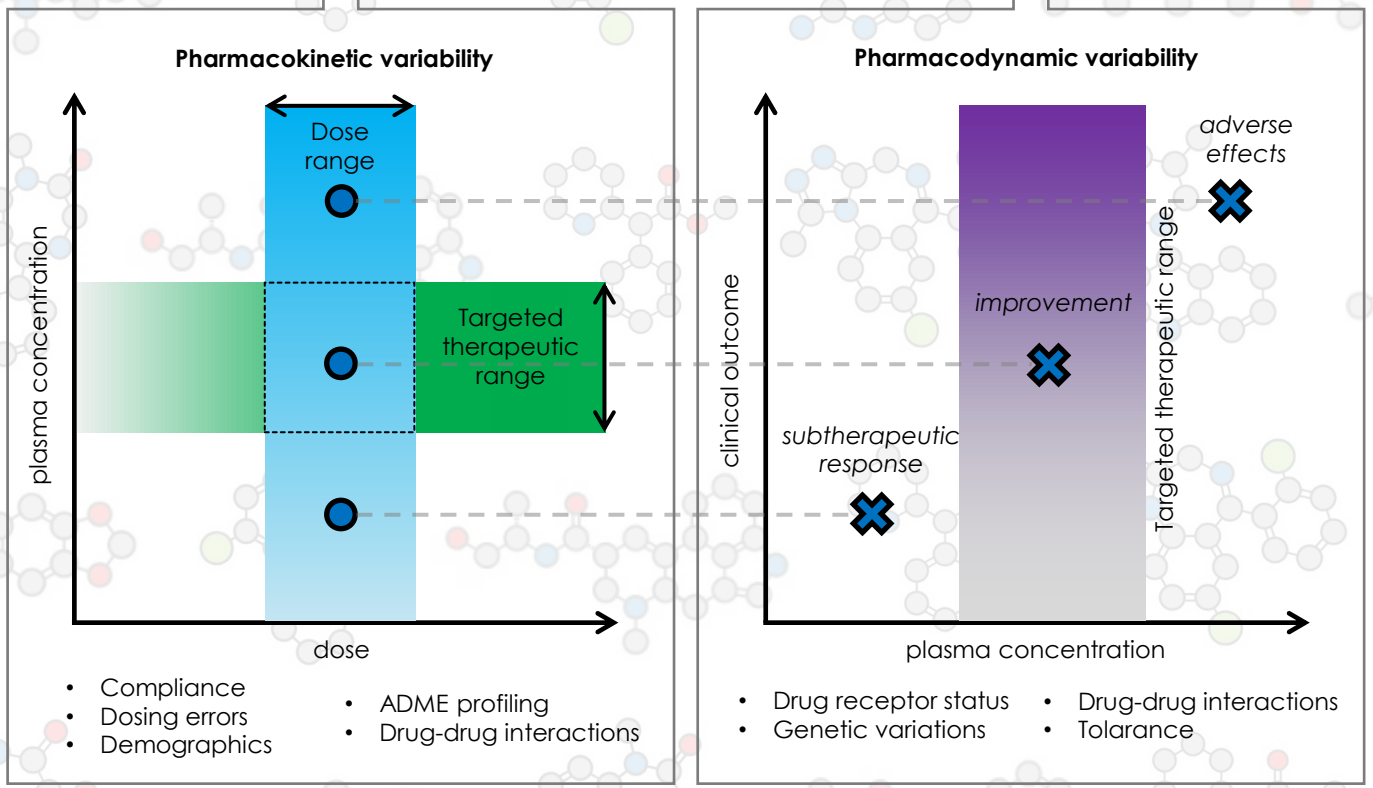
→ **TDM**

C. Hiemke et al., Pharmacopsychiatry. 51 (2018) 9-62
L. Mercolini et al., Int Clin Psychopharmacol. 38 (2023)121-122.

Modern therapeutic drug monitoring

TDM

Drug dosage schedule → Drug plasma concentrations → Clinical outcomes



More frequent time points

Therapy personalisation

Precision medicine

Patient-centric approach

M. Protti et al., Med. Res. Rev. 40 (2020) 1794-1832
V.P. Gaspar et al., J. Mass Spectrom. 56 (2021) e4788

Aims of modern TDM

SUSTAINABILITY

- Reduced use of solvents
- Better waste management
- Logistical savings

ETHICS

- Less invasive procedures
- Increased patient compliance
- Reduce patient discomfort

POINT-OF-CARE

- Home-sampling
- Self-sampling
- Remote collection

PRECISION MEDICINE

- Frequent, effective, feasible patient monitoring for therapy personalisation

MICROSAMPLING for patient-centric TDM

H.Y. Tey et al., J. Chromatogr. A. 1635 (2021) 461731
L. Mercolini, Front. Psychiatry. 13 (2022) 1056380

Microsampling

conventional sampling
> 200 µL

In-tube fluid samples

Wet microsamples

Dried matrix spots

Advanced, volumetric microsampling

microsampling
< 100 µL

L. Mercolini et al., J. Pharm. Biomed. Anal. 130 (2016) 202-219.
V. Avataneo et al., J. Pharm. Biomed. Anal. 166 (2019) 40-51.

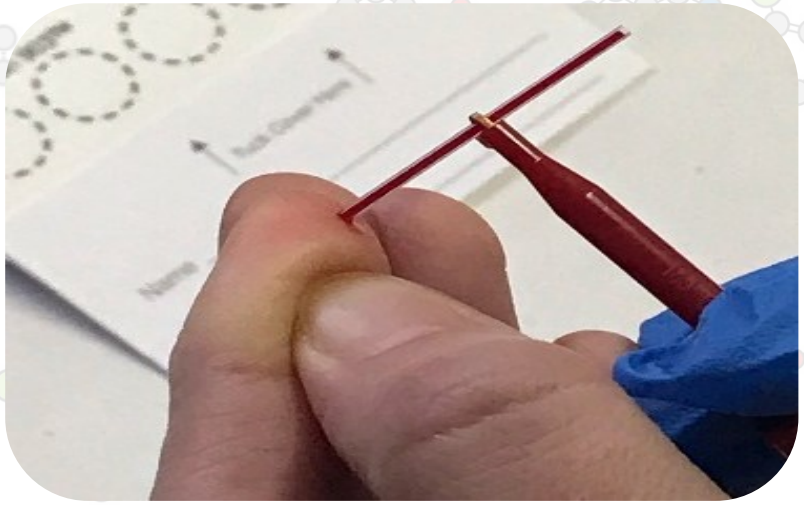
Advantages of microsampling

When properly developed and validated

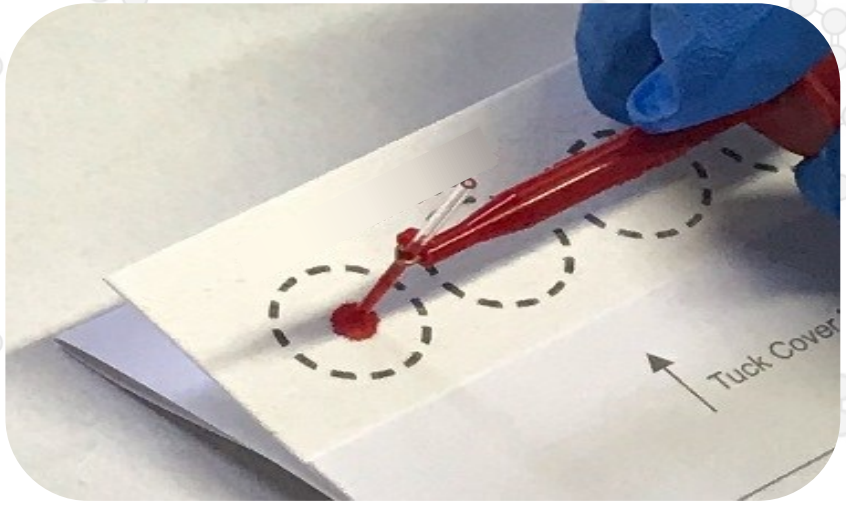
Minimal invasiveness	Sample storage and transport at RT	Minimal sample handling	Stop of bacterial and enzymatic reactions	Fast and simplified procedures	Point-of-care testing
• Finger / heel prick sampling • Increased patient complinace	• No freezer • No dry ice • Overall analysis time and costs considerably reduced	• Lower risk of sample contamination • Lower biohazard • Simplified chain of custody	• Increased analyte stability • Possibility of delayed analysis	• Lower analysis times • High throughput • Cost-effective	• Home-sampling • Self-sampling • Simplified logistic requirements

