First results from standardized oral fluid contamination experiments using Diphenhydramine as a model substance

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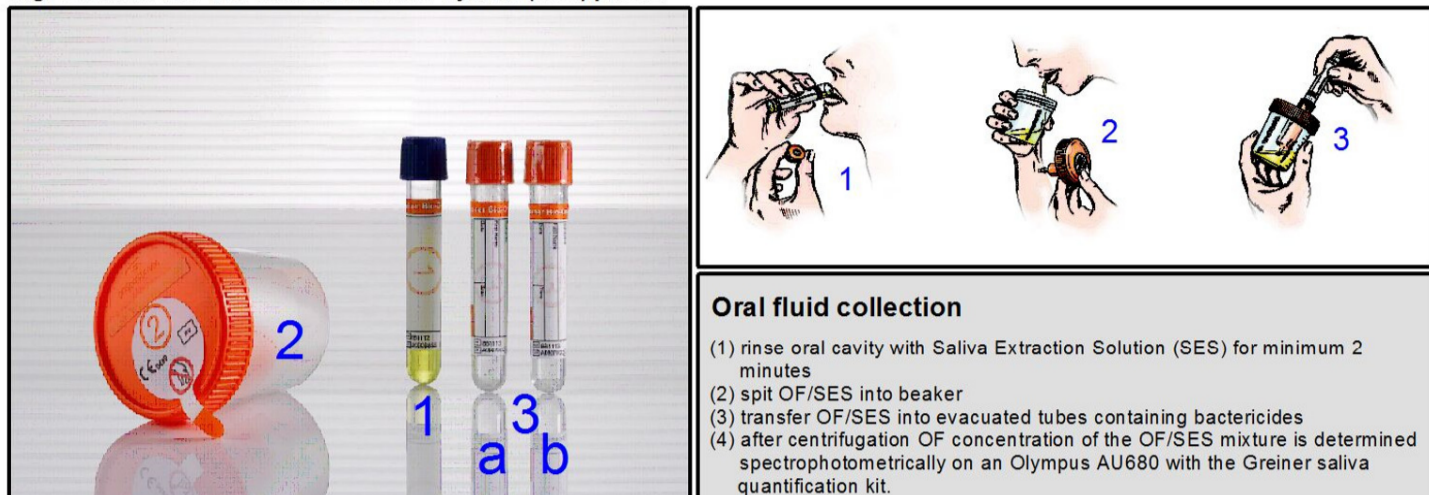### MVZ Labor Dessau GmbH, Germany

Introduction

Oral fluid (OF) is getting increasingly accepted as a suitable alternative matrix for drugs of abuse testing in clinical, drug treatment, workplace and other settings. This is mostly because of ease of collection and less risk for adulteration. In addition compliance monitoring in pain management or psychopharmacotherapy, which is normally based on serum level quantification could possibly be performed in OF in the future. However, little is known about the probability and extend of OF contamination with drugs shortly after ingestion or after unintended/ accidental oral contamination (e.g. kissing, passive smoking) what could be cited by patients to explain their positive test results. OF contamination could lead to false positive or false high results and hence to interpretation problems. To get an idea of the influence OF contamination could have on analytical results an OF contamination simulating experiment was conducted with the non-prescription drug Diphenhydramine as a model substance. Uncoated tablets of the drug were applied.

Fig. 1 Greiner Bio-One Saliva Collection System (SCS) pH 4.2

Oral fluid collection

(1) rinse oral cavity with Saliva Extraction Solution (SES) for minimum 2 minutes
(2) spit OF/SES into beaker
(3) transfer OF/SES into evacuated tubes containing bactericides
(4) after centrifugation OF concentration of the OF/SES mixture is determined spectrophotometrically on an Olympus AU680 with the Greiner saliva quantification kit.

