PTA Lab
Pharmaco Toxicological Analysis Laboratory

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

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EBF

7th YSS
(in collaboration with the Universities of Bologna and Ghent

7th Young Scientist Symposium

Reconnecting after Quarantine
10-11 June 2021

Haematic volumetric microsampling for TDM of patients under treatment with lurasidone

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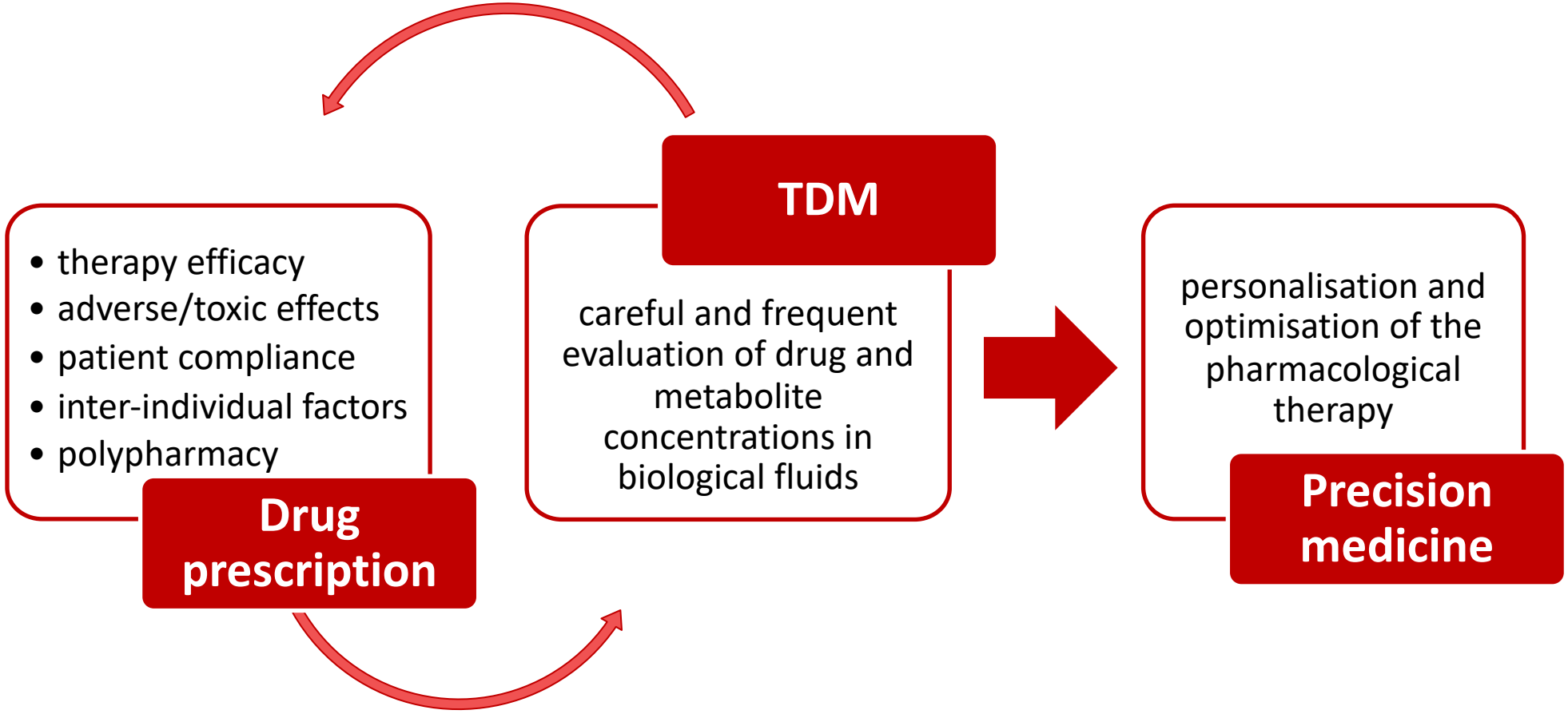
THERAPEUTIC DRUG MONITORING (TDM)



L. Mercolini, M.A. Saracino, M. Protti
Bioanalysis 7(15) (2015) 1925-42.
E. Eliasson, J.D. Lindh, R.E. Malmström, O. Beck, M.L. Dahl.
Eur J Clin Pharmacol. 69 (2013) 1:25-32.

