Drug Testing in Oral Fluid—
Evaluation of Sample Collection Devices

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Abstract

Nine different oral fluid (OF) collection devices were studied to evaluate their suitability for collecting samples for drug analysis. The devices were Greiner Bio-One, Orasure Intercept®, Immunalysis Quantisal™, StatSure Saliva-Sampler™, Cozart®, Sarstedt Salivet™, Malvern Medical OraCol, Acro Biotech Salicule, and Varian OraTube™. For comparison, OF was also collected into plastic tubes. The volume of collected OF was quantified for samples collected both in vitro and from volunteers. Drug recovery was studied by collecting OF fortified at 1000 ng/mL with amphetamine, 3,4-methylenedioxymethamphetamine, cocaine, δ9-tetrahydrocannabinol, morphine, codeine, diazepam, and alprazolam with the devices in vitro and analyzing the samples with gas chromatography-mass spectrometry. Recovery of ethanol was measured from 0.2% in OF by headspace gas chromatography-flame-ionization detection. The stability of drugs in the samples was studied by analyzing the samples after 0, 14, and 28 days storage. The study shows that there are substantial differences between the OF collection devices on the market. Some are well suited for collecting samples for toxicological analysis, but some give quite poor results.

Introduction

Oral fluid (OF) has many advantages over the other biological matrices used in drug testing. Collection is quite easy and far less invasive than taking a urine or a blood sample (1,2), and it also carries a smaller risk of spreading infection (3). In addition, OF collection can be directly supervised without the intrusion of privacy thus decreasing the chance of adulterating or substituting the sample (1,4). Finding drugs in an OF sample is generally considered a better indication of recent use and possible impairment than detecting them in a urine sample (1,3,4). This could be very useful in driving under the influence of drugs cases, when evidence of intoxication is generally needed.

Although OF has many advantages, there are still some issues that limit its use in drug testing. The collection method has been shown to have a significant effect on drug concentrations in OF (2,5,6), and the overall drug recoveries after the whole toxicological procedure are highly affected by the selection of the collection method (7). OF collection is usually done by expectoration or with special collection devices. Some of the devices on the market have been studied in terms of drug recovery (1,7–11) and stability (1,10,12). Also, quantification of the volume of collected OF has been done for some devices (7,8,11,13). The differences between the results of different devices have been remarkable and this should be taken into account when choosing a device for collecting samples for drug analysis. Some devices absorb most of the drugs, thus resulting in poor recoveries, and with some devices it is very difficult to get enough OF for the analysis. In addition, the buffer solutions used in some of the devices to elute and preserve the sample can complicate the analysis (14,15), and the materials used in the devices can cause some interference with the analysis (16). There have also been some problems in getting quantitative results for the drug recoveries from devices including a buffer (17,18).

This study was conducted in order to evaluate the suitability of nine commercially available collection devices for collecting OF samples for drug analysis. For comparison, an ordinary capped plastic tube was also tested. The parameters studied include volume of OF collected; recovery and stability of amphetamine, 3,4-methylenedioxymethamphetamine (MDMA), δ9-tetrahydrocannabinol (δ9-THC), cocaine, morphine, codeine, diazepam, and alprazolam; recovery of ethanol; and practical aspects in use of the devices.

Experimental

Collection devices

The collection devices studied were the Quantisal saliva collection device (Immunalysis, Pomona, CA), the Saliva-Sampler (StatSure Diagnostic Systems, Framingham, MA), the Cozart Laboratory Screening Kit (Cozart Bioscience, Oxfordshire, U.K.), the Intercept oral specimen collection device (OraSure Technologies, Bethlehem, PA), the Greiner Bio-One Saliva Collection System and the Greiner Bio-One Saliva Quantifica-
tion (Greiner Bio-One GmbH, Kremsmünster, Austria), the OraCol test kit (Malvern Medical Developments, Worcester, U.K.), the Salivette (Sarstedt AG & Co., Nümbrecht, Germany), the OnTrack OraTube (Varian, Lake Forest, CA), the Salicule saliva sampler (Acro Biotech, Rancho Cucamonga, CA), and a 50-mL capped polypropylene centrifuge tube from Sarstedt (REF 62.548.004). (The names used later in the article for the devices are Quantisal, Statsure, Cozart, Intercept, Greiner, OraCol, Salivette, OraTube, Salicule, and plastic tube.)