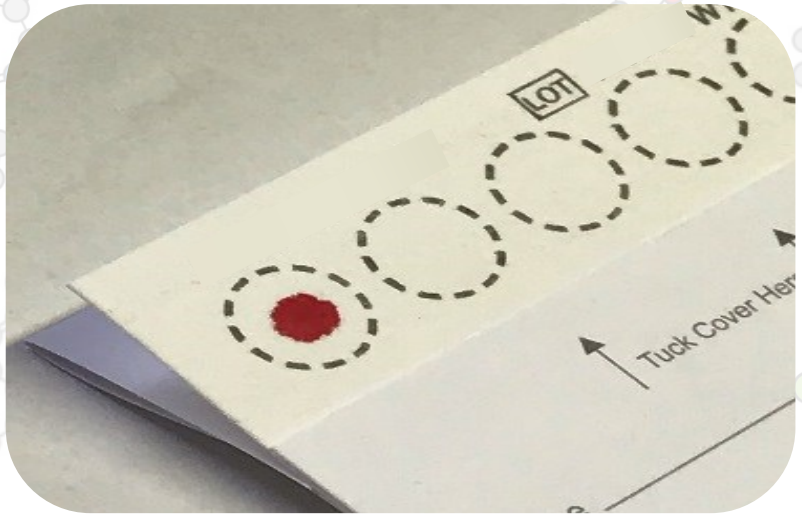
I.R. Müller et al., Expert Rev. Clin. Pharmacol. 16 (2023) 691-701.
M.G.M., Kok et al., J. Pharm. Biomed. Anal. 147 (2018) 288-296.

Early microsampling
Dried blood spots (DBS)



Capillary blood from finger pricking, adsorbed on dedicated support





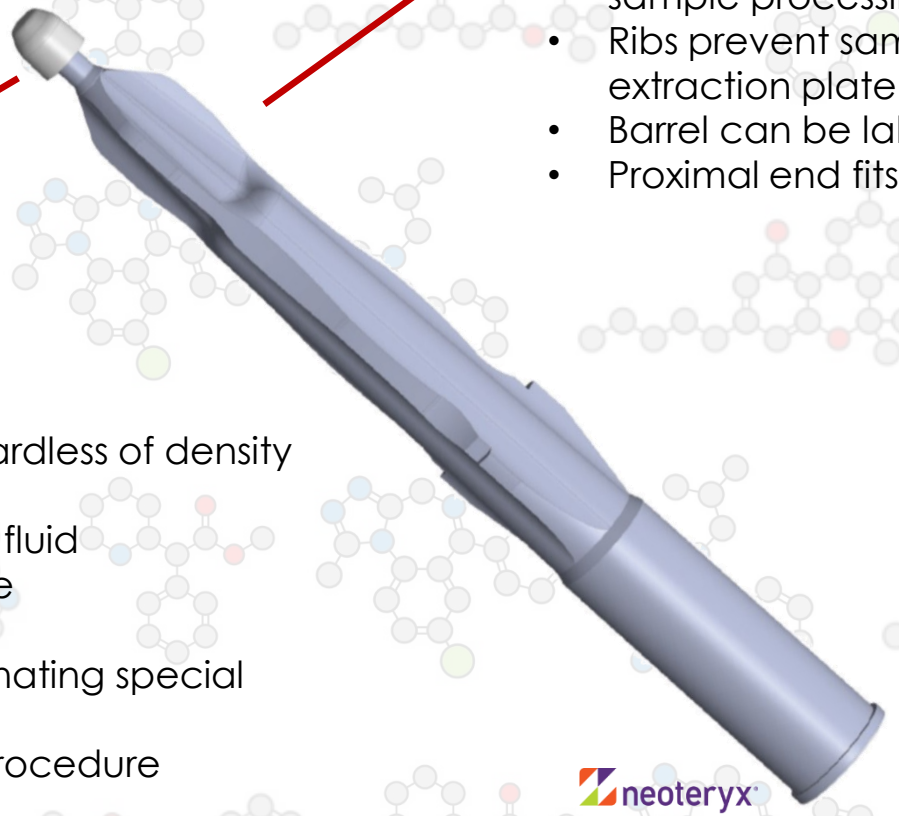
DBS can be stored for months at room temperature with minimal risk of sample degradation: most enzymatic and non-enzymatic reactions are blocked due to water loss

In the 1960s: first use of DBS testing for neonatal screening
In the early 2000s: extension of the use of DBS to TDM

M. Protti et al., J. Pharm. Biomed. Anal.152 (2018) 204-214
X. Xiaoyong et al., Eur. J. Clin. Pharmacol. 79 (2023) 183-193

Advanced microsampling
Volumetric absorptive microsampling (VAMS)

2014: first scientific paper about VAMS technology
2015: first VAMS device marketed

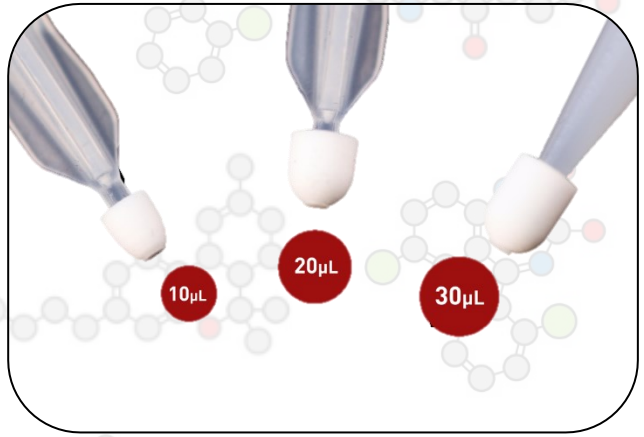


SAMPLER HANDLE

- Leverage existing liquid handler systems to increase sample processing throughput
- Ribs prevent sample from contacting walls of extraction plate and clamshell
- Barrel can be labeled or written on
- Proximal end fits standard 20-200 µL pipette head

SAMPLER TIP

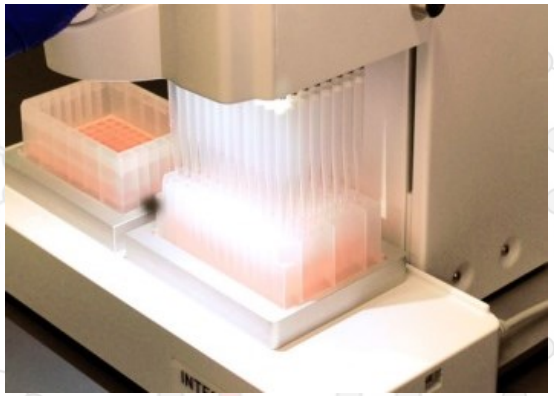
- Collect 10-20-30 µL in a few seconds regardless of density (e.g. blood HCT)
- Hydrophilic porous material rapidly wicks fluid
- Fixed, highly reproducible internal volume
- Dries in ~1 hour or less
- Dried samples are not a biohazard, eliminating special transportation needs and costs
- Extraction follows simple, automatable procedure



M. Protti et al., Anal. Chim. Acta 1046 (2019) 32-47.
V. Londhe et al., J. Pharm. Biomed. Anal. 182 (2020) 113102.

Advanced microsampling

Volumetric absorptive microsampling (VAMS)



- 2/3/4/96 x unique samples (depending on format)
- No haematocrit volumetric bias
- No volcano effect
- Patient-friendly
- Point-of-care testing
- Home- and self-sampling

M.G.M., Kok et al., J. Pharm. Biomed. Anal. 147 (2018) 288-296.
M. Protti, et al., Anal. Chim. Acta 1046 (2019) 32-47.

