First experiments on the possible use of diphenhydramine as a model substance for the evaluation of oral fluid sample collection

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Introduction

Oral fluid (OF) is gaining increasing interest in drugs of abuse and compliance testing as a less intrusive matrix compared to serum or urine. However, little is known about the influence of the collection process on analyte recovery depending on different collection devices. In addition contamination of OF with the corresponding drugs shortly after drug ingestion may lead to interpretation problems. Furthermore unintended oral contamination (eg. "kissing") could be cited (falsely or correctly) by patients / clients to explain their positive drug testing results. A proper model substance to investigate possible ways of oral contamination and the OF sampling process with its influencing factors is therefore needed. Pharmacological and physicochemical properties (pKa: 9.0 , plasma protein binding: 80%, oral bioavailability: 50% - 70%, $t_{1/2}$: 4h), relative safety and availability (non-prescription drug) makes Diphenhydramine (DPH) a candidate substance for evaluation. This approach is complemented by the ease of capillary whole blood sampling.

Methods

Patients: All volunteers were between 23 and 47 years of age, some participated in more than one experiment.
Sample collection: OF samples were collected using the liquid based Greiner Bio-One (GBO, Kremsmünster, Austria) SCS pH 4.2 device or the Quantisal (Immunalysis, Pomona, USA) device according to the manufacturer. Capillary whole blood (CB) was collected with the Minivette POCT (Sarstedt, Nümbrecht, Germany) according to the manufacturer. Sample collection was performed by the volunteers themselfes. All collection devices used in the study are approved for self application by the manufacturer. Amylase and oral fluid concentration in GBO samples were measured with an Olympus AU680.
Sample preparation: 20µl CB or OF/SES fortified with 20 µL internal standard (100 ng/mL DPH-d_3 in MeOH), was protein precipitated with 60 µL MeOH/ACN (50:50, v/v). After centrifugation 20 µL of the supernatant was diluted with 130 µL MeOH/H_2O (60:40, v/v) + 0.1% FA. 5 µL were injected into the UPLC system. CB and OF/SES matrix calibration was performed from 1 to 1000 ng/mL (LoD: OF = 0.34 ng/mL, CB = 0.37 ng/mL; LoQ: OF = 0.42 ng/mL , CB = 0.48 ng/mL).
UPLC-MS/MS: Gradient separation was conducted on a Waters Acquity UPLC connected to a Xevo-TQ-S with a BEH Phenyl column (1.7 µm, 2.1x100 mm), kept at 40°C within 2.5 min. MoPh A was 0.1% FA in H_2O and MoPh B was 0.1% FA in MeOH. The instrument operated in ESI positive mode and two transitions for each analyte were recorded.

Conclusion

- The detection times of DPH in OF and CB are comparable.
- The DPH OF/CB ratio of the first two hours after ingestion (mean: 5.96, CV: 113%, range: 1.13 - 23.8, n= 12) was significantly higher than during elimination (4 - 58 h; mean: 1.62, range: 0.27 - 4.09; CV: 60%, n = 47) indicating OF contamination from the uncoated tablet.
- After induced contamination of OF with DPH, high drug concentrations could be detected (49947 - 296553 ng/mL OF/SES). Even after rinsing the mouth with 248 ml rinsing solution in twenty steps, within 45 minutes, 0.04 - 0.1% of the initial drug concentration was detectable in the rinsing solution (20.5 - 285 ng/mL OF/SES; see Fig. 5, 6).
- Both collection devices showed reproducible recovery after consecutive OF sampling in different individuals with the same collection device (Fig. 3). Consecutive sampling with the two different OF collection devices in the same individuals gave comparable concentrations (Fig. 4).
- DPH fulfills the key criteria for a good model substance: long detectability in both matrices, "sufficient" elimination half-life and a correlation between OF and blood concentrations.

Results

Experimental design: Elimination of DPH in OF and CB

Step 1:	before drug ingestion, sampling of OF and CB
Step 2:	ingestion of one uncoated tablet of DPH-HCl 50 mg ("Dorm", Berco, Kleve, Germany)
Step 3:	susequently, sampling of OF and CB
Step 4:	rinsing mouth and lips with water for one minute
Step 5:	subsequently, sampling of CB and OF
Step 6 - 8:	after 1h, 2h and 4h, sampling of OF and CB
Step 9 - 11:	sampling of all three matrices every 4 hours until 16h after drug ingestion
Step 12 - 16:	sampling of all three matrices every 8 hours until 56h after drug ingestion