**Q**uantisal. The Quantisal device consists of an absorptive cellulose pad with a polypropylene stem and a plastic tube containing a buffer solution. The collection pad has a volume adequacy indicator based on a blue dye that is dissolved when the aqueous medium (OF) migrates along the cellulose pad by capillary action. When 1 mL ± 10\% of OF is collected, the dye becomes visible in the window of the stem. The supplier states that the volume of the buffer solution is 3 mL, and it contains a non-azide preservative.

For sample collection, the collection pad is placed under the tongue. When the indicator window turns blue, the pad is removed from the mouth and placed into the collection/transport tube.

**S**tatsure. The Statsure device consists of an absorptive cellulose pad with a polypropylene stem and a plastic tube containing a buffer solution. The window in the stem of the collection pad turns blue when 1 mL of OF is collected. The supplier states that the volume of the buffer solution is 1 mL.

For sample collection, the level of buffer solution must be checked against a line on the side of the tube before collection begins. OF is allowed to gather in the mouth and the collection pad is placed under the tongue. When the indicator window turns completely blue, the pad is removed from the mouth and placed into the collection/transport tube. In the laboratory, the collection pad is disconnected from the stem and dropped to the bottom of the tube, and a filter is inserted into the tube to recover the OF-buffer solution for testing.

**C**ozart. The Cozart device consists of an OF collection pad with a volume adequacy indicator and a collection/transport tube with a preservative buffer. The indicator area in the stem of the collection pad turns blue when 1 mL of OF has been collected. The volume of the buffer solution is stated to be 2 mL, and the solution contains salts, preservatives, and detergents.

For sample collection, the collector is actively swabbed around the gums, the tongue, and the inside of the cheek, and then held inside the mouth until the sample presence indicator turns blue. The pad is placed into the collection tube bud-end first.

**I**ntercept. The Intercept device consists of a treated absorbent cotton fiber pad attached to a nylon stick and a preservative solution in a plastic container. The pad is impregnated with a mixture of common salts (sodium chloride 3.5\%) and gelatin. The amount of preservative solution is stated to be 0.8 mL.

For sample collection, the collection pad is placed between the lower cheek and gums and gently rubbed back and forth until it is moist. The pad is kept still for 2 min (maximum 5 min) and then placed into the blue liquid at the bottom of the container. The pad handle is snapped against the side of the container to get the cap in place, and the sample is sent to the laboratory. The collected specimen should be stored at 4–37°C and tested within 21 days of collection or within 6 weeks if frozen. In the laboratory, the device is centrifuged to recover the OF-buffer solution for testing.

**G**reiner. The Greiner device consists of a rinsing solution tube (6 mL), an OF extraction solution tube (4 mL), an OF collection beaker with a cap, and two OF vacuum transfer tubes that contain stabilizers and preservatives. The extraction solution contains a yellow food dye, tartrazine, which serves as an internal standard enabling spectrophotometric quantification of collected OF. It also contains citrate buffer at a certain pH to increase salivation. The Quantification Kit contains five calibrators, two controls, OF extraction solution, and artificial OF for spectrophotometric measurements.

For sample collection, the oral cavity is cleaned with the rinsing solution and OF is collected by rinsing the mouth with the extraction solution for 2 min. The extracted OF, along with the rinsing solution, is spat into the collection beaker. A vacuum transfer tube is connected to the beaker and the solution is sucked into the tube (or two tubes if necessary). The sample(s) is shaken and shipped to the laboratory, where it is stored at refrigerated temperature and centrifuged before analysis. The quantification of the collected OF is done spectrophotometrically (450 nm) from the aliquots of the centrifuged samples using the calibrators and controls provided.

**O**raCol. The OraCol device consists of a centrifuge tube with a cap and an absorbent foam swab designed to collect up to 1 mL of OF.

For sample collection, OF is collected by rubbing the sponge firmly along the gum at the base of the teeth until the sponge swab is wet. This should take about 1 min. The swab is then removed, placed into the collection tube, and shipped to the laboratory. In the laboratory, the tube is centrifuged to extract the sample from the swab.

**S**alivette. The Salivette device consists of a cotton swab in a plastic tube with an insert and a cap.

For sample collection, the swab is placed into the mouth and chewed for approximately 45 s. It is removed, placed into the insert in the collection tube, and shipped to the laboratory. In the laboratory it is centrifuged and the insert with the swab is removed to recover clear OF.

