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Introduction

The collection of oral fluid as matrix for drug testing is easy, non-invasive and can be easily supervised. Preconditions for a routine use in drug testing and for pharmacokinetic measurements are the reproducibility of the collection process and that the collection always reflects the equilibrium in the oral cavity. Furthermore analyses must not be discriminated due to unspecific absorption in or on the collection system.

A new, commercially available saliva collection system was tested which is based on rinsing the oral cavity with a *Saliva Extraction Solution (SES)*.

In saliva samples, obtained by this procedure from healthy individuals, the amount of saliva, pH and - as marker for the integrity of a saliva sample - amylase activity was determined. Furthermore a possible absorption of drugs of abuse on or in the collection system was tested by using radioactive labelled drugs.



Fig. 1: The Greiner Bio-One Saliva Collection System

- a) Rinsing Solution (for optional use)
- b) Saliva Collection Solution (SES)
- c) Saliva Collection Beaker
- d) Saliva Transfer Tube

Method

The *Saliva Collection System* (Greiner Bio-One, Fig. 1) was used for saliva collection from 102 healthy volunteers (51 males, 51 females) by rinsing the oral cavity with the *Saliva Extraction Solution (SES)* according to the manufacturer's protocol. The collected oral fluid is voided into the *Saliva Collection Beaker* and then transferred to the *Saliva Transfer Tube* where the liquid will be preserved and, after centrifugation, is ready for testing. The *SES* contains a non-toxic food dye, which is needed to quantify photometrically the amount of saliva in the collected sample. Amylase activity in saliva was determined with a modified urine assay (Olympus) on an Clinical Analyzer (e.g. Olympus AU640).

The pH value was determined by direct measurement with a pH electrode and by a modified Microgenics urine assay on a Clinical Analyzer.

For absorption studies saliva samples were spiked with approx. 10,000 cpm of the different radioactive labelled drugs (³H-tetrahydrocannabinol (THCdiol), ³H-cocaine, ³H-nicotine, ¹⁴C-morphine and ¹⁴C-codeine [American Radiolabeled Chemicals Inc.]). Spiked saliva samples were transferred from *Saliva Collection Beaker* to *Saliva Transfer Tube* and to *Greiner Bio-One Cryo storage vials*. In each vessel the sample was shaken at room temperature for at least 30 minutes. Radioactivity (cpm) was measured with an Wallac 1414 counter.

Results

When healthy volunteers use the collection system, the amounts of saliva in the collected fluid varies inter-individually from 39 to 89% (mean 67%) and is easily but very accurately (<1.5% CV) determined by photometric measurement (in the range between 20 to 80%, Fig. 2).

The inter-individual pH value in the collected oral fluid was found in the range between pH 4.7 to 6.3 (mean pH 5.1), showing good correlations to photometrical determined values.

Amylase activity showed a broad inter-individual range between 3,500 U/L and 350,000 U/L (mean 70,000 U/L; Fig. 3).

No absorption was detected on the utilized collection devices when using oral fluid samples spiked with radioactively labelled drugs (Fig. 4), even not after storage under various conditions up to 14 days.

Discussion

Results show, that the new oral fluid collection principle is easy and reproducible to perform. There are no indication of unspecific binding of a number of drugs to the collection device, even not after prolonged storage. The amount of collected oral fluid can be accurately and easily determined together with other parameters. As an important advantage to other collection techniques, the pH conditions in the oral cavity are defined during the collection process, due to the pH and buffering capacity of the rinsing fluid *SES*. Amylase is always detected in native saliva and thus is a good marker for the integrity of saliva and therefore can be used as a possible adulteration marker.

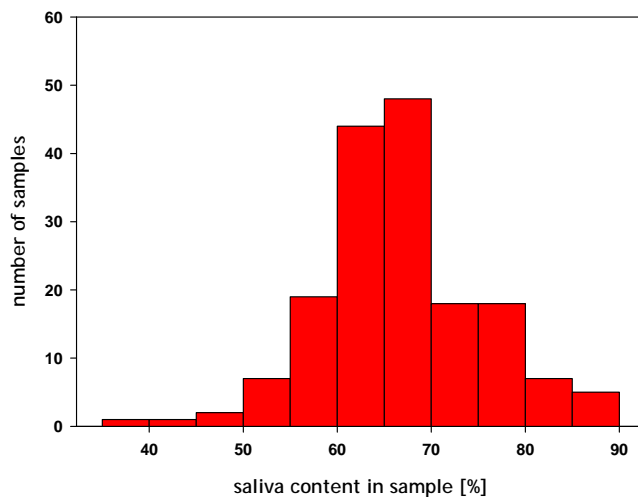


Fig. 2: Saliva concentration in oral fluid samples

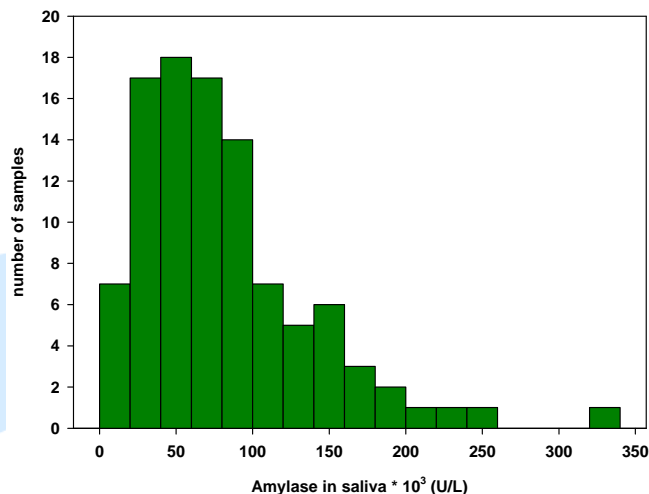


Fig. 3: Amylase activity in oral fluid

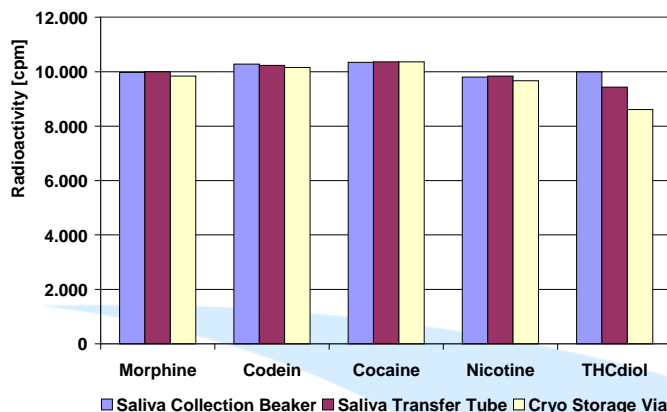


Fig. 4: Loss of drugs during the different sample collection steps tested with radio-labelled drugs: Saliva samples were spiked with approx. 10,000 cpm of radioactive labelled drug and transferred from one vessel to the next. In each vessel the samples were shaken at room temperature for at last 30 min. After every step radioactivity in the sample was measured.