Individuals:	Collecting devices:
-- male: 4, 23 - 28 years	-- CB: Sarstedt Minivette POCT
-- female: 3, 23 - 29 years	-- OF: Greiner Bio-One

Fig. 1 Capillary whole blood elimination

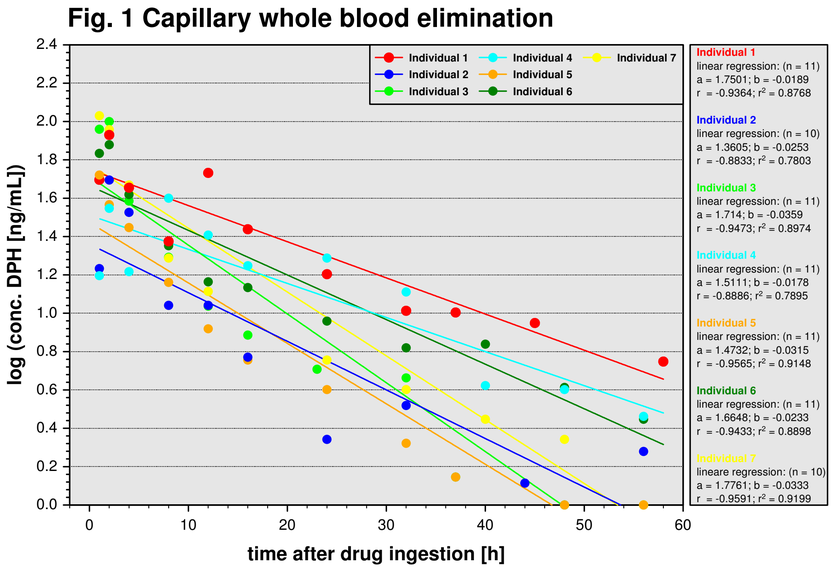
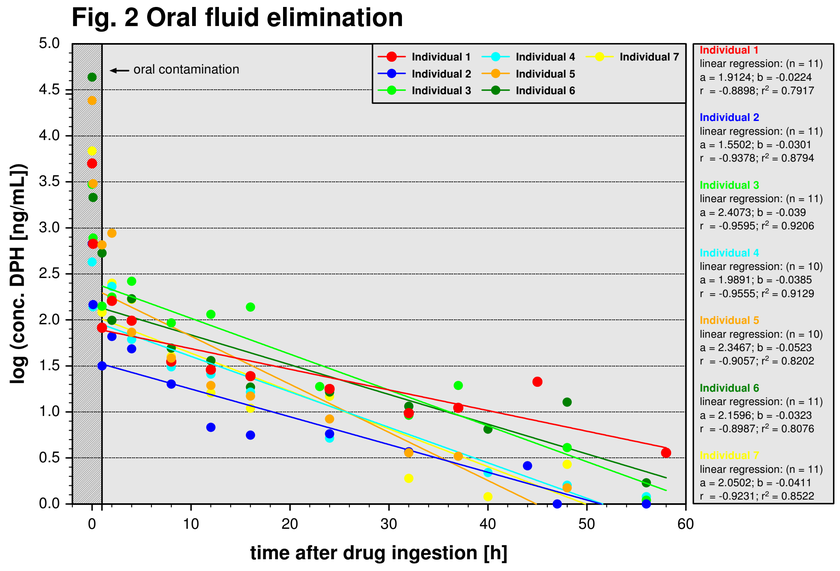


Fig. 2 Oral fluid elimination



Experimental design: Analyte recovery - Greiner vs. Quantisal

Step 1:	ingestion of one uncoated tablet of DPH-HCl 50mg ("Dorm", Berco, Kleve, Germany)
Step 2:	after 12 h collection of 3 OF samples with the Quantisal collection device (Group a) or with the Greiner Bio-One collection device (Group b) within 30 minutes maximum
Step 3:	90 min later one collection of one OF samle with the Greiner Bio-One collection device and one with the Quantisal collection device (Group a) respectively the other way around (Group b)

Individuals:	Collection devices:
-- male: 4, 23 - 28 years	-- a): Immunalysis Quantisal
-- female: 4, 23 - 29 years	-- b): Greiner Bio-One