Individuals and sampling

20 healthy volunteers (12 male, 8 female) between 23 and 37 years of age, were devided into four groups with five individuals each. All individuals placed an uncoated tablet with 50 mg Diphenhydramine-HCl (Dorm, Berco-Arzneimittel, Germany) on their tongue and spat it out after 5 seconds. Oral fluid samples were collected with the Greiner Bio-One saliva collection system (Greiner Bio-One, Austria) immediately after the tablet was spat out ("time zero" sample) and then OF samples were collected every hour. Each group stopped sampling after a defined period of time: Group A after one, Group B after two, Group C after three and Group D after eight hours. Every individual from Group A - C drank 250 mL water within <2 min after their last sampling and subsequently took a final oral fluid sample. Food intake was prohibited for Group A-C during the experiment, individuals from Group D were allowed to eat and drink normally after one hour.
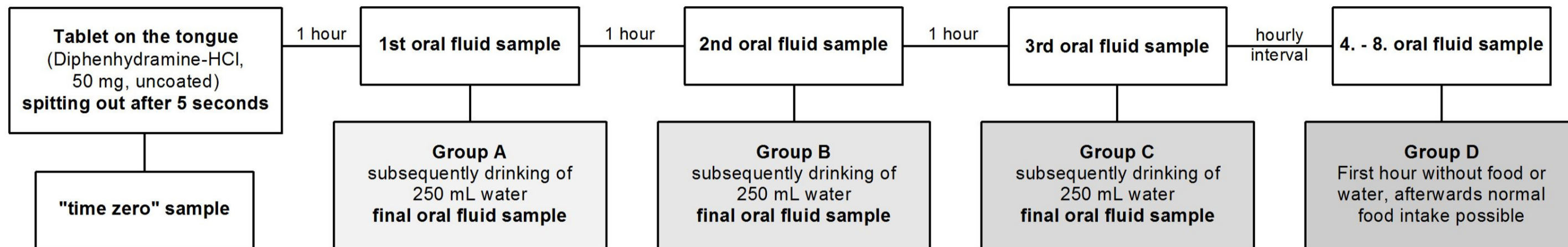
Sample preparation and LC-MS/MS method