Advanced microsampling
Volumetric absorptive microsampling (VAMS)

Analytica Chimica Acta 1046 (2019) 32–47

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Tutorial

Tutorial: Volumetric absorptive microsampling (VAMS)

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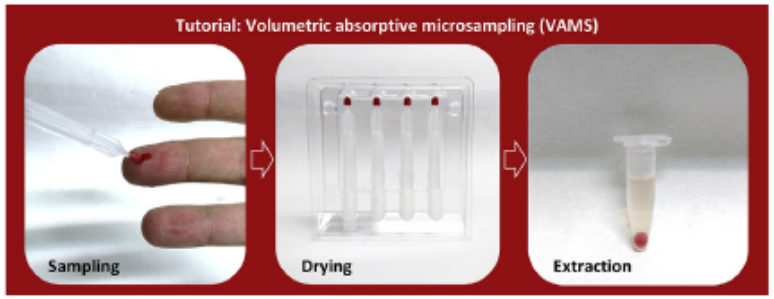
[b] Department for Life Quality Studies, Alma Mater Studiorum - University of Bologna, Rimini, Italy

HIGHLIGHTS

- VAMS is a novel dried microsampling approach for bioanalysis that promises to be more feasible and reliable than DBS.
- VAMS is increasingly used, but applications, limitations and best practices are still not widely studied and known.
- This tutorial aims to explain in detail all stages of VAMS procedure, with many real use cases.

GRAPHICAL ABSTRACT



ABSTRACT

Volumetric absorptive microsampling (VAMS) is a recent microsampling technique used to obtain dried specimens of blood and other biological matrices for application to a plethora of bioanalytical purposes. As such, it can be likened to dried blood spot (DBS) technique that has been in wide use for the last 40 years. However, VAMS promises to bring some significant advantages over DBS, related to sampling volume accuracy, haematocrit (HCT) dependence, pre-treatment and automation. Although some aspects still need to be investigated in depth, VAMS is increasingly recognised as a viable alternative to DBS and other dried microsampling techniques.

In this tutorial, different aspects of VAMS approach are described and discussed, presenting the procedures adopted and the results obtained by those authors who have developed this kind of analytical workflow in the last few years. Hopefully, this will help other scientists to find new solutions to old and recent problems related to microsampling and to produce new, sound and interesting science in this field.

Microsampling and TDM
Monitoring of patients treated with ADAs

Aim

To design, develop and apply VAMS microsampling coupled to LC-MS/MS for TDM of patients undergoing treatment with antidepressant agents (ADAs)

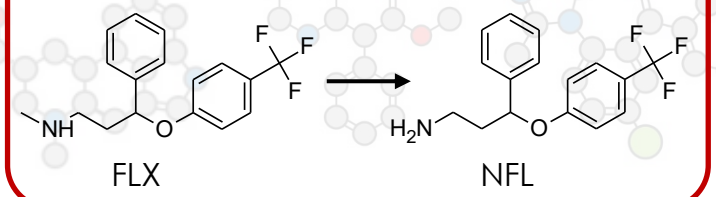
Agenda

- LC-MS/MS analysis and target compounds
- VAMS development and method validation:
  - Selectivity
  - Extraction yield
  - Matrix effect
  - Carryover
  - Calibration
  - Precision
  - Accuracy
  - HCT assays
  - Storage conditions
- Application to patient samples and clinical implications
- Conclusion

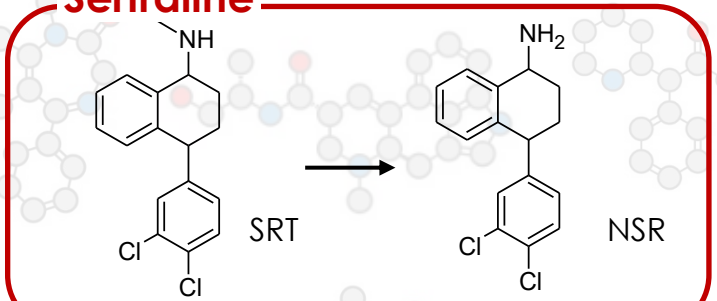
VAMS-LC-MS/MS for the TDM of patients treated with ADAs

Target analytes and LC-MS/MS

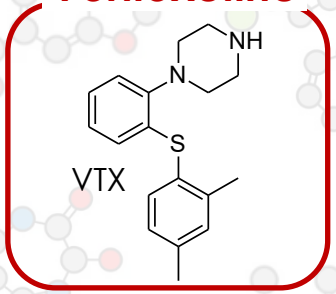
Fluoxetine



Sertraline

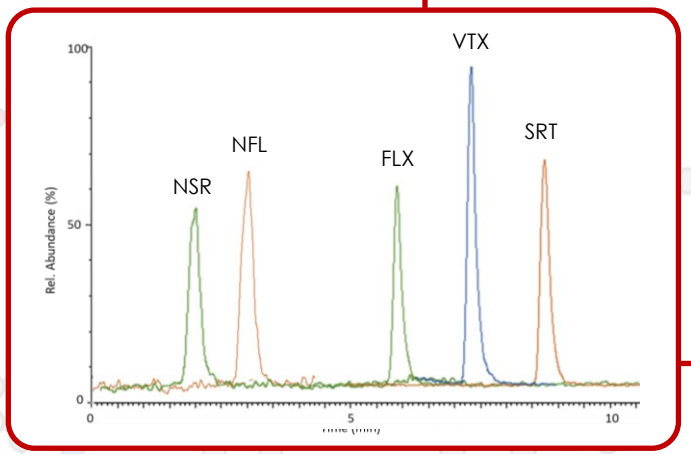


Vortioxetine



Selective serotonin reuptake inhibitors (SSRIs)
Effective against
- Major depressive disorder (MDD)
- Obsessive-compulsive disorder (OCD)
- Panic disorder

Serotonin modulator and stimulator (SMS)
Effective against major depressive disorder (MDD) with efficacy on cognitive symptoms



LC-MS/MS system

Stationary phase: RP C18 + guard column

Mobile phase: 0.1% FA in H_2O / 0.1% FA in ACN gradient elution

Flow rate: 0.25 mL/min

Injection volume: 10 µL

Analyser: Triple quadrupole (QqQ, MS/MS)

Ionisation: ESI+

Acquisition: MRM

FLX	310.13	→	148.0
		→	*44.2*
NFL	296.11	→	134.9
		→	*30.3*
SRT	307.34	→	275.2
		→	*159.1*
NSR	293.17	→	159.0
		→	*129.1*
VTX	299.15	→	150.1
		→	*109.0*

