PART II: SAMPLING AND SALIVA SAMPLE PRETREATMENT

PROJECT TEAM

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The aim

- The aim of this study was to develop microextraction suitable for isolation of eight tricyclic antidepressants (TCAD) from oral fluid:

Imipramine (D)

Desipramine (D/M)

Doxepin (D)

Norclomipramine (M)

Nortriptyline (D/M)

Nordoxepin (M)

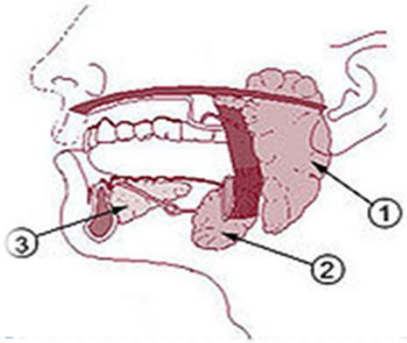
Clomipramine (D)

Amitriptyline (D)

D – parental drug, M - methabolite

Oral fluid in toxycology

- saliva is produced constantly (1-2 L per day)
- sample collection is non-invasive, simple, painless and child or older person friendly
- saliva collection is inexpensive
- saliva can be collected in any place
- density ~1.01 g/ml, viscosity ~ 0.8 mP·s
- composition:
  - 94 – 99% of water
  - protein substances (enzymes, mucins, immunoglobulins)
  - non-protein (uric acid, cholesterol, some vitamins)



1, 2, 3- Salivary glands

Oral fluid sample collection

- PROCEDURE:
  - at least one hour after eating, drinking, smoking, rinsing mouth and brushing teeth
  - movements of volunteer's face and mouth should be minimalized about 5 minutes before collecting samples to get only the unstimulated ones
  - saliva was collected in the morning to disposable test tubes from healthy volunteers
- samples were stored in a refrigerator (+4°C) and used the same/next day

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Sample pretreatment

Pretreatment of saliva samples moved out proteins, remains of food, air bubbles and other interferences:

- oral fluid samples were spiked with TCDA drugs (c=2 ng·mL^{-1})
- samples were sonicated for 50 min and then centrifuged for 10 min, 10000 rpm at (-1°C)
- supernatant was collected and diluted with phosphate buffer pH 7.4 (2:1, v/v)

Optimization steps – achievements

HPLC-UV	• optimization MEPS extraction procedure: C_8/SCX sorbent, type and volumes of solutions
CE-UV	• saliva sample preparing: centrifugation and direct injection to the capillary
LC-MS	• the lowest limit of quantification which allow to examinated real saliva samples

Micro**e**xtraction by **P**acked **S**orbent

- MEPS is the miniaturization of conventional SPE packed bed devices
- the MEPS Barrel Insert and Needle Assembly (BIN), contains the stationary phase, is built into the syringe needle

eVol: SEMI-AUTOMATIZATION:

•improves MEPS extraction because of possibility to programming the extraction procedure including controlling the speed and volume of each step

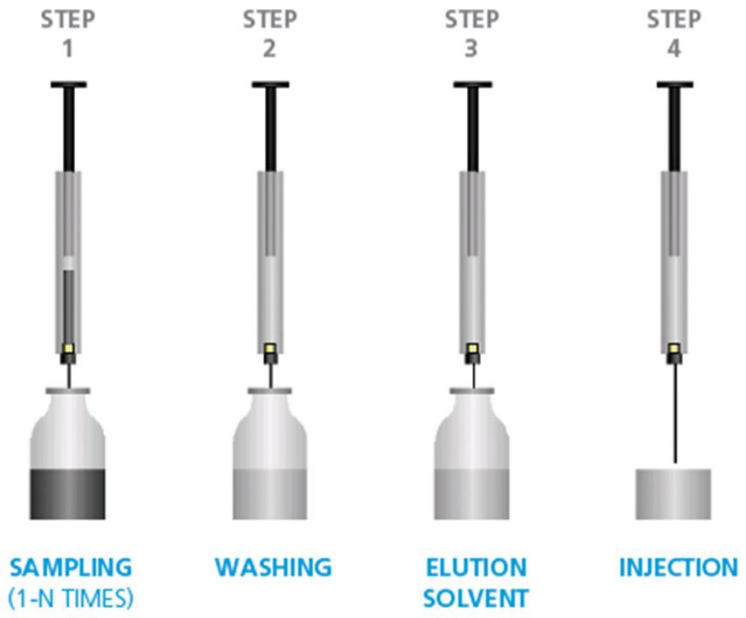
•received results are more reproducible ($RDS_{eVol} < RSD_{manual\ MEPS}$)

