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Comparison of the drug concentrations in oral fluid collected by two sampling methods (Varian OraLab and Statsure Saliva•Sampler)

Comparaison des concentrations de drogues dans la salive collectée par deux méthodes de prélèvement (Varian OraLab et Statsure Saliva•Sampler)

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Abstract – Objective: To determine the influence of oral fluid sampling methods on drug concentrations. **Methods:** Oral fluid was obtained from 249 subjects by Varian OraLab and Statsure Saliva•Sampler. The OraLab consists of foam-tipped oral fluid collector. The sponge contains a salt that stimulates salivation. The Saliva•Sampler consists of a collector with a blue indication when 1 mL of oral fluid is collected. After sampling, the collector is transferred to a tube that contains 1 mL of buffer. Oral fluid was analysed for seven drugs with UPLC-MSMS. **Results:** For all the tested drugs, the concentrations in the oral fluid collected with OraLab were 37–76% compared to Statsure. Possible explanations are: a buffer and surfactants could explain a better extraction recovery with Statsure (particularly for THC) and the stimulation of salivation by a salt could explain lower concentrations in OraLab. A comparison of the concentrations in both samples showed a wide scatter with relatively low correlation coefficients (0.56–0.90). **Conclusions:** For all tested drugs, the concentrations measured in the oral fluid collected by OraLab are lower. This could have consequences for the determination of legal cut-offs.

Key words: Saliva, point-of-collection testing, street drugs/analysis

Résumé – Objectif : Déterminer l'influence de la méthode d'échantillonnage dans la salive sur les concentrations des drogues. **Méthodes :** La salive a été obtenue chez de 249 sujets par Varian OraLab et Statsure Saliva•Sampler. L'OraLab se compose d'une tige avec une éponge qui absorbe la salive. L'éponge contient un produit salé qui stimule la salivation. Le Saliva•Sampler se compose d'un collecteur avec une indication bleue quand 1 mL de salive a été collecté. Après le prélèvement, le collecteur est transféré dans un tube qui contient 1 mL de tampon. Sept drogues ont été analysées dans la salive par UPLC-MSMS. **Résultats :** Pour toutes les drogues testées, les concentrations dans la salive recueillie avec OraLab étaient de 37–76 % par rapport à Statsure. Les explications possibles de ces différences sont la présence d'un tampon et de surfactants qui pourrait expliquer une meilleure extraction avec le Statsure (en particulier pour le THC) et la stimulation de la salivation par un sel dans l'OraLab pourrait également expliquer les plus faibles concentrations. La comparaison des concentrations dans les deux échantillons montre une grande dispersion avec des coefficients de corrélation relativement faibles (0,56-0,90). **Conclusions :** Pour toutes les drogues, les concentrations mesurées dans la salive collectées par OraLab sont plus basses. Cela pourrait avoir des conséquences pour la détermination des seuils médico-légaux.

Mots clés : Salive, tests rapides, analyse de drogues

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Table I. Cut-offs (in µg/L) of the different drugs in the different legislations on driving under the influence of drugs.

Analyte	Belgium (screening)	Belgium (confirmation)	France (screening)	Victoria (confirmation)
Amphetamine	50	25	50	5*
MDMA	50	25	50	5
THC	25	10	15	2
Cocaine	20	10	10	
Benzoylecgonine	20	10	10	
6-acetylmorphine	10	5	10	
Morphine	10	5	10	

* Methamphetamine

1 Introduction

Several countries (*e.g.* Australia, Belgium, France and Spain) use oral fluid for drug screening at the roadside in order to detect drivers under the influence of drugs. The screening and confirmation cut-offs for the different drugs are often mentioned in the law. The cut-offs used in the different countries are given in Table I.

The literature has shown that drug concentrations in oral fluid are affected by many variables. First, oral contamination can occur after the drug was taken orally or intranasally. It usually takes about one to two hours for the oral contamination to disappear [1]. When the oral fluid flow is stimulated, the pH increases and the drug concentrations decrease [2].