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ANTIPSYCHOTIC TREATMENTS

Antipsychotic drugs (APDs) are widely used to treat psychotic symptoms in the context of schizophrenia, schizoaffective, bipolar or psycho-organic disorders. There are two groups of APDs:

- **neuroleptics** (e.g. haloperidol, fluphenazine and perphenazine)
  → currently are used much less frequently because of their severe side effects (extrapyramidal symptoms, tardive dyskinesia, neuroleptic malignant syndrome)

- **atypical (or second generation) antipsychotics** (e.g. amisulpride, aripiprazole, clozapine, olanzapine, risperidone, quetiapine, ziprasidone)
  → low acute extrapyramidal symptoms and tardive dyskinesia, but also other schizophrenia symptoms (cognitive, aggressive and depressive symptoms)
  → risk of metabolic effects, cardiovascular and cerebrovascular adverse events

TDM is a valid tool for all central nervous system (CNS) drugs, especially for APDs.
Most of these drugs show:
- high interindividual variability in kinetics
- narrow therapeutic range → high risk of toxic/adverse effects

R. Mandrioli, M. Protti, L. Mercolini.
Current Drug Metabolism 16(2) (2015) 141-151.

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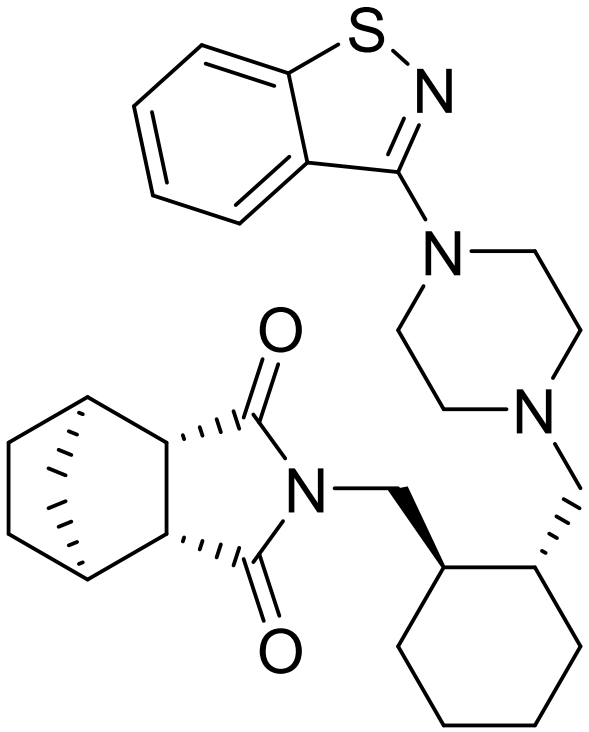
ANTIPSYCHOTIC TREATMENTS



Lurasidone

Lurasidone is an antipsychotic agent used for the treatment of schizophrenia in adults and is approved by the US Food and Drug Administration (US FDA) in 2010 and in 2014 in the European Union.

Unlike other atypical antipsychotics (e.g., risperidone, olanzapine, clozapine), lurasidone is **safe and tolerable** with higher affinity for dopamine D_2 and serotonin 5-HT_{1A} receptors but has no affinity towards muscarinic M_1 and histamine H_1 receptors.

R. Mandrioli, M. Protti, L. Mercolini.
Current Drug Metabolism 16(2) (2015) 141-151.
F. Corponi, C. Fabbri, I. Bitter, S. Montgomery, E. Vieta, S. Kasper, S. Pallanti, A. Serretti.
European Neuropsychopharmacology 29(9) (2019) 971-985.