•reduced possible mistakes during the manual MEPS procedure

eVol® XR

Micro**e**xtraction by **P**acked **S**orbent

MEPS procedure

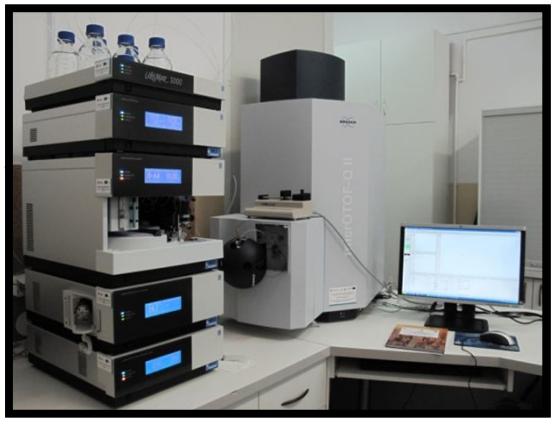


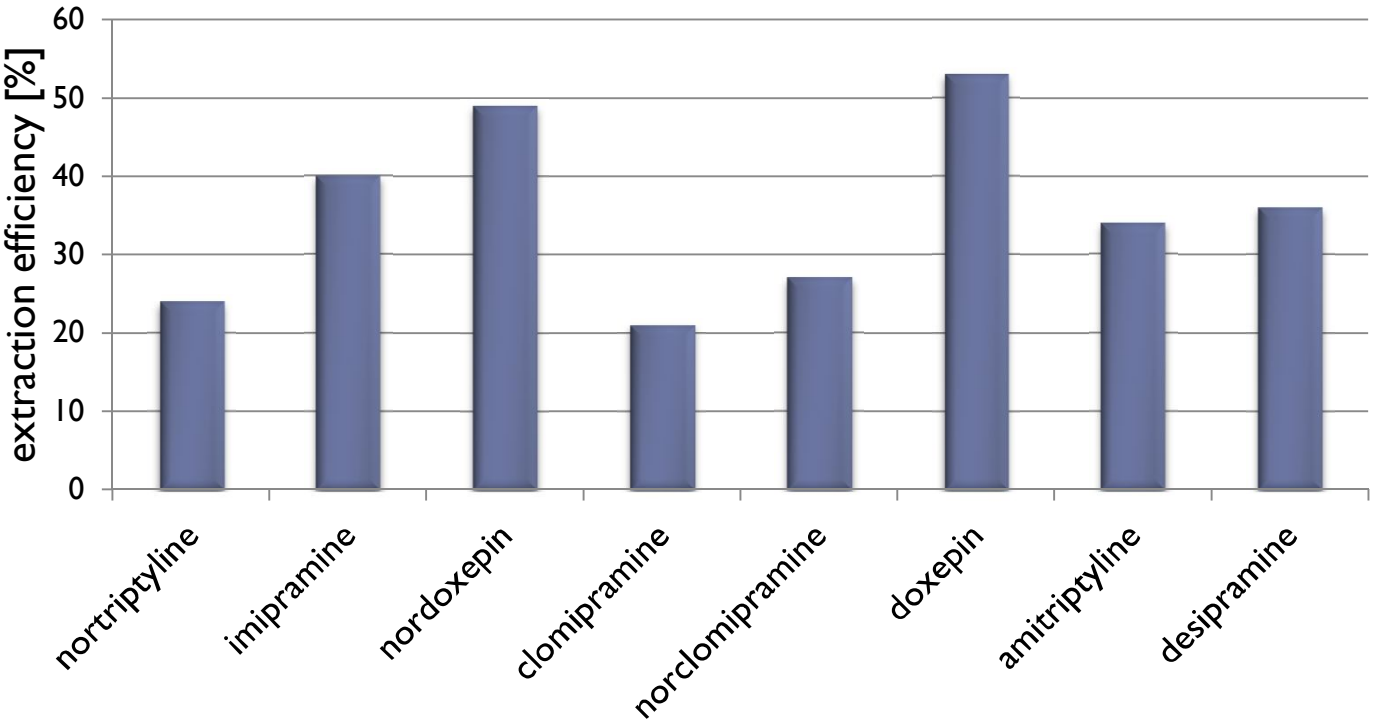
Steps of extraction

- Conditioning:
  - methanol
  - 0,1% formic acid
- Sampling: *3x50 µL*
- Washing:
  - 0,1% formic acid
- Drying
  - air
- Elution: *4x50 µL*
  - methanol:water:$NH_{3(aq)}$
- Washing:
  - methanol

Results

- analysis by Liquid Chromatography coupled with Mass Spectrometry LC-MS





%RSD
2 – 15%

Validation of MEPS/LC-MS method

- oral fluid samples were spiked with TCADs and their metabolites at three concentration levels:
  - 1 ng·mL^{-1}
  - 2 ng·mL^{-1}
  - 3 ng·mL^{-1}

