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 attention in drug abuse testing of medication maintenance patients and within other clinical settings. The issue of non-invasive sampling under obese supervision decreases the chances for dilution or substitution of the sample by the patient. Recovering OF samples from non-smokers and individuals with opiate addiction without stimulating saliva flow can be challenging. It is assumed, however, that stimulating OF collection devices would reduce the drug concentration due to an increase of oral flow rate and pH. In this study, we compared the performance of non-stimulating devices Salyte® (SA), Bardex® and Quantifoam® (QF), compared to the stimulating cloud-based Breath Collection System (R) GITraider® (QF), using the pH remains constant during the collection process. Methylene Blue (MB), Nicotine (COT) and 3-Hydroxy-2-nicotinamide (HCOT) in OF from smokers acted as model analytes. NC was excluded from the study due to oral contamination. Aims of this study were: (1) to select an UPLC-MS/MS method for the analysis of COT, HCOT, and COT in OF and to compare the influence of different collection devices on COT and HCOT values in OF.

Methods

Subjects: 10 volunteers each classified in 2 of the following OF collection devices: A-UPLC-MS/MS, B-UPLC-MS/MS, C-QF, D-QF. Samples were collected in the following conditions: A-UPLC-MS/MS, B-UPLC-MS/MS - oral stimulation, C-QF - no oral stimulation; D-QF - no oral stimulation. A total of 30 samples were collected (3 samples/group/device) from each subject. Sample collection: OF samples were collected using the Salyte® (SA) and Quantifoam® (QF) devices according to the manufacturer’s instructions. NC and COT were quantified spectrophotometrically from OF. Nicotine content using the MB-coupled spectrophotometric method and COT and HCOT were quantitated using an automated UPLC-MS/MS method. Sample preparation is described elsewhere. All measurements were performed in triplicate. The samples were analyzed by an Agilent 1290 UPLC instrument coupled to an Agilent 6490 Triple Quad mass spectrometer. Chromatographic parameters: injection volume 10 μL, flow rate 0.4 mL/min, column temperature 40°C, and an electrospray ionization source under negative mode. The mobile phase was a mixture of 70% water/methanol/methanol/formic acid 90:9:0.1 (v/v) at a flow rate of 0.4 mL/min. The OF samples were analyzed as 15 μL volume samples in the positive mode. QF and SA devices were operated in triplicate. Nicotine content was assessed using the MB-coupled spectrophotometric method and COT and HCOT using the UPLC-MS/MS method. All measurements were performed in triplicate. The samples were analyzed by an Agilent 1290 UPLC instrument coupled to an Agilent 6490 Triple Quad mass spectrometer. Chromatographic parameters: injection volume 10 μL, flow rate 0.4 mL/min, column temperature 40°C, and an electrospray ionization source under negative mode. The mobile phase was a mixture of 70% water/methanol/methanol/formic acid 90:9:0.1 (v/v) at a flow rate of 0.4 mL/min. The OF samples were analyzed as 15 μL volume samples in the positive mode. QF and SA devices were operated in triplicate. Nicotine content was assessed using the MB-coupled spectrophotometric method and COT and HCOT using the UPLC-MS/MS method. All measurements were performed in triplicate.

Results

A. Consecutive sampling with same collection device

B. Consecutive sampling with different collection devices

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.