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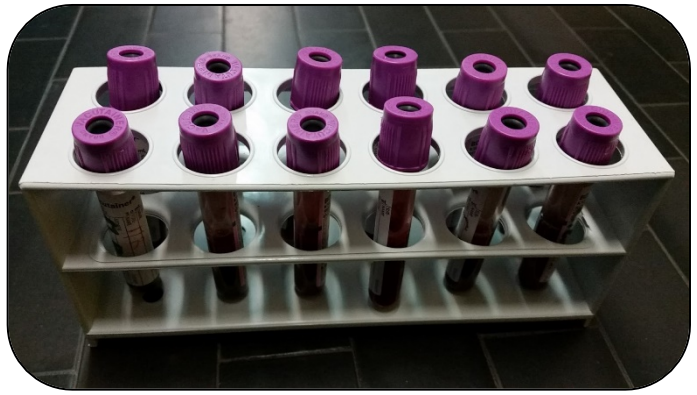
MICROSAMPLING



Conventional in-tube wet samples

dried microsampling

- sample volume decrease (> 200 mL → < 50 µL)
- minimally invasive capillary blood collection
- home- and self-sampling procedures
- storage and transport at RT, cryopreservation is not required
- analyte stability is potentially higher
- lower biological/cross contamination risks
- high-throughput pretreatment and analysis
- implementation in automated systems

C. Marasca, M. Protti, R. Mandrioli, A.R. Atti, A. Armirotti, A. Cavalli, D. De Ronchi, L. Mercolini. Journal of Pharmaceutical and Biomedical Analysis 188 (2020) 113384.
H.Y. Tey, H.H. See. J Chromatogr A. 1635 (2021) 461731.

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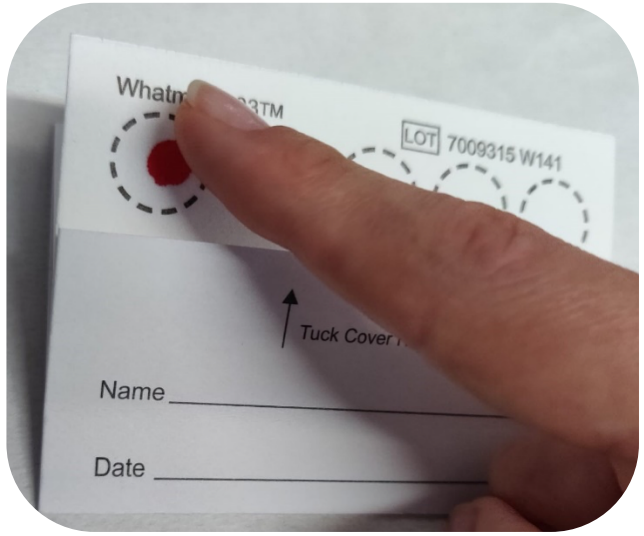
DRIED BLOOD SPOT (DBS)