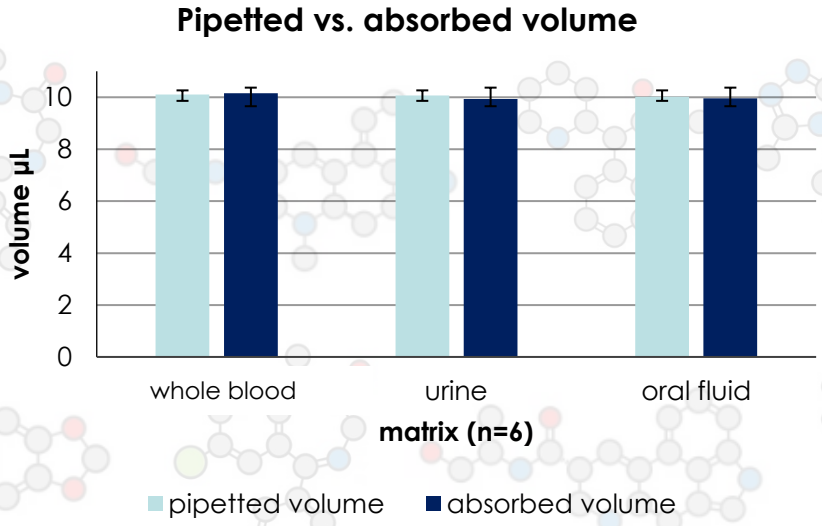
+ deuterated ISs

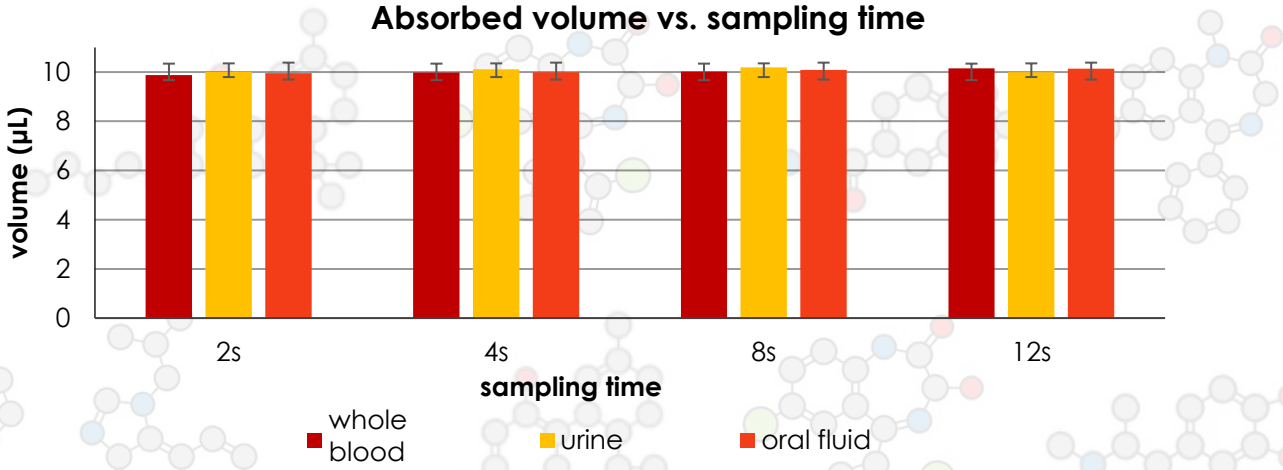
M. Protti et al., Analyst 145 (2020) 5744-5753.

