Oral Fluid Levels of Nicotine and Metabolites in Smokers as a Function of Collection Device

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Introduction

Oral fluid (OF) attracts increasing intention in drugs of abuse testing of methadone maintenance patients and within other clinical settings. The ease of non-invasive sampling under close supervision decreases the chances for adulteration or substitution of the sample by the patient. Recovering OF samples from xerostomic individuals (eg. opiate addicts) without stimulating saliva flow can be difficult. It is assumed however, that a stimulating OF collection device would reduce the drug concentration due to an increase of salivary flow and 14. In this study we compared the seemingly non-stimulating devices Salivette (SA; Sarstedt) and Quantisal (QS; Immunalysis) to the stimulating liquid-based pH 4.2 Saliva Collection System (SCS; Greiner-Bio-One (GBO)). Using SCS the pH remains constant during the collection process. Nicotine (NIC), Cotinine (COT) and trans-3-Hydroxycotinine (HCOT) in OF from smokers acted as model analytes. NIC was excluded from the study due to oral contaminations. **Aims of this study: 1.** to establish an UPLC-MS/MS method for the detection of NIC, COT, HCOT. **2.** to verify that OF collection at short intervals (3x within <=20 min) with the same collection device gives comparable values **3.** to investigate the influence of the different collection devices on COT and HCOT values in OF.

Methods

Subjects: 15 volunteer smokers (see results section (Fig.6)) took part in 2 series of OF collection: series A: five volunteers each collected 3 consecutive OF samples using the same collection device (n = 45 samples); series B: all 15 individuals collected 3 consecutive OF samples using the 3 different devices in different order (n = 45 samples). In both series total collection time never exceeded 20 min. **Sample collection:** OF samples were collected using the SCS, SA and QS device according to the manufacturer. OF concentration [%] of the OF/buffer mixture recovered with the SCS was quantified spectrophotometrically on an Olympus AU880 using the GBO saliva quantification kit. **Instrument:** Waters Acquity UPLC system connected to a Waters XEVO TQ-detector. **Sample preparation:** to 200 µL urine 2 µL internal standard containing 2ng NIC-d4, COT-d3 and HCOT in ACN was added followed by 200µL extraction buffer (0.1 M ammonium formate, pH 9.3) and 1 mL EtAc/IPA/DCM (2/2/1 by vol.). After vortexing and centrifugation the supernatant was spiked with 35 µL formic acid and evaporated to dryness under nitrogen at 35 °C. The residue was redissolved with 100 µL MeOH/water (50/50 with 5% NH4). Five µL was injected into the UPLC. **UPLC conditions:** separation was performed on a Waters 2.1 × 100 mm BEH Phenyl 1.7 µm column kept at 50 °C. Mobile phase A consisted of 5mM formic acid and mobile phase B was MeOH. Isocratic separation was conducted within 2.5 min at a flow rate of 0.4 mL/min with 95% A and 5% B. **Detector settings:** data were aquired with an ESI source operating in the positive ionization, MRM mode. Capillary voltage was set to 0.6 kV, ion source temperature was 150 °C, and desolvation gas was heated to 600 °C and delivered at a flow rate of 850 L/h. Cone gas (N2) was set to 20 L/h and the collision gas (Ar) was maintained at 0.25 mL/min. Cone voltages: NIC 12V, COT: 30V and HCOT: 32V. Three transitions were monitored for each analyte including internal standards (Fig.2).

Conclusion

-- The UPLC-MS/MS method is sensitive, proved to be robust and allowed high throughput for routine analysis.

-- COT and HCOT are promising "model analytes" for the evaluation of new OF collection devices.

-- **Consecutive OF sampling in an individual seems not to "exhaust the system"**

-- **Consecutive OF sampling with one stimulating and two non-stimulating devices gave comparable concentrations for COT and HCOT**
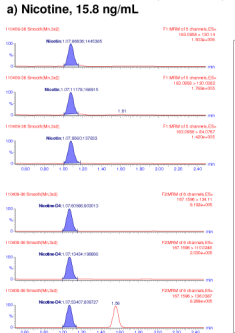
UPLC-MS/MS method