Concentration in real saliva samples – examples:
Ami[1]: 20,2 – 33,1 ng·mL^{-1}
Dox[2]: 3,7 – 14,0 ng·mL^{-1}

Parameter	Imi	Nort	Clo	Nord	Norc	Dox	Ami	Des
LOD [ng·mL^{-1}]	0,2	1,5	0,5	0,2	0,2	0,2	0,3	0,3
LOQ [ng·mL^{-1}]	0,6	4,9	1,6	0,8	0,6	0,6	0,9	0,9
Precision, RSD [%]	5,4	8,4	6,7	6,8	2,9	5,9	9,1	10,3
Accuracy, RE [%]	11,6	13,0	11,6	12,7	11,3	6,4	-3,3	12,7

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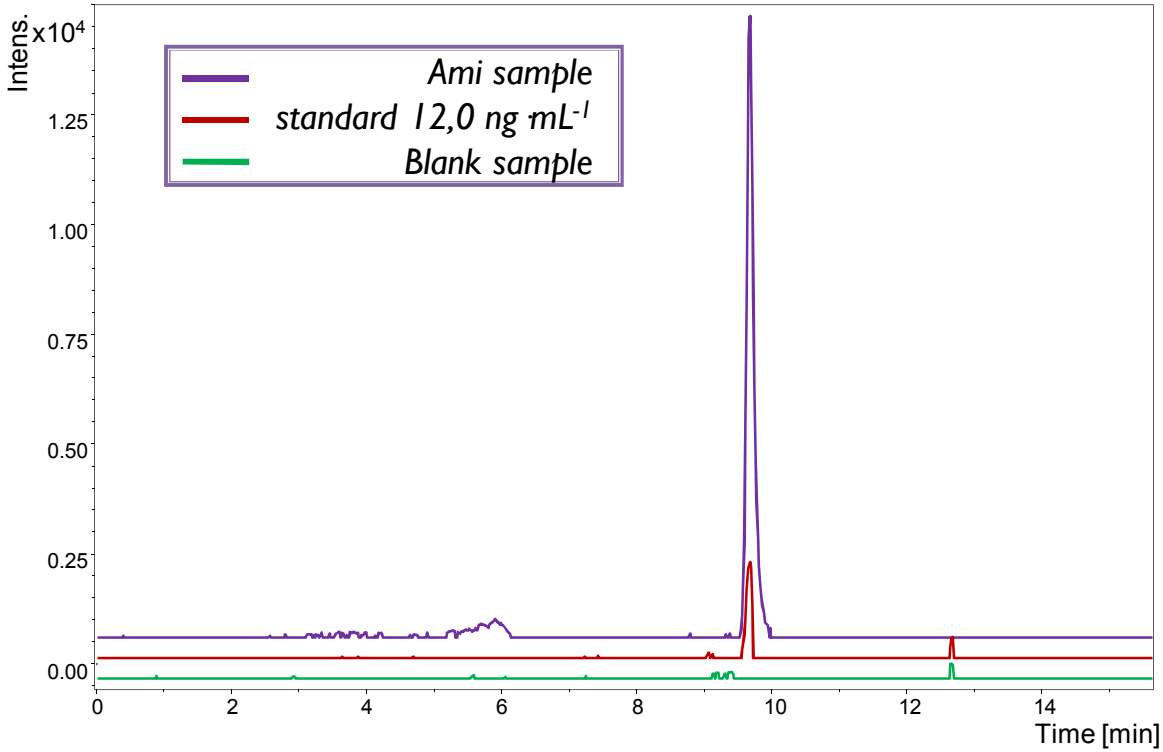
[1]de Castro A et al., Journal of Pharmaceutical and Biomedical Analysis 2008, 183-193
[2]Chudzikiewicz E. et al., Problems of Forensic Science 2005, LXII, 166-177

Analysis of real samples

- real oral fluid samples containing Amitriptyline were collected from volunteer and stored in a refrigerator (+4°C) until LC-MS analysis
- calibration curve was prepared from 0,5 to 12,0 ng·mL^{-1}



- obtained results shown that the concentration of Amitriptyline was higher than the range of the curve

Conclusions

- SAMPLE PRETREATMENT:
  - the method of sample preparation is quite short and simple compared to usual SPE extraction
- MEPS EXTRACTION:
  - the BIN sorbent can be used about 25 times (SPE column – 1 time)
  - time of extraction of one saliva sample is about 5 minutes
  - eVol improves MEPS extraction
- GENERAL CONCUSIONS:
  - developed method was found to be a robust and efficient approach to analyse TCDA drugs in oral fluid samples
  - low limit of quantification was obtained (examinated real saliva samples is possible)
  - using eVol semi-automation and miniaturization were achieved

Thank you for your attention

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