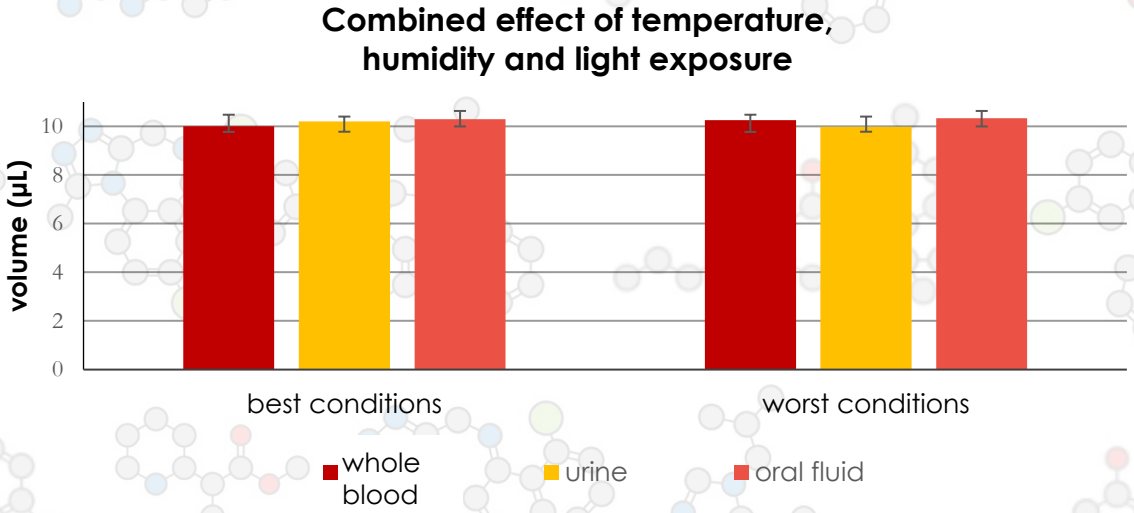
PTA Lab **Michele Protti**

VAMS-LC-MS/MS for the TDM of patients treated with ADAs

VAMS process optimization





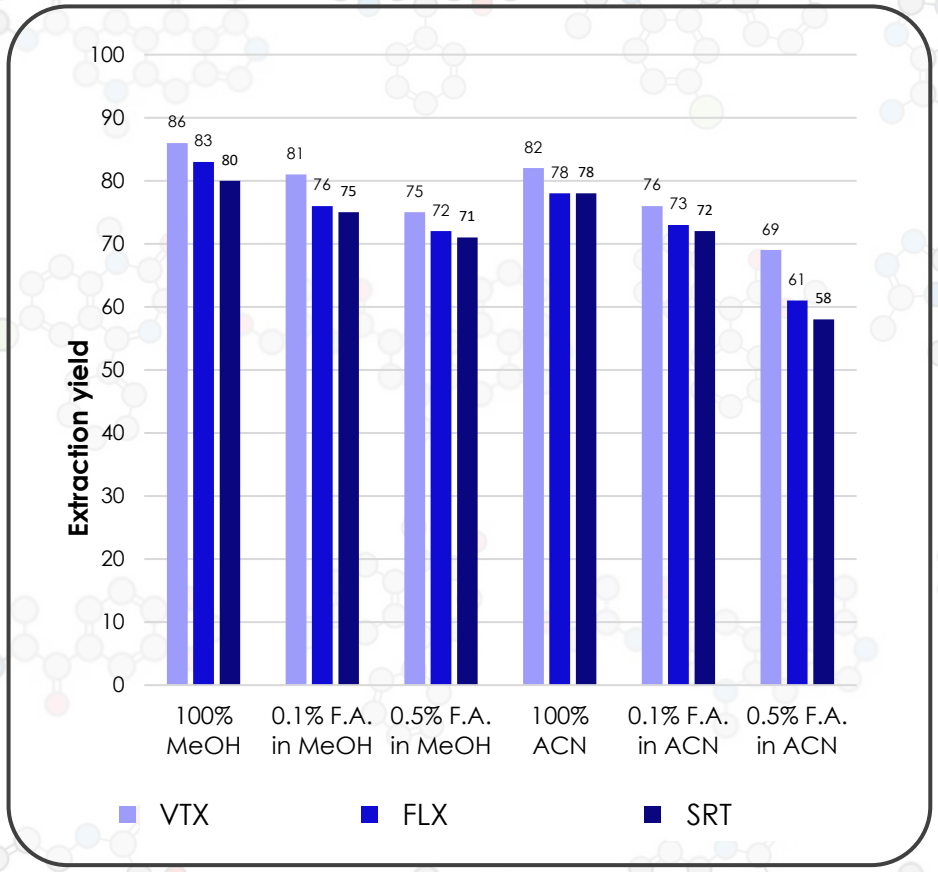


L. Mercolini, et al., J. Pharm. Biomed. Anal. 123 (2016) 123-186.

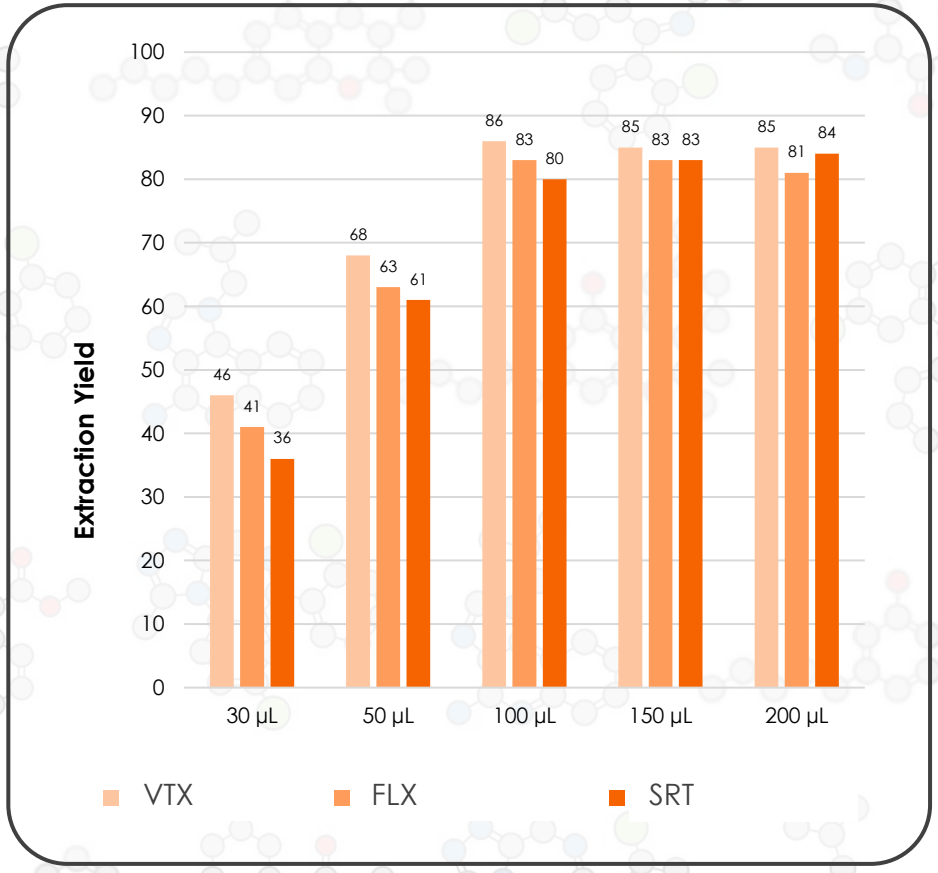
VAMS-LC-MS/MS for the TDM of patients treated with ADAs

VAMS process optimization and validation

Extraction solvent



Extraction volume



Matrix effect
< 4.7% ✓

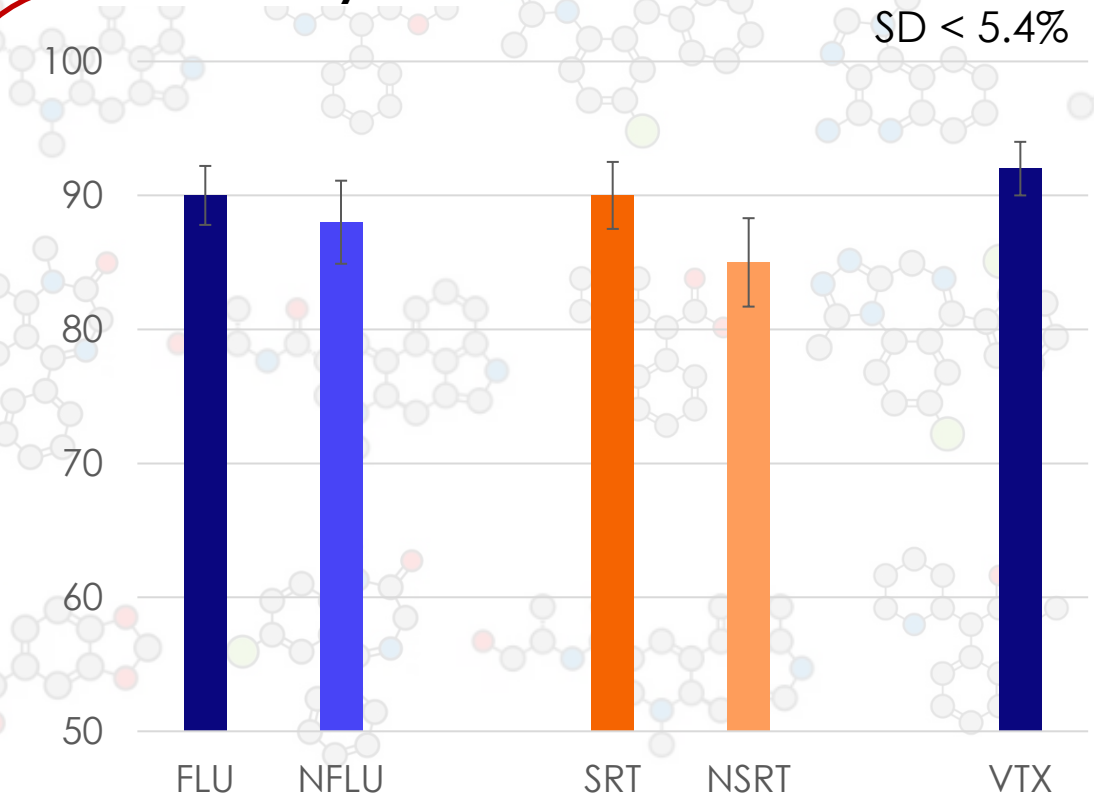
Accuracy
94-103% ✓

Precision
RSD% < 7.6
(< 9.4 for LLOQ) ✓

VAMS-LC-MS/MS for the TDM of patients treated with ADAs

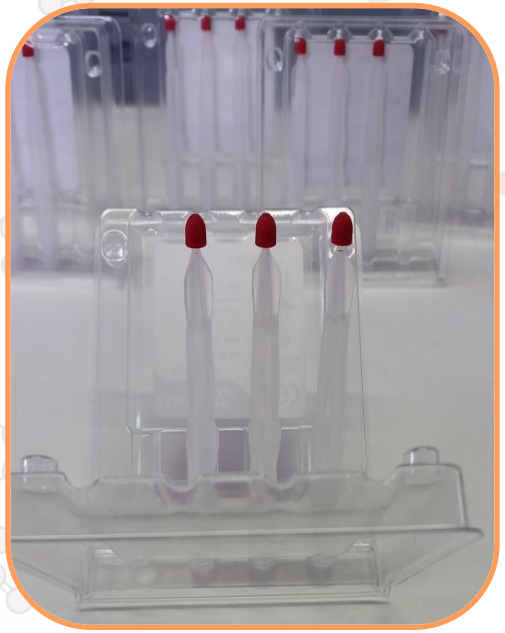
VAMS process optimization and validation