Fig. 3 Consecutive sampling with same collection device

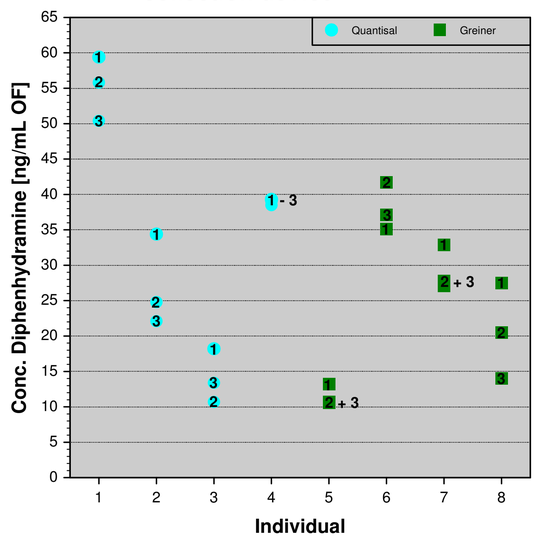
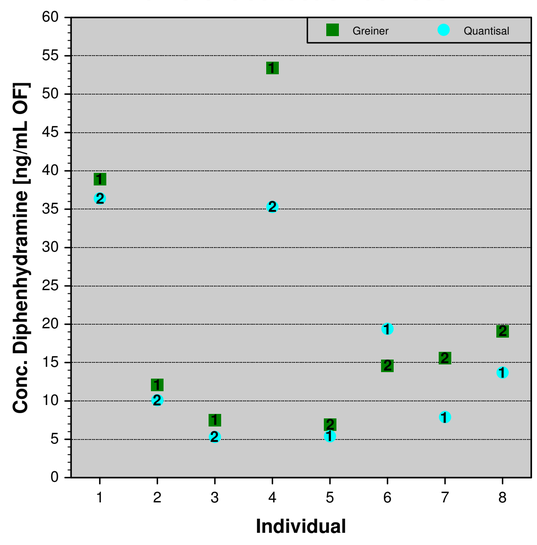


Fig. 4 Consecutive sampling with different collection devices



-- C_{max} CB: 85.2 - 107 ng/mL (after 1 - 2 h)

-- C_{max} OF: 427 - 43402 ng/mL (0h)
due to oral contamination!

-- C_{max} OF 2nd: 66.0 - 263 ng/mL (after 2 - 4 h)

-- detection time CB: 37 - 58 h, mean: 49.7 h

-- detection time OF: 47 - 58 h, mean: 53.9 h

-- OF/CB ratio 1 - 2 h after drug ingestion*:
mean: 5.96, CV: 113%, n = 12, range: 1.13 - 23.8

-- OF/CB ratio 4 - 58 h after drug ingestion*:
mean: 1.62; CV: 60%; n = 47, range: 0.27 - 4.09

*individual 3 excluded due to implausible results

Experimental design: Oral contamination

Step 1:	sampling of OF before drug ingestion
Step 2:	putting one uncoated tablet of DPH-HCl 50mg ("Dorm", Berco, Kleve, Germany) into the mouth for 30 seconds
Step 3:	subsequently sampling of OF with RS a
Step 4:	rinsing mouth with RS b for 30 seconds
Step 5 - 12:	repeat step 3 and 4 nine times
Step 13:	sampling of OF (RS a)

Individuals:	Rinsing solutions (RS):
-- male: 1, 34 years	-- RS a: 4 mL SES (GBO) (pH 4.2)
-- female: 4, 23 - 47 years	-- RS b: 20 mL tap water (pH 6)

Fig. 5 Cumulative rinsing volume vs. DPH conc.

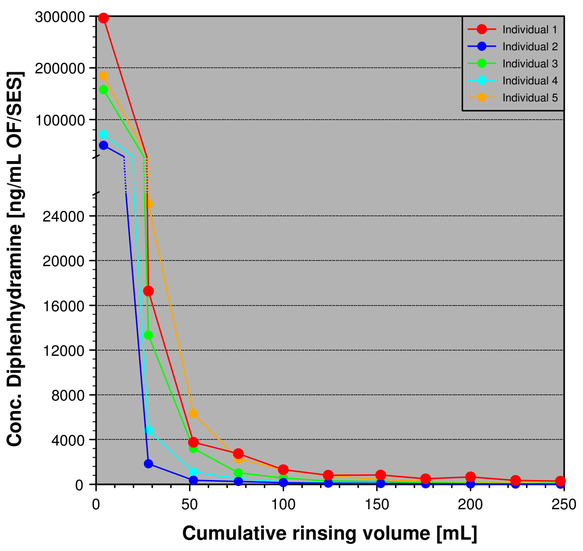
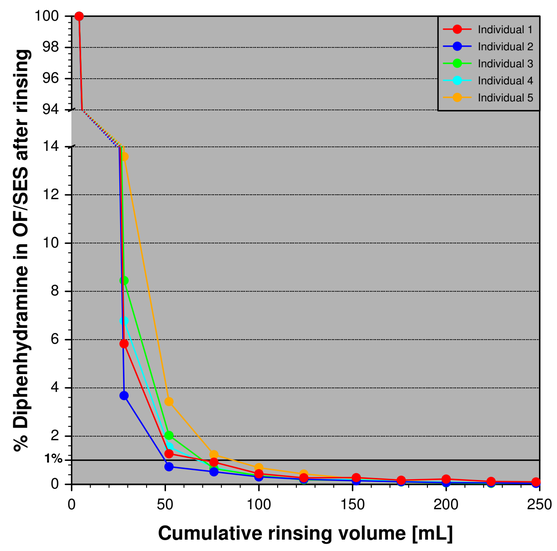