20 µL OF/SES fortified with 20 µL internal standard (100 ng/mL Diphenhydramine-d3 in MeOH), was protein precipitated with 60 µL MeOH/ACN (50:50 v/v). After centrifugation 20 µL of the supernatant were diluted with 130 µL MeOH/H_2O (60:40, v/v) + 0.1% FA. 5 µL were injected into the UPLC system. OF/SES matrix calibration was performed from 1 to 1000 ng/mL (LoD = 0.34 ng/mL; LoQ = 0.42 ng/mL).
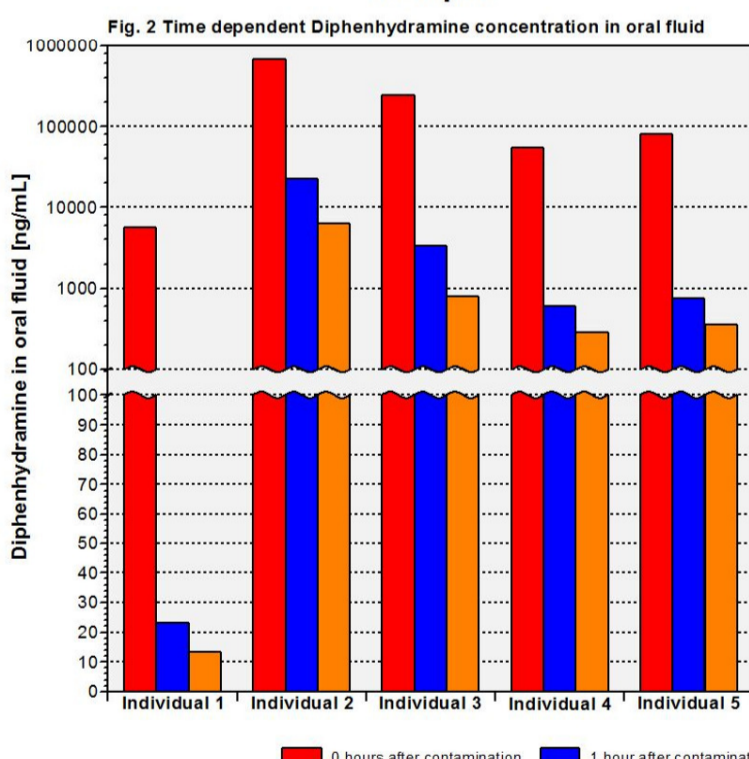