Many basic drugs are present in oral fluid in much higher concentrations than in blood [3]. This is explained by the phenomenon of ion trapping. When a basic drug reaches the oral fluid where the pH is a little bit lower, it becomes ionised and gets a positive charge [2]. Thus, it will not diffuse back into the blood.

O'Neal [4] *et al.* have shown that concentrations in oral fluid vary according to the sampling method. The highest concentrations were observed in oral fluid that was collected by spitting. These concentrations averaged 3.6 times higher than concentrations in specimens collected by acidic stimulation and 1.3 to 2.0 higher than concentrations in specimens collected by nonacidic stimulation or collection using either the Salivette or the Finger Collector devices.

The collection device has an influence on the drug concentration that is found in the oral fluid [2]. There is variation in collection volume, volume recovered from the device and drug recovery from the device [2]. In one study of 5 devices, between 18% and 83% of the collected oral fluid volume was recovered [2]. Two important phenomena are adsorption of the drug on different parts of the collection device, and the variable recovery of the drugs from the device. For instance, THC binds to the Salivette and very little is found in the supernatant after centrifugation of the Salivette. Extraction of the Salivette with an organic solvent however releases the THC [5]. Langel *et al.* [6] have compared ten sampling devices and found large differences in recovery among them. Another issue is the stability of the drugs once they have been collected by the device. It is well-known that cocaine is not stable and is hydrolysed to benzoylecgonine. In some devices, stabilizers and buffers have been added in order to preserve the molecules.

The objective of this study was to determine if various oral fluid sampling methods yield different concentrations for the main recreational drugs. This study was part of the evaluation of the sensitivity and specificity of the OraLab [7].

2 Material and methods

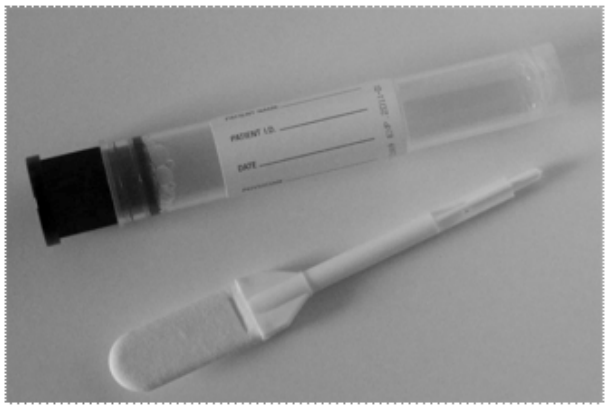
Oral fluid was obtained from 249 subjects (who had given informed consent) by Varian OraLab and Statsure Saliva•Sampler. Fifty subjects were selected during roadside surveys and 199 samples were obtained in a rehabilitation centre for drug addicts. Two oral fluid samples were collected, one with the Varian Oralab® 6 (Agilent Technologies, Santa Clara, CA, USA), and the other with the StatSure™ Saliva•Sampler™ (Saliva Diagnostic Systems, Framingham, MA, USA), an oral fluid collector. The latter was chosen because of the high recovery for all drugs [6].

The Oralab® 6 test consists of 3 elements: an oral fluid collector, a test tube and a test card, as shown in Figure 1A. The oral fluid collector is a swab with salty taste (to increase oral fluid production), the test tube contains the oral fluid when it is squeezed out of the swab and it is shaped to hold the test card. The test card is the lateral-flow immunoassay to detect drugs in the oral fluid.

The oral fluid for the sample with the Oralab® 6 was collected by keeping the swab under the tongue for about three minutes, which normally is sufficient to collect a minimum of 1mL oral fluid. After collection of the oral fluid with the swab, the latter was squeezed out in the test tube. The test card was inserted in the tube to perform the test and removed within 15 min to read the results. The test tube with the remaining oral fluid was stored at −20 °C for later analysis.