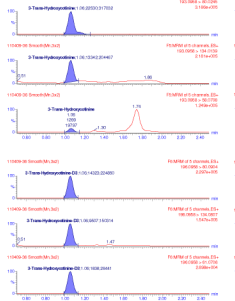**Fig.1** OF/SES calibration for NIC, COT, HCOT -- working- and cutoff-range
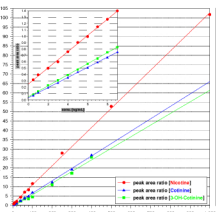
Nicotine:	LoD: 0.25 ng/mL;	
LoQ: 1.42 ng/mL		
Cotinine:	LoD: 0.25 ng/mL;	**Cotinine**
LoQ: 0.61 ng/mL		**Cutoff oral fluid:**
3-OH-Cotinine:	LoD: 0.30 ng/mL;	**10 ng/mL**
LoQ: 0.73 ng/mL		

Fig.2 MRM chromatograms of OF/SES spl. from volunteer 6 (2nd series)
a) Nicotine, 15.8 ng/mL

b) 3-Hydroxycotinine, 19.6 ng/mL
c) Cotinine, 99.9 ng/mL

Results
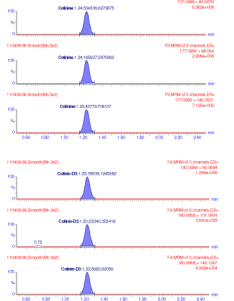
A. Consecutive sampling with same collection device

Fig.3a Cotinine, 3 OF devices (SCS, QS, SA)
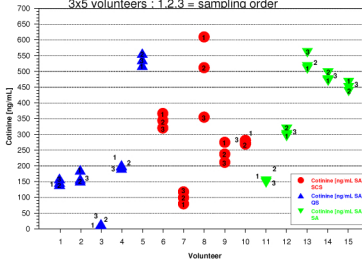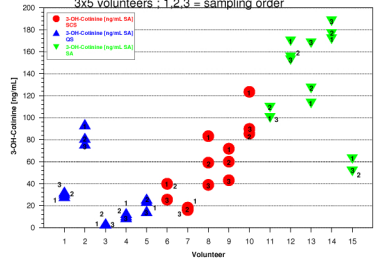3x5 volunteers ; 1,2,3 = sampling order

Fig.4a 3-OH-Cotinine, 3 OF devices (SCS, QS, SA)
3x5 volunteers ; 1,2,3 = sampling order

B. Consecutive sampling with different collection devices

Fig.3b Cotinine, 3 OF devices (SCS, QS, SA)
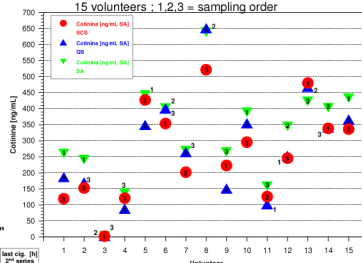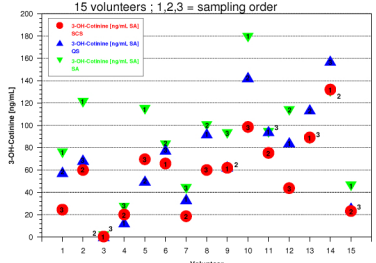15 volunteers ; 1,2,3 = sampling order

Fig.4b 3-OH-Cotinine, 3 OF devices (SCS, QS, SA)
15 volunteers ; 1,2,3 = sampling order

Fig.6 Volunteers and "nicotine burden"

volunteer	sex	age [years]	cig. id	last cig. [h] 1st series	last cig. [h] 2nd series
1	f	27	15	1.5	0.25
2	f	47	15	1.0	0.25
3	m	50	25	17	16.0
4	m	22	15	2.5	1.0
5	m	29	10	4.0	1.0
6	m	22	10	3.0	0.75
7	m	22	12	0.5	0.25
8	f	24	13	2.3	0.75
9	f	26	15	1.5	0.75
10	m	24	15	0.25	1.0
11	f	24	8	0.5	1.0
12	m	25	10	2.5	0.75
13	m	31	30	0.25	0.25
14	m	32	10	5.0	1.5
15	m	33	17	1.5	0.25

Fig.3c Cotinine:
agreement of QS with SCS

agreement of SA with SCS
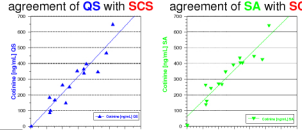
Fig.4c 3-OH-Cotinine:
agreement of QS with SCS

agreement of SA with SCS

OF collection devices

Fig.5a SCS, pH 4.2

Fig.5b SA

Fig.5c QS