Gradient separation was conducted on a Waters Acquity UPLC connected to a Xevo-TQ-S with a BEH-Phenyl column (1.7 µm, 2.1x100mm), kept at 40°C within 2.5 min. Mobile phase A was 0.1% FA and mobile phase B was 0.1% FA in MEOH. The instrument operated in ESI positive mode and two transitions for each analyte were recorded, Diphenhydramine: **256.1>167.1**; 256.1>152.0 and Diphenhydramine-d_3: **259.2>167.1**; 259.1>152.0.
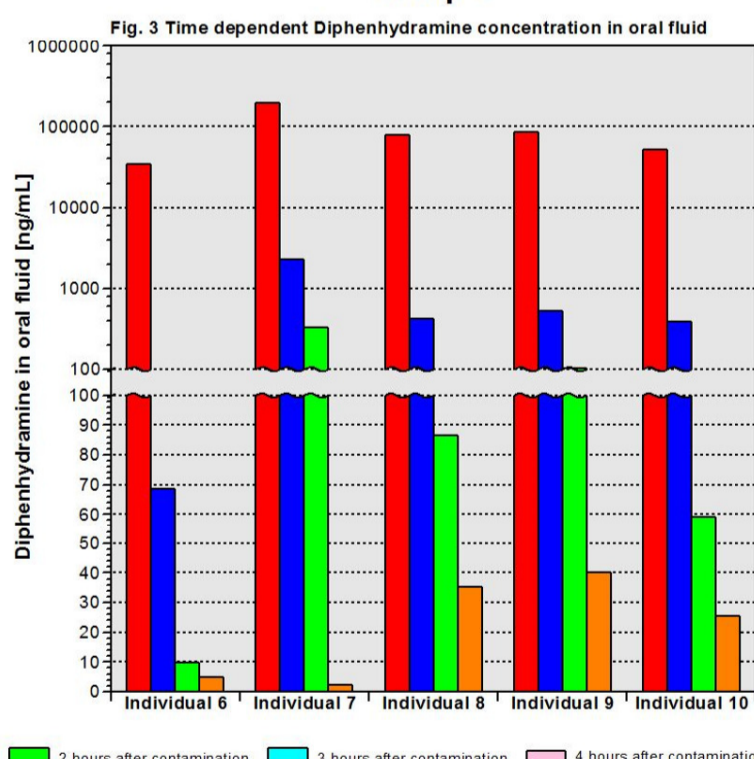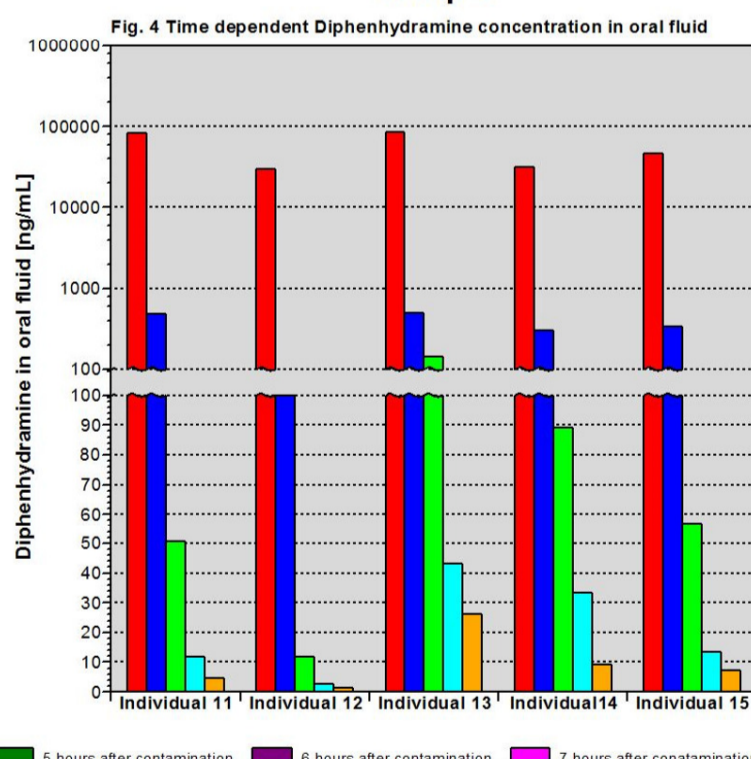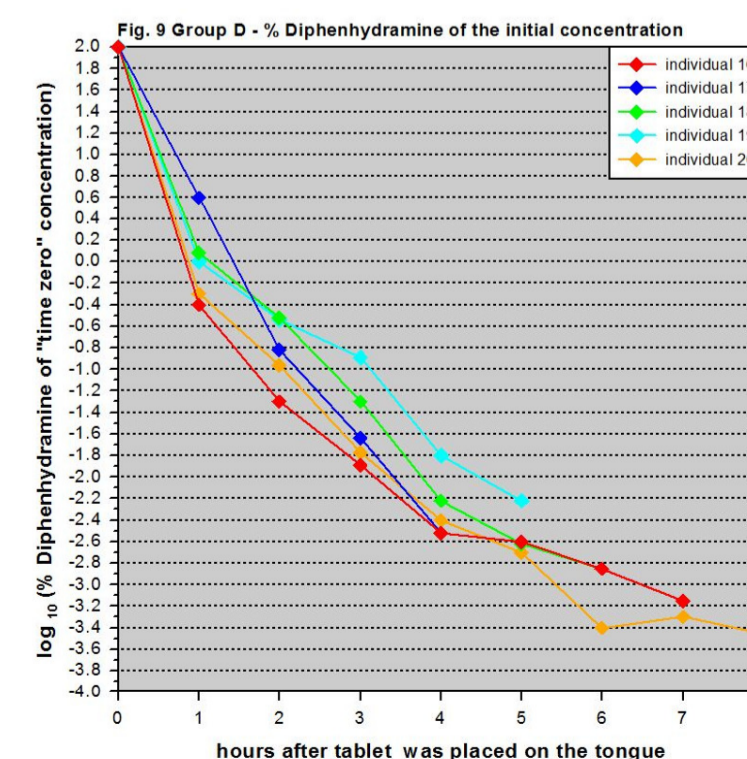
Sampling Schedule