A second oral fluid sample was collected with the StatSure™ Saliva•Sampler™ (Figure 1B). The StatSure™ oral fluid collector is a cellulose swab that has to be kept under the tongue to absorb oral fluid. An indicator in the plastic handle turns blue when 1 mL oral fluid is collected. The transport tube contains 1 mL of buffer that dilutes the oral fluid sample. The concentrations of drugs found in this second sample after analysis with LC-MS/MS were adjusted for volume, based on the weight of the StatSure™ device after collection.

(A)



(B)

Fig. 1. A: Varian OraLab: stem with a sponge that absorbs oral fluid. The sponge contains an acid product that stimulates salivation. B: Statsure: collector with a blue indication when 1 mL of oral fluid is collected. After sampling, the collector is transferred to a tube that contains 1 mL of buffer.

Oral fluid was analysed with UPLC-MSMS on a Quattro Premier™ XE (Waters, Zellik, Belgium), with an Electrospray Ionisation-source in positive mode after liquid-liquid extraction with heptane/ethyl acetate (1:4). The oral fluid collected with the OraLab was diluted 1:1 with Statsure buffer prior to extraction. The UPLC method has been described elsewhere [7].

Passing-Bablok regression was used for calculation of the slope and intercept (Medcalc, Mariakerke, Belgium). Samples were included in the calculation if both concentrations were above the lower limit of quantitation.

3 Results

The median concentrations with both sampling methods, the Passing-Bablok slope and intercept, and the correlation coefficient for the different drugs are given in Table II. With the exception of amphetamine (confidence interval 0.5–17), the intercept was not significantly different from zero. For all the drugs, the concentrations in the oral fluid collected with OraLab were 37–76% (based on the slope) of those in the samples obtained with Statsure. The lowest recovery with OraLab (in comparison to Statsure) was observed for cocaine (37%), the highest was observed for morphine (76%).

The correlation coefficients varied between 0.56 (cocaine) and 0.90 (THC). Figures 2–4 show the scatter diagrams and regression lines for 6-acetylmorphine, codeine and THC.

Table III shows the number of samples that would have been positive in comparison to the confirmation cut-offs for the individual drugs in the Belgian legislation. If oral fluid is sampled with Statsure, there would be 25% more positives (10–54% depending on the drug).

4 Discussion

Our study showed that the concentrations in oral fluid sampled with OraLab were lower than with Statsure. Possible explanations for the lower recovery with OraLab are: adsorption of the drugs to the device with OraLab and the presence of a buffer that could explain the better extraction recovery with Statsure, particularly for THC, while the absence of a stabilising buffer in the OraLab could explain the lower cocaine concentrations. The stimulation of salivation by a salt in the OraLab could also explain the lower concentrations observed for all drugs. The differences however were of the same magnitude as the differences that O'Neal *et al.* [4] observed for codeine: with spitting the codeine concentrations were 3.6 times higher than with acidic stimulation, 2 times than with nonacidic stimulation, and 1.3 times than with Salivette or Finger collector. As in our study the oral fluid was first sampled with OraLab, and a few minutes later with Statsure, one would have expected that most concentrations would have been lower with the latter.

The correlation coefficients in the OraLab-Statsure comparison were relatively low (0.56–0.90) and the scatter plots (Figs. 2–4) show a wide scatter. This also confirms previous findings that the drug concentrations in oral fluid are not as constant as in urine or blood, where the sampling method does not influence the concentration. These large differences could again be explained by decreasing concentrations when the oral fluid production is stimulated, or, in the case of THC, variations in the adsorption of the THC that is deposited on the mucosa.

Recently, Gjerde *et al.* [8] compared zopiclone concentrations in oral fluid sampled with Intercept® and Statsure in a clinical study where 5 and 10 mg of zopiclone were administered. The concentrations of zopiclone were approximately 2 times higher with the Intercept than with Statsure (no slope was reported in their paper). They also found that the correlation between the concentrations with the two devices was poor (r^2 = 0.35).