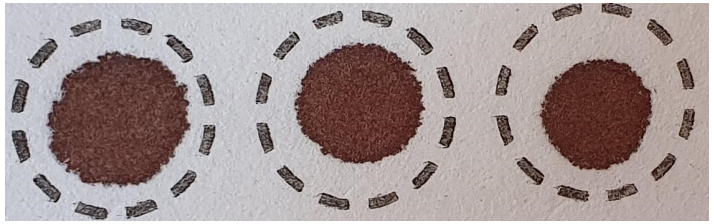


Original pictures from PTA Lab

→ **sampling inaccuracy**
of the collected blood volume

→ **haematocrit (HCT) issue**
influences size and homogeneity of the spot

HCT 20 % **HCT 45 %** **HCT 70 %**



VOLUMETRIC BIAS

UNREPRODUCIBLE SAMPLING

UNRELIABLE QUANTITATIVE ANALYTICAL DATA

M. Protti, R. Mandrioli, L. Mercolini.
Analytica Chimica Acta 10476 (2019) 32-47.

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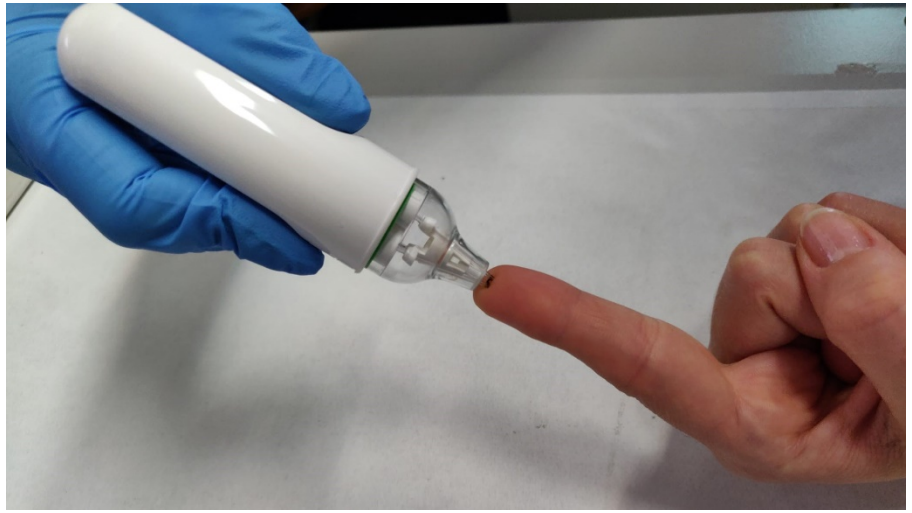
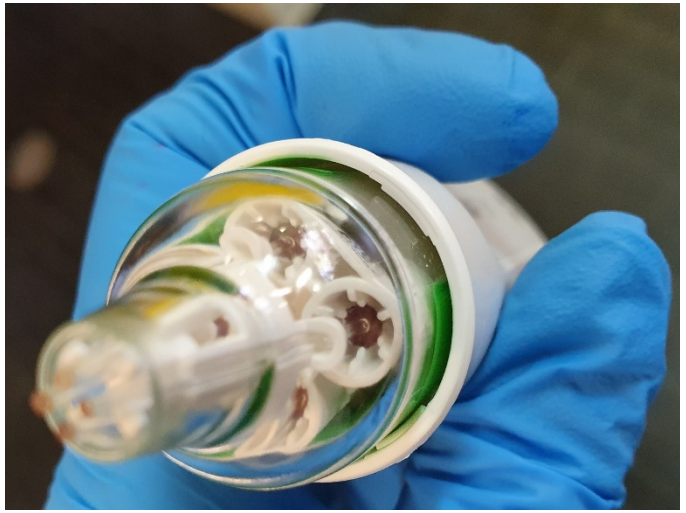
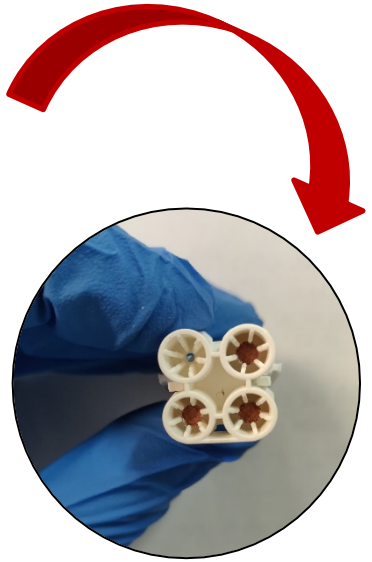
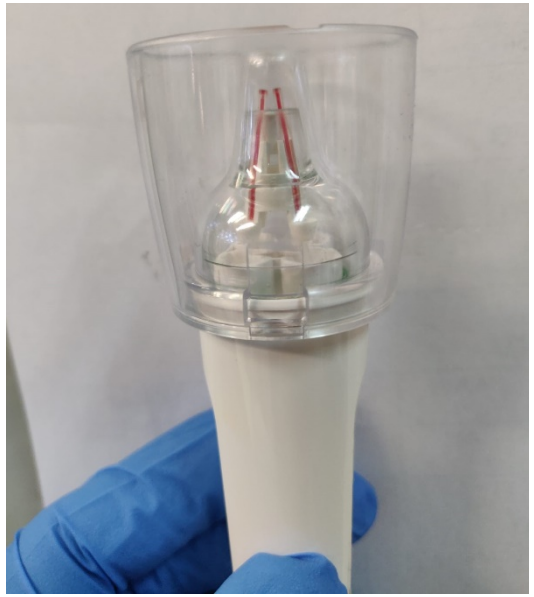
CAPILLARY VOLUMETRIC MICROSAMPLING TECHNOLOGY