Extraction yield



extraction procedure

- 10-µL detached tip extracted with 100 µL of 0.1% FA in MeOH
- 30 min by ultrasonic-assisted extraction (UAE)
- LC-MS/MS analysis



Matrix effect

< 4.7% ✓

Accuracy

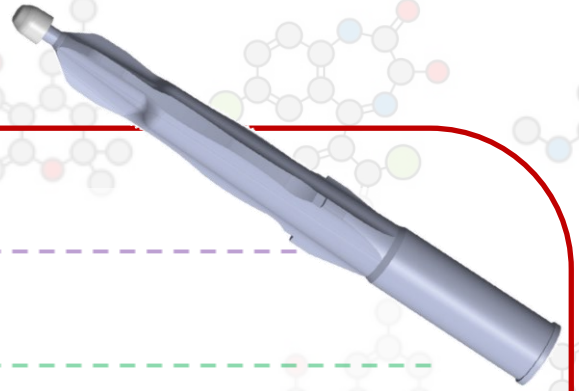
94-103% ✓

Precision

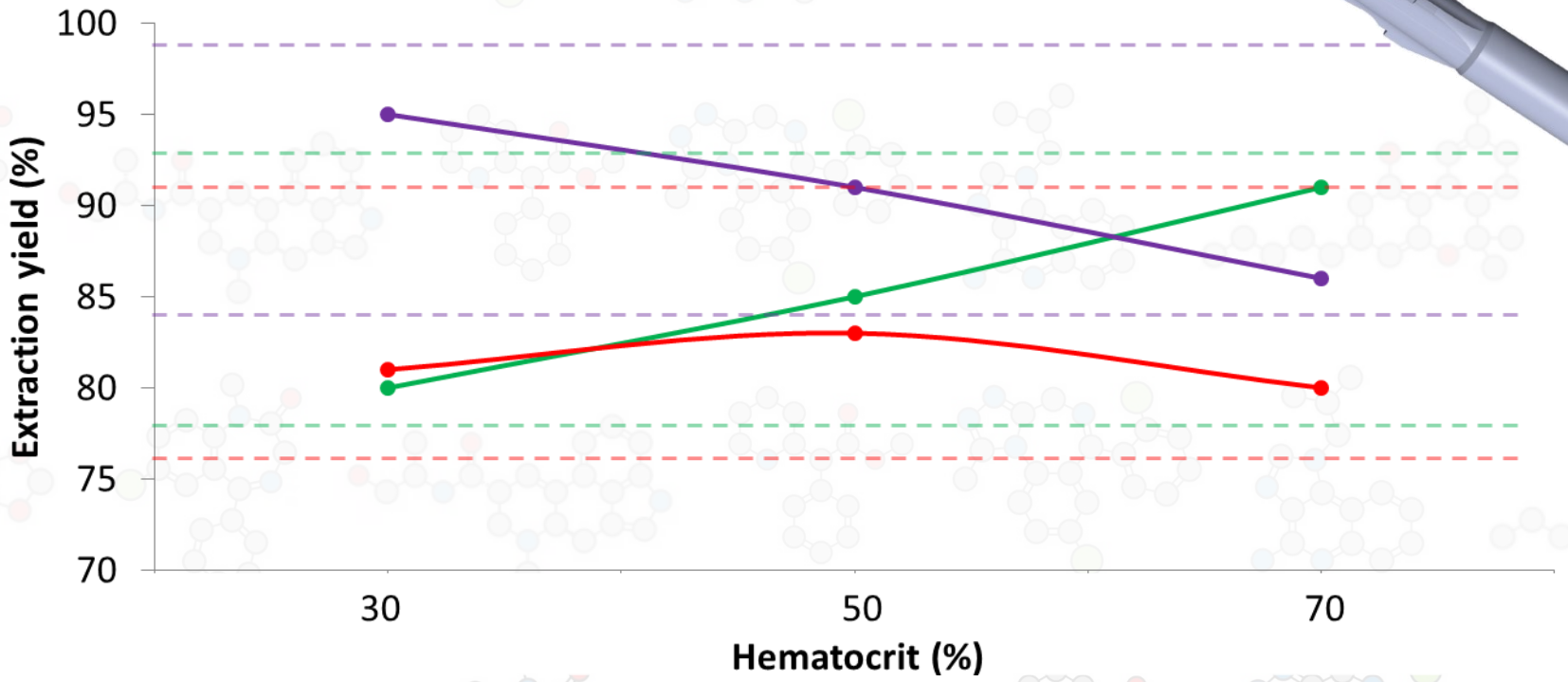
RSD% < 7.6 (< 9.4 for LLOQ) ✓

VAMS-LC-MS/MS for the TDM of patients treated with ADAs

VAMS HCT study



HCT influence on extraction yield



FLU/NFLU