Table II. Median concentrations, regression line and correlation coefficient of drug concentration measured in oral fluid collected with Statsure and OraLab.

Drug	COC	BE	THC	AMP	6-AM	MOR	COD
n	39	37	97	32	88	121	111
Median concentration Statsure (ng/mL)	104	191	40	241	128	98	37
Median concentration OraLab (ng/mL)	50	57	17	282	48	52	21
Regression:							
Slope	0.37	0.52	0.65	0.69	0.47	0.76	0.75
Intercept	8	5	–2	6	4	0	0
r	0.56	0.83	0.90	0.83	0.75	0.83	0.69

COC: cocaine, BE: benzoylecgonine, THC: tetrahydrocannabinol, AMP: amphetamine, 6-AM: 6-acetylmorphine, MOR: morphine, COD: codeine.

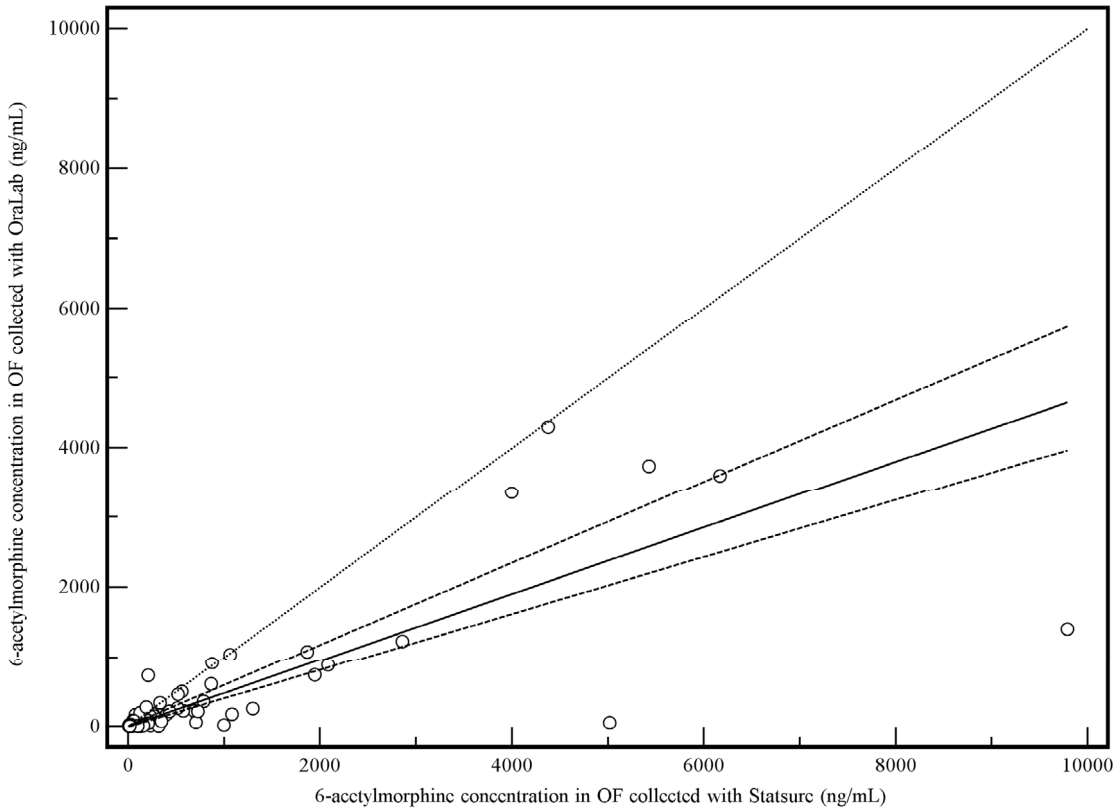


Fig. 2. Scatter-plot and regression line for the concentrations of 6-acetylmorphine in oral fluid collected with OraLab (Y-axis) and Statsure (X-axis). $Y = 0.47X - 4$, $r = 0.75$.