Original pictures from PTA Lab

- pen-like design facilitates a patient-friendly capillary whole blood microsampling
- volumetric accuracy by end-to-end EDTA-coated glass microcapillaries
- simultaneous collection of 4 replicates
- generation of DBS on pre-punched disks
- whole blood collection HCT-independent

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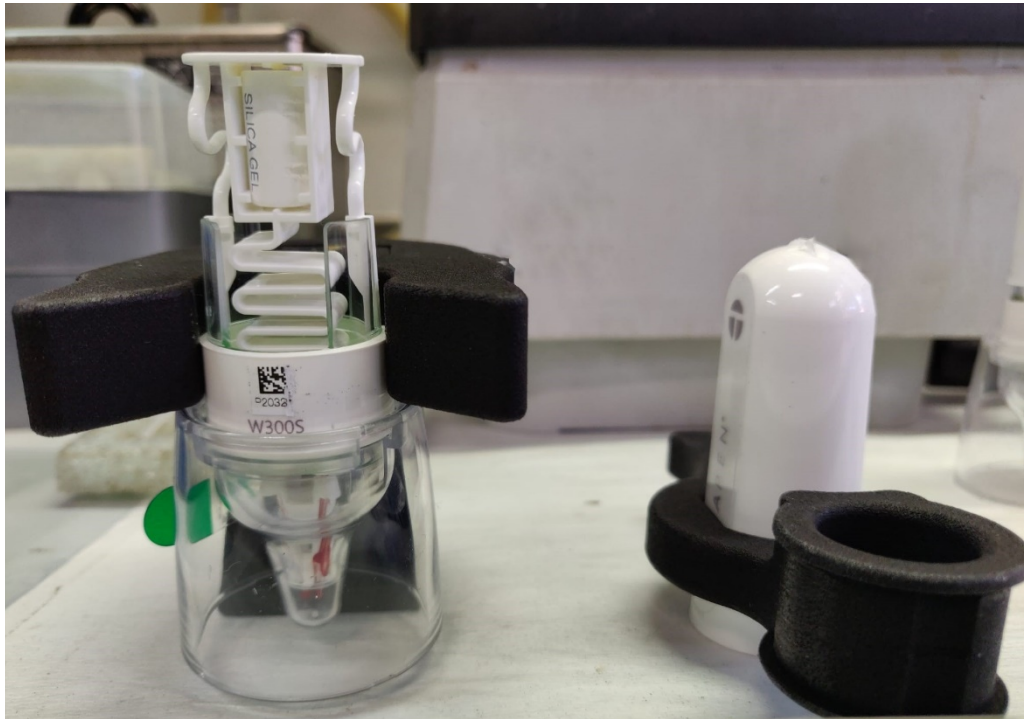
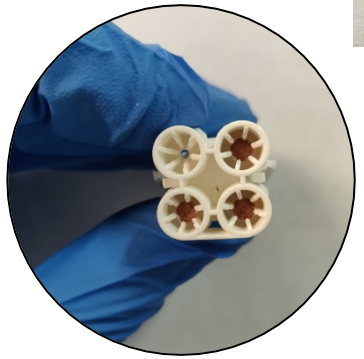
CAPILLARY VOLUMETRIC MICROSAMPLING TECHNOLOGY





Original pictures from PTA Lab

- single-use device
- tamper-proof, needing a specific opening tool for the retrieval of DBS samples
- integrated storage, desiccant included for granting sample integrity and preventing any contamination.