Tablet on the tongue (Diphenhydramine-HCl, 50 mg, uncoated) **spitting out after 5 seconds**	1 hour →	1st oral fluid sample	1 hour →	2nd oral fluid sample	1 hour →	3rd oral fluid sample	hourly interval →	4. - 8. oral fluid sample
"time zero" sample		**Group A** subsequently drinking of 250 mL water **final oral fluid sample**		**Group B** subsequently drinking of 250 mL water **final oral fluid sample**		**Group C** subsequently drinking of 250 mL water **final oral fluid sample**		**Group D** First hour without food or water, afterwards normal food intake possible

Results

Group A
Fig. 2 Time dependent Diphenhydramine concentration in oral fluid

Group B
Fig. 3 Time dependent Diphenhydramine concentration in oral fluid

Group C
Fig. 4 Time dependent Diphenhydramine concentration in oral fluid

Group D
Fig. 5 Time dependent Diphenhydramine concentration in oral fluid

Legend: 0 hours after contamination | 1 hour after contamination | 2 hours after contamination | 3 hours after contamination | 4 hours after contamination | 5 hours after contamination | 6 hours after contamination | 7 hours after conatamination | 8 hours after contamination | after drinking 250 mL water

Fig. 6 Group A - % Diphenhydramine of the initial concentration
Fig. 7 Group B - % Diphenhydramine of the initial concentration
Fig. 8 Group C - % Diphenhydramine of the initial concentration
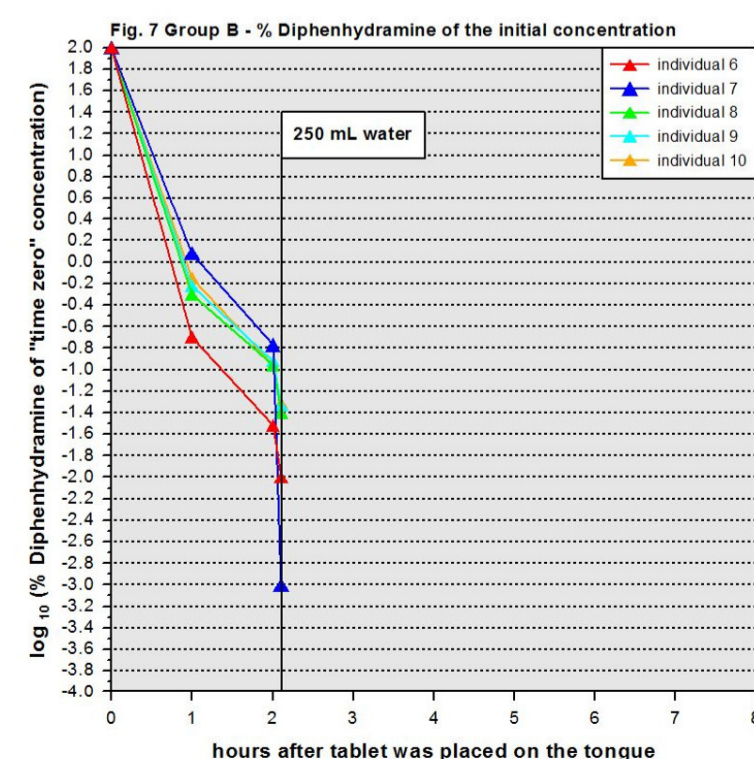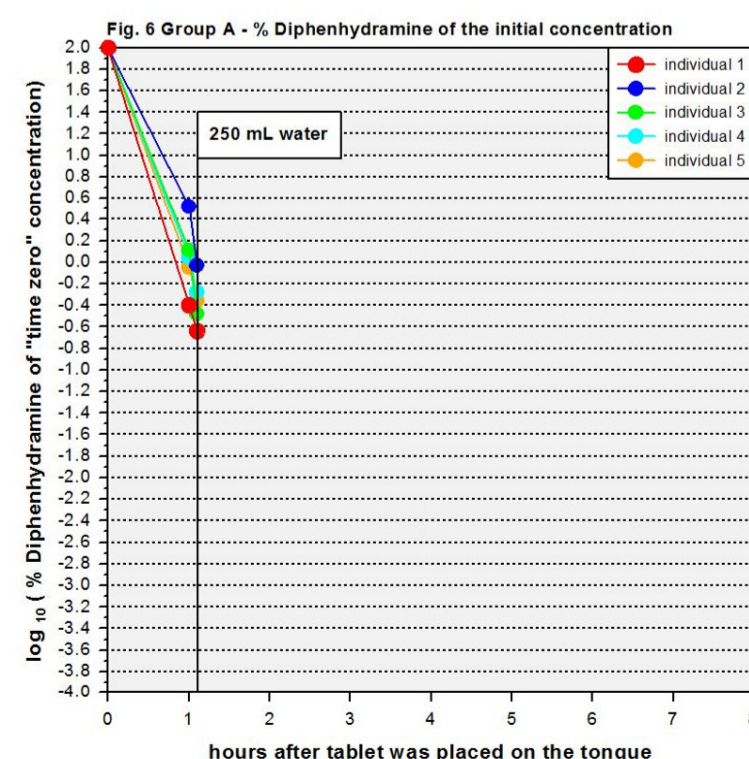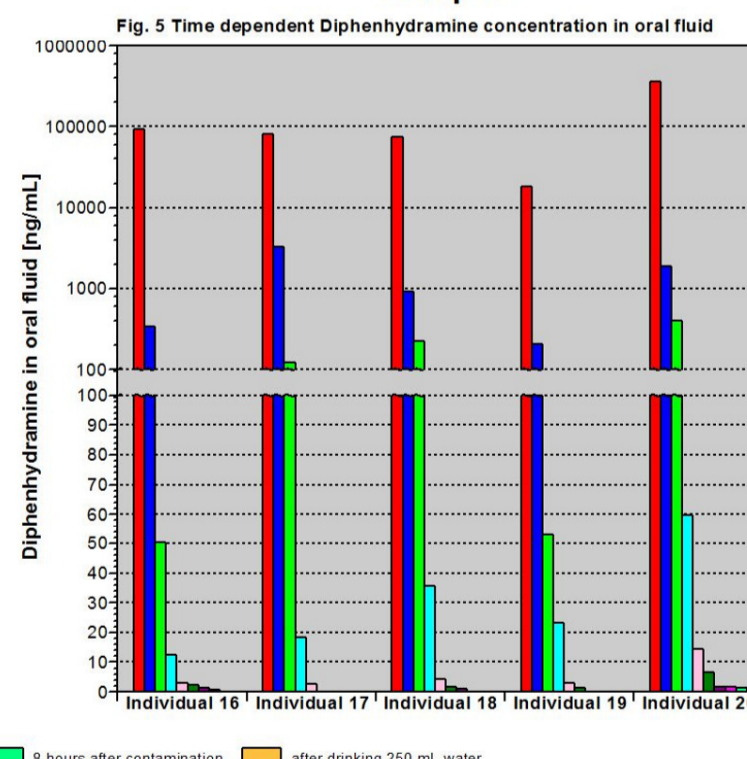Fig. 9 Group D - % Diphenhydramine of the initial concentration

Results - Summary

Diphenhydramine concentration range (DPH-CR*) in "time zero" samples: 5.6 - 679 µg/mL (n = 20)

One hour (n = 20)	Two hours (n = 15)	Three hours (n = 10)
DPH-CR: 23.1 - 22763 ng/mL % DPH *: 0.2 - 4.0 MCD**: 99% (CV = 1%)	DPH-CR: 9.9 - 396 ng/mL % DPH *: 0.03 - 0.94 MCD**: 81.3% (CV = 9%)	DPH-CR: 2.7 -59.9 ng/mL % DPH *: 0.009 - 0.13 MCD**: 74.7% (CV = 14%)

Mean concentration decrease (MCD**) after drinking 250 mL water: 59.2% (n=15; CV = 27%)

* referring to initial ("time zero") concentration
** referring to previous concentration

Conclusion

1. The OF contamination was unexpected high and interindividually variable.

2. Diphenhydramine was detectable in OF in Group A - C during the complete sampling period and at least 4 hours minimum in all individuals from Group D.

3. Drinking of at 250 mL liquid is recommended even though it probably does not completely remove the remaining drug residues in every case.

4. In case of a very recent contact of the oral cavity with a drug we would recommend at least a 4 hour delay before sampling.