Very few studies have measured the reproducibility of sampling oral fluid. The only study we are aware of is that of Niedbala [9], who sampled with the Intercept to the left and right of the mouth. We calculated the correlation base on the data in the publications, and found a slope of 0.97 and a r^2 of 0.85 ($n = 172$).

These differences could have consequences for the determination of legal cut-offs, as was shown in Table III. With a sampling method that has a greater recovery, more people will test positive for drugs. More studies are needed on intra-individual variability of drug concentrations in oral fluid. In the USA, when the draft SAMHSA guidelines for workplace drug testing were written, this was a concern and in the 2004 draft [10], it was recommended to collect oral fluid by spitting, despite the fact that this collection method is not very practical nor hygienic.

5 Conclusion

We have shown differences in drug concentrations when oral fluid was sampled with two different techniques. With the OraLab, the concentrations were 37–76% compared to Statsure. These differences were of the same order of magnitude as in another study. The variation in concentration ratio of the

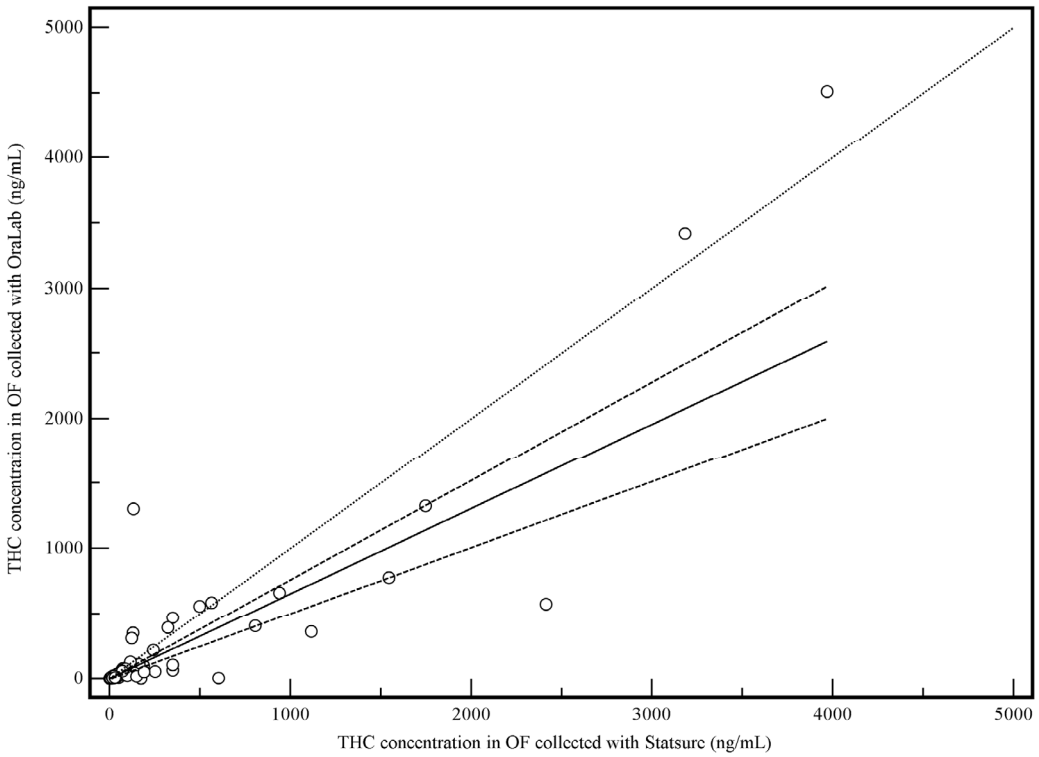


Fig. 3. Scatter-plot and regression line for the concentrations of THC in oral fluid collected with OraLab (*Y*-axis) and Statsure (*X*-axis). $Y = 0.65X - 2$, $r = 0.90$.