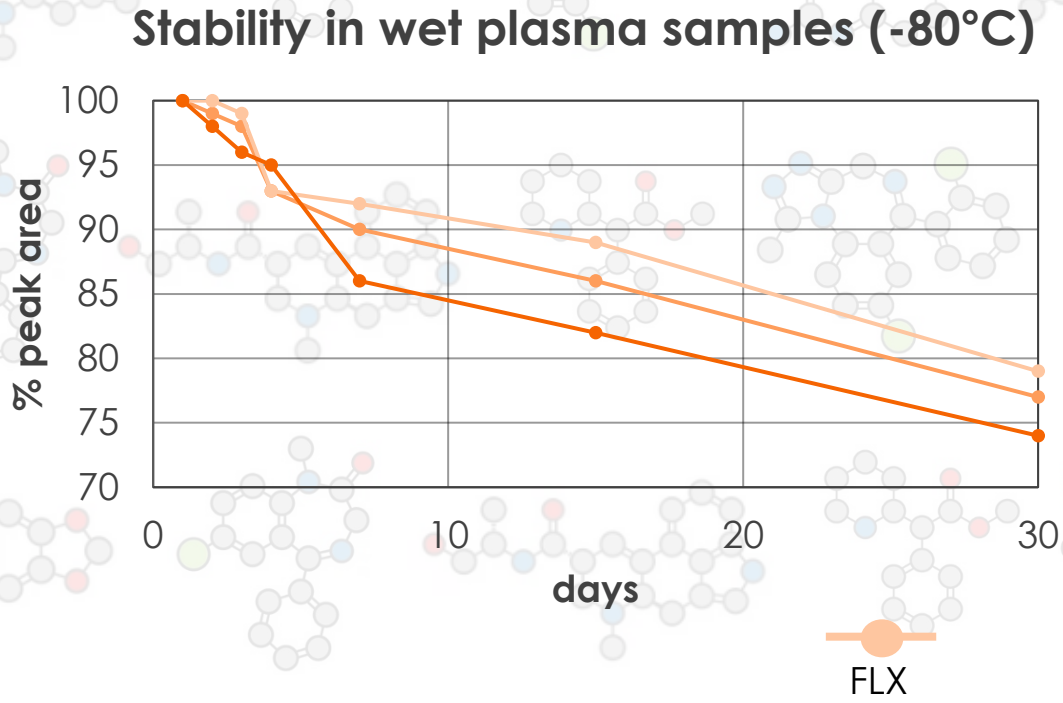
SRT/NDSRT

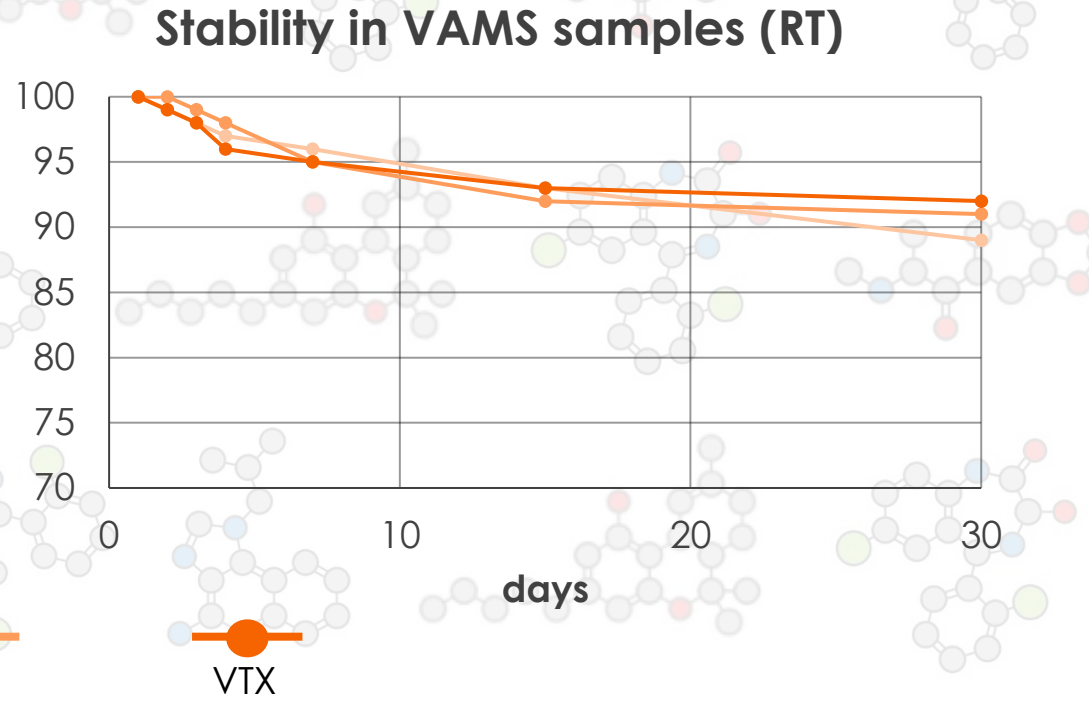
VTX

± 15% acceptance criteria

VAMS-LC-MS/MS for the TDM of patients treated with ADAs

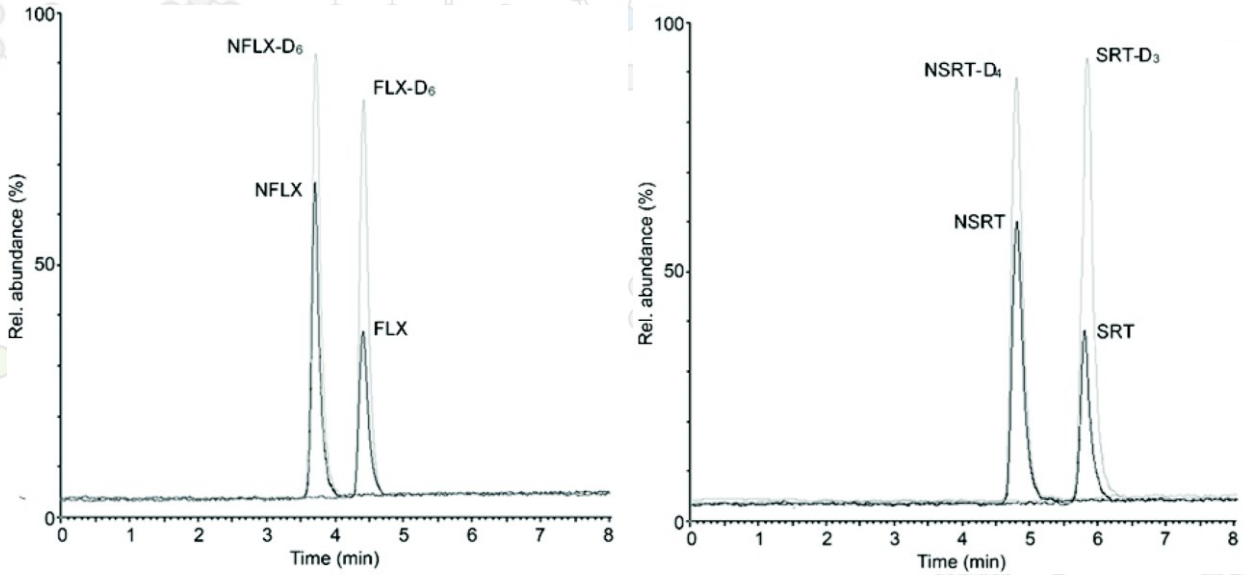
VAMS stability study





VAMS-LC-MS/MS for the TDM of patients treated with ADAs

Method application



LC-MS/MS chromatograms of VAMS samples

Patients treated with **FLX** (60 mg/day) and **SRT** (75 mg/day)

Observed concentrations :