Fig. 6 Cumulative rinsing volume vs. % DPH of initial conc.



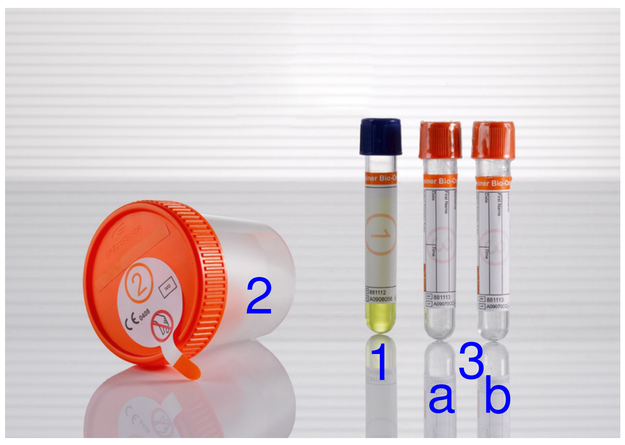
-- Initial DPH conc. (100 %) range:
49947 - 296553 ng/mL OF/SES

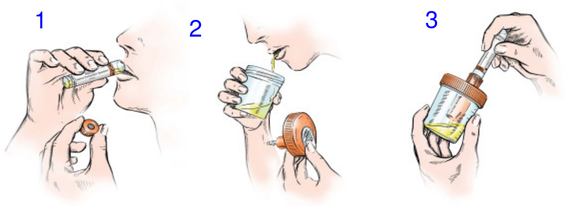
-- DPH conc. below 1%: 72 - 96 mL (5 - 7 rinsings)

-- DPH conc. after 248 mL:
0.04 - 0.1 % of the initial DPH conc.
(range: 20.5 - 285 ng/mL OF/SES)

-- Amount of time: ~ 45 min

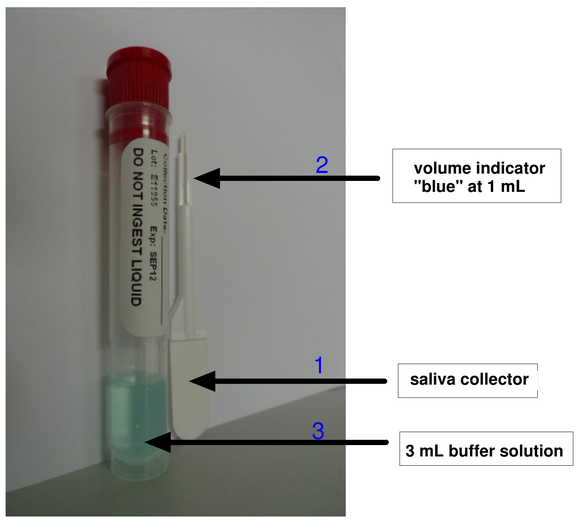
Fig. 7 Greiner Bio-One Saliva Collection System pH 4.2

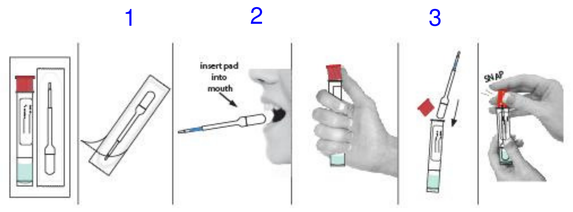




Saliva collection
(1) rinse oral cavity with Saliva Extraction Solution (SES) for minimum 2 minutes
(2) spit OF/SES into beaker
(3) transfer OF/SES into evacuated tubes containing bactericides and send to lab
(4) after centrifugation Amylase and OF concentration are determined on an Olympus AU680

Fig. 8 Quantisal collection device





Saliva collection
(1) put the salica collector under the tongue
(2) wait until the volume indicator turns "blue"
(3) transfer the saliva collector in the buffer solution and send it the lab
(4) OF concentration is 25%