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AIM OF THE RESEARCH WORK

Sampling

optimisation of capillary whole blood collection and drying time

Pretreatment

design of a fast and straightforward extraction procedure

Analysis

development of a sensitive but feasible LC-UV-MS/MS method for lurasidone analysis

Validation

extraction yield
precision
linearity
matrix effect, carry over
selectivity
accuracy

Application

TDM of psychiatric patients under lurasidone therapy

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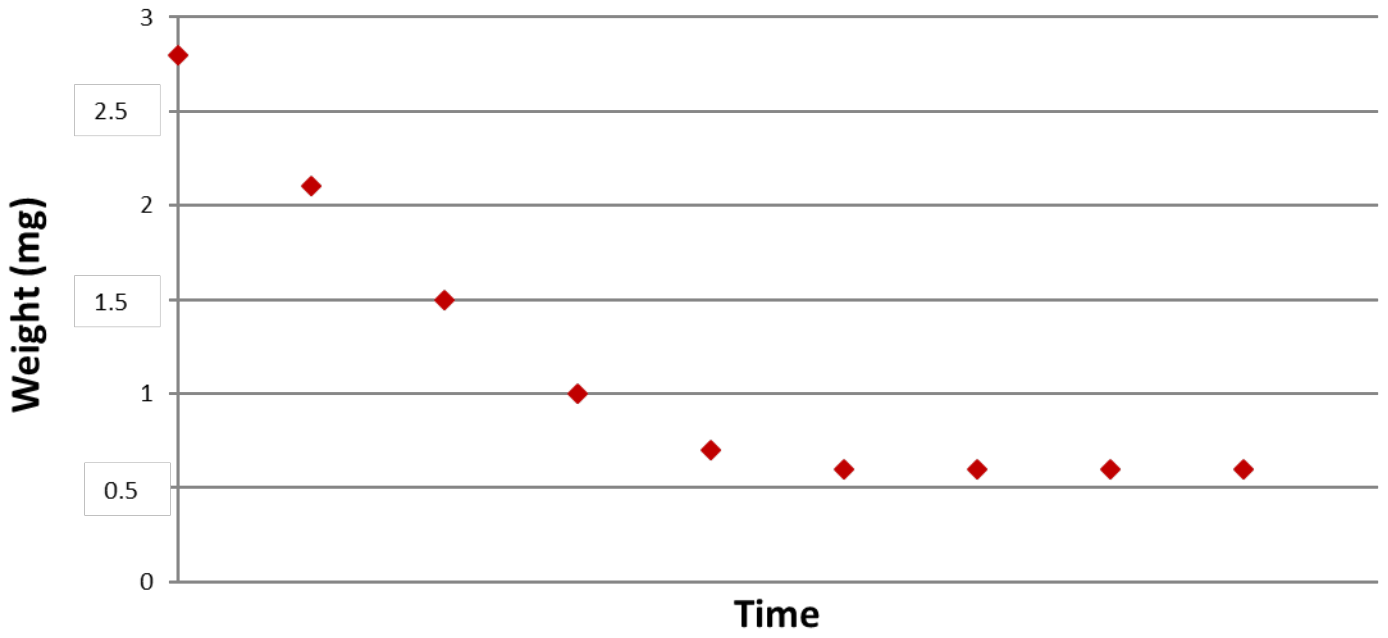
DRYING TIME

Optimisation of drying time of the blood spots



→ After collection, each spot was weighed at regular intervals until a constant weight was reached

Manuscript in preparation, May 2021

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PRETREATMENT & LC-UV-MS/MS SYSTEM

Extraction procedure

- Extraction with organic solvent
- Ultrasound-assisted extraction (UAE)
- Injection into the LC system

Method parameters

- **Stationary phase:** RP C18 + guard column
- **Mobile phase:** FA in H_2O/FA in ACN
- **Flow rate:** 0.3 mL/min
- **Injection volume:** 10 µL
- **MS Analyser:** triple quadrupole (MS/MS)
- **Ionisation:** ESI+