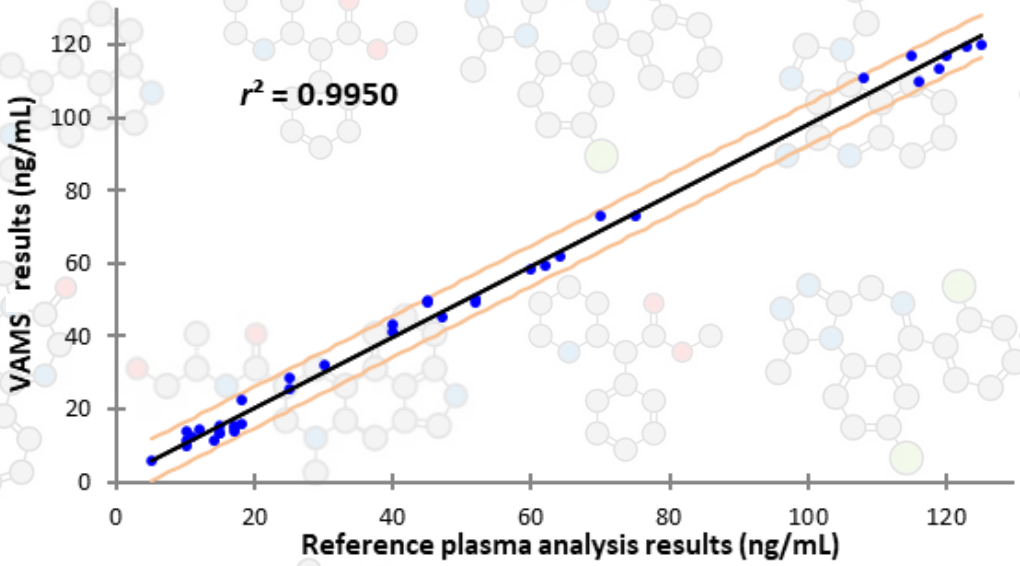
FLX 86.9 ng/mL and NFLX 127.1 ng/mL

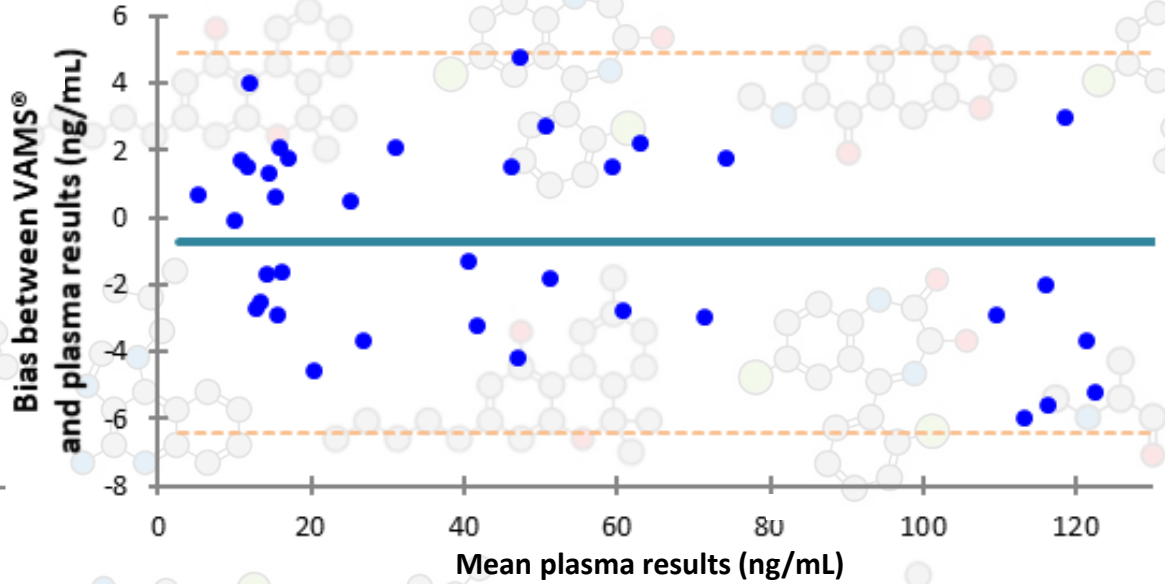
SRT 41.3 ng/mL and NSRT 53.2 ng/mL

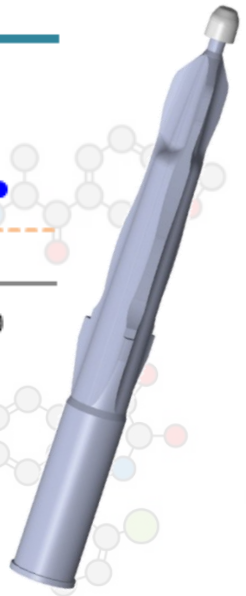
Patient	Treatment, dose (mg/d)	Blood concentration (ng/mL)	
		Parent drug	Metabolite
1	SRT, 75	41.3	53.2
2	SRT, 100	88.6	116.1
3	FLX, 50	49.8	48.3
4	FLX, 60	86.9	127.1
5	VTX, 7.5	9.6	/
6	VTX, 10	13.3	/

VAMS-LC-MS/MS for the TDM of patients treated with ADAs

Method application







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

Perspectives:
New-generation microsampling technologies / 1

Capillary microsampling

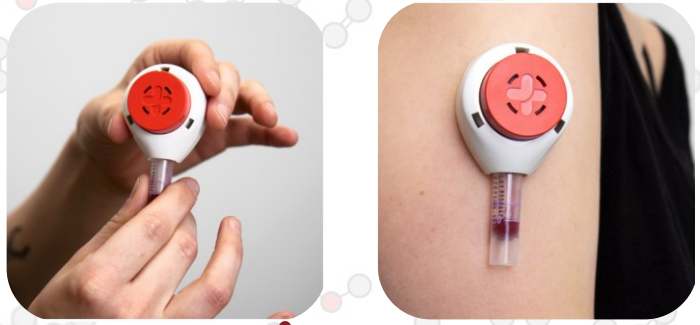
M. Protti et al. Analyst. 145 (2020) 5744-5753

Microfluidic microsampling

C. Marasca et al. Front Psychiatry. 13 (2022) 794609

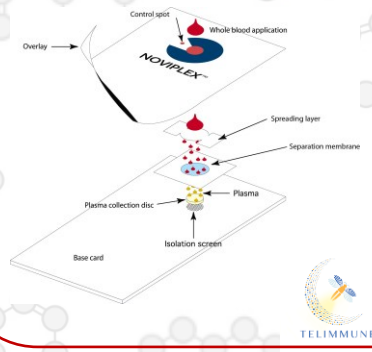
Perspectives:
New-generation microsampling technologies / 2

Microneedle + microfluidic



Tasso

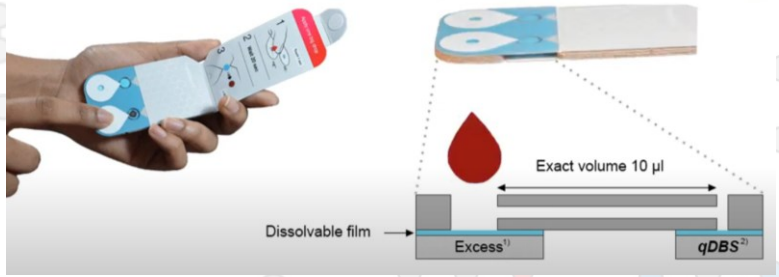
In-situ dried plasma spots



Fluid blood microsampling

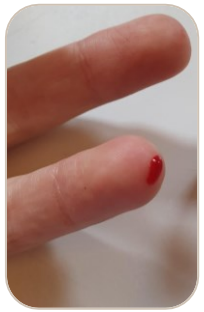
Winnoz

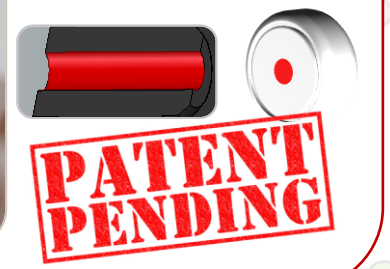
Microfluidic DBS



Capitainer

Lab-made 3D printed devices





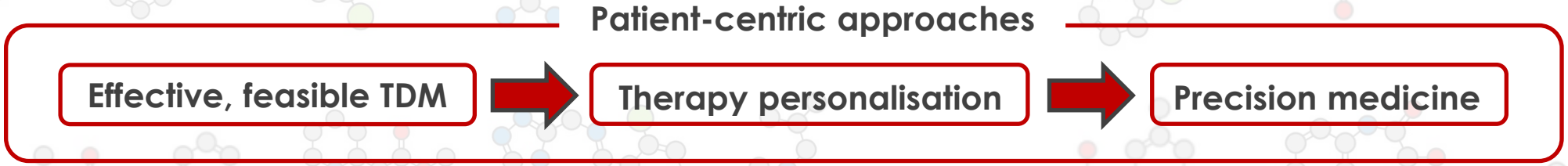
Take-home messages

Advantages of microsampling over classic fluid matrix analysis

- **Higher compound stability** due to water loss: samples are dried, stored and shipped at RT
- **Lower contamination risks** (operator-sample, sample-sample and sample-operator)
- **Lower analysis costs** and **logistical savings** by storage and shipping without the need of cryopreservation and by the small sample volumes
- **Analysis feasibility** even when **small sample amounts** are available and extraction is performed by using **small solvent volumes**
- Significant **simplification of sampling and pretreatment** steps:
  no sub-sampling, centrifugation, isolation, nor further clean-up is needed
- **High-throughput**, **automated** sample handling and analysis
- **Minimal training** required for proper use

Take-home messages

- **Microsampling** coupled to **LC-MS/MS** analysis represents a great opportunity and a potentially big improvement in **TDM practice**



- The proposed VAMS protocol for capillary blood sampling and analysis is **feasible**, **straightforward** and **reliable**
- VAMS proved to be a **suitable and promising strategy** for the **TDM of antidepressant drugs** and their metabolites in whole blood with satisfactory analytical performances
- VAMS allow **accurate collection** of microvolumes of biological matrices with **minimal invasiveness** and in a feasible way, suitable for a **frequent, point-of-care sampling**
- **Reduced sample volumes** make **mass spectrometry** an essential ally of microsampling

Acknowledgements

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