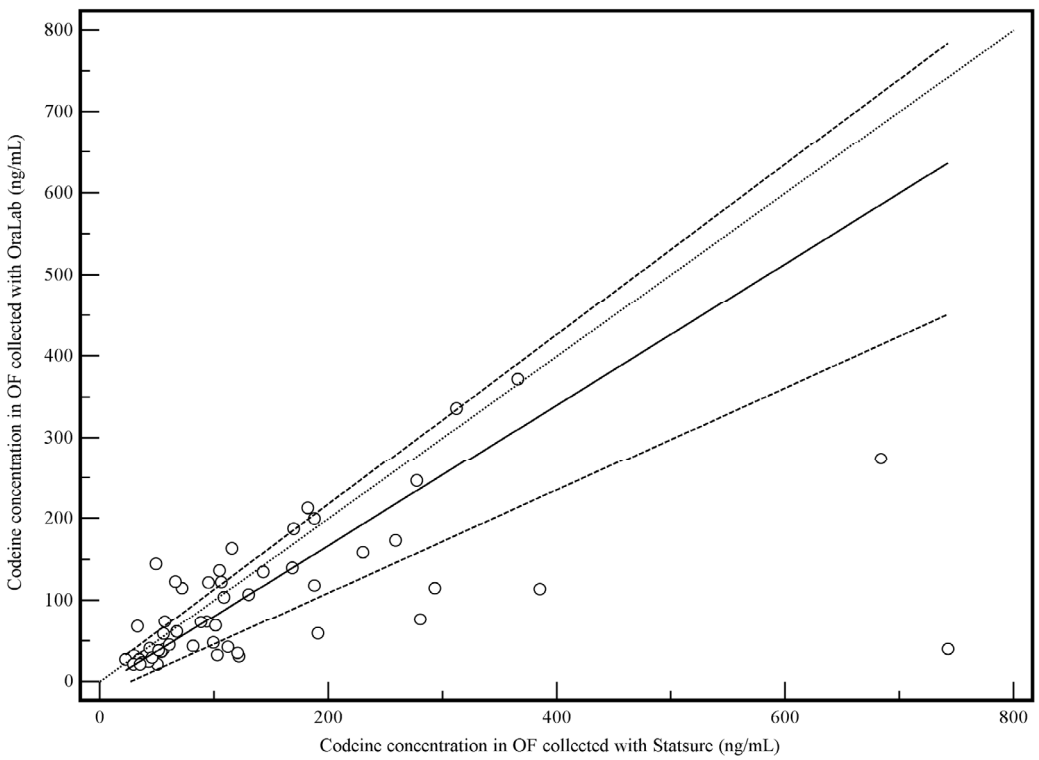


Fig. 4. Scatter-plot and regression line for the concentrations of codeine in oral fluid collected with OraLab (*Y*-axis) and Statsure (*X*-axis). $Y = 0.75X$, $r = 0.69$.

Table III. Number of subjects (n = 249) that would have been positive above the Belgian confirmation cut-offs in oral fluid, when sampled with the respective devices.

Substance	OraLab	Statsure	Difference in positive cases
Amphetamine	25	32	+28%
Cannabis (THC)	66	88	+33%
Cocaine	35	54	+54%
Benzoylecgonine	34	48	+41%
6-acetylmorphine	90	108	+20%
Morphine	104	114	+10%

two sampling techniques was also large. If oral fluid is sampled with the Statsure device approximately 25% more people will be positive for driving under the influence of drugs under the Belgian legislation. Only few studies have been performed on the reproducibility of oral fluid sampling, and more are needed in order to gain a better understanding of all the variables involved in sampling oral fluid.

Disclaimer

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