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VALIDATION RESULTS

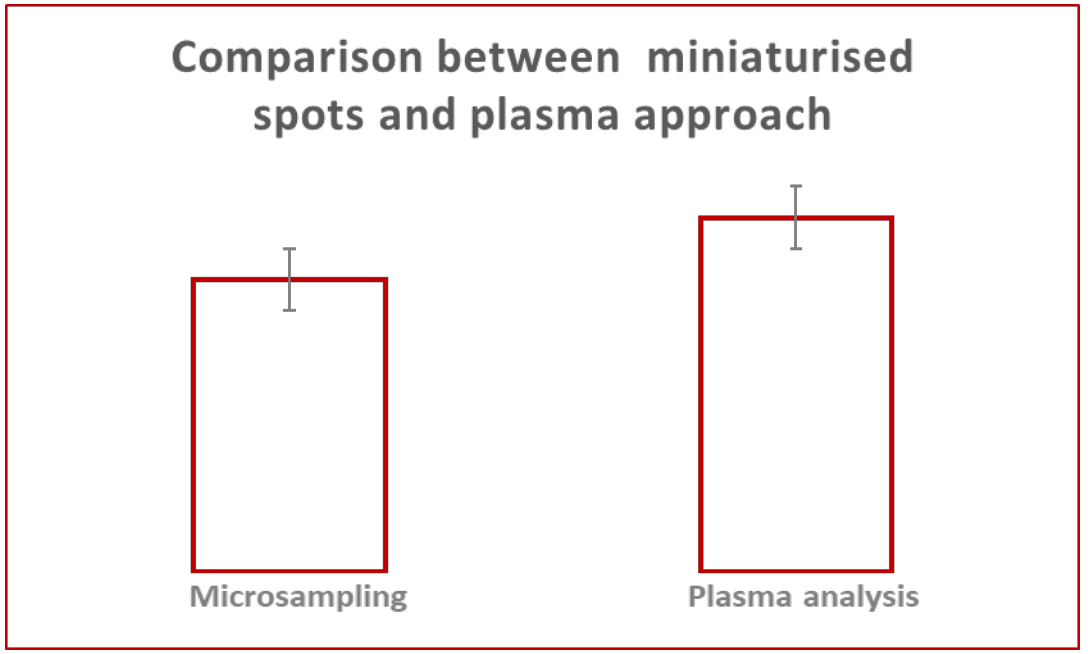
Selectivity (n=6)
Extraction yield (n=3)
Matrix effect (n=3)
Carryover (n=3)
Calibration (5 conc., n=3)
Precision (n=6)
Accuracy (n=3)

Original pictures from PTA Lab

Manuscript in preparation, May 2021

MICROSAMPLING APPROACH APPLICATION

- Capillary volumetric technology was exploited for TDM of psychiatric patients under treatment with lurasidone
- Simultaneous collection of 4 samples was carried out in order to obtain 4 identical DBS, regardeless of whole blood density
- To demonstrate the reliability of the proposed miniaturised strategy, the quantitative results of the novel microsampling approach were compared with those of reference plasma analysis, showing accordance



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CONCLUSION

- The miniaturisation of sampling, pretreatment and analysis steps represents a reliable approach to be able to streamline the whole analytical methodology and develop high-throughput procedures

- The capillary volumetric platform allows an accurate whole blood collection of 4x2.74 µL replicates, HCT-independent, directly by fingerprick, demonstrating to be a suitable and patient-friendly tool for TDM purposes

- The original miniaturised strategy based on an innovative volumetric blood microsampling technology, fast and feasible pretreatment and a sensitive LC-MS/MS method was designed for the analysis of the antipsychotic drug lurasidone. The proposed methodology showed consistent quantitative results when they are compared with those obtained from reference plasma analysis

- Microsampling proved to be a great opportunity to improve TDM practice and a promising alternative to classical plasma procedure thanks to the reduced invasiveness of blood sampling, straight-forward sample handling and processing, in order to perform a more frequent TDM reaching a personalisation of pharmacological therapy in the perspective of precision medicine

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