**O**raTube. The OraTube device consists of a collection pad and a plastic collection tube with an expresser.

For sample collection, the foam collector is kept in the mouth until it is thoroughly soaked (up to 3 min). The collector is then placed foam-first into the expresser in the tube and pushed to the bottom of the expresser so that the sample flows to the bottom of the tube. Finally, the collector and the expresser are thrown away and the sample is shipped to the laboratory.

**S**alicule. The Salicule device is a collection vial with an expectoration straw and a two-piece cap.

For sample collection, the cap of the collection vial is removed to pull out the expectoration straw. OF is expectorated via the straw into the vial until enough fluid is collected (there is a scale on the side of the vial). In the laboratory, the top-cap is removed, and the sample can be poured to a test tube for
Table I. Ions Monitored

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Ions Monitored(m/z)</th>
<th>Analyte</th>
<th>Ions Monitored(m/z)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphetamine</td>
<td>116, 117, 192</td>
<td>(±)-Amphetamine-(d_6)</td>
<td>120, 203</td>
</tr>
<tr>
<td>MDMA</td>
<td>130, 131, 250</td>
<td>MDMA-(d_5)</td>
<td>134, 255</td>
</tr>
<tr>
<td>Δ²-THC</td>
<td>371, 343, 315</td>
<td>Δ²-THC-(d_3)</td>
<td>374, 389</td>
</tr>
<tr>
<td>Cocaine</td>
<td>182, 303, 272</td>
<td>Cocaine-(d_3)</td>
<td>185, 306</td>
</tr>
<tr>
<td>Morphine</td>
<td>429, 414, 401</td>
<td>Morphine-(d_6)</td>
<td>435, 420</td>
</tr>
<tr>
<td>Codeine</td>
<td>371, 234, 372</td>
<td>Codeine-(d_6)</td>
<td>377, 378</td>
</tr>
<tr>
<td>Diazepam</td>
<td>256, 283, 221</td>
<td>Diazepam-(d_5)</td>
<td>289, 261</td>
</tr>
<tr>
<td>Alprazolam</td>
<td>279, 308, 204</td>
<td>Alprazolam-(d_5)</td>
<td>284, 313</td>
</tr>
</tbody>
</table>

* Quantification ions are bolded.

**Reagents and instrumentation**

Diazepam and alprazolam were from Orion Pharma (Espoo, Finland) and codeine phosphate from Leiras (Turku, Finland). Cocaine hydrochloride and amphetamine sulfate were from Sigma Chemical (St. Louis, MO), and MDMA hydrochloride was donated by the United Nations Narcotics Laboratory (Vienna, Austria). Morphine sulfate pentahydrate was from RBI (Natick, MA). Ampoules of Δ²-THC (1 mg/mL) and deuterated standards in water were from Cerilliant. A solution of 100 g/L ethanol standards in water were from Cerilliant. A solution of 100 g/L ethanol in water was prepared in the laboratory and concentration verified by a testing laboratory. Methanol and acetonitrile were from BDH and the internal standard for ethanol, 2-methyl-2-propanol, and ethyl acetate were from Merck (Darmstadt, Germany), all analytical grade or higher. N-Methyl-(trimethylsilyl)trifluoroacetamide (MSTFA) was from Sigma-Aldrich (St. Louis, MO). Drug-free OF was collected from volunteers working in the laboratory by spitting into a plastic cup. It was frozen, thawed, and centrifuged to give a clear and easy to handle liquid.

Stock solutions of the drugs were used (prepared by weighing the analyte compounds and dissolving them in methanol to obtain a solution with a final concentration of 1 mg/mL). All weighing was done using a calibrated Sartorius Research R 180 D balance. The pH of 0.5 mol/L Na₂HPO₄·2H₂O was adjusted to a value of 10 with 10 mol/L KOH using an IQ Scientific Instruments 150 pH meter. The oven temperature program started at 48°C increased to 58°C, and then lowered back to 48°C. The detection ionization was performed using Agilent ChemStation software.

**Ethanol**

Ethanol was analyzed with an adaptation of the principles described by Machata (19) and Lillsunde et al. (20) with a PerkinElmer Autosystem XL GC equipped with a PerkinElmer Turbomatrix 110 headspace. The GC column was an Elite-BAC2 from PerkinElmer (30-m length, 0.53-mm i.d., and 2-μm stationary phase thickness). The parameters of the method are comparable to those described by Musselman et al. (21). The oven temperature program started at 48°C, increased to 58°C, and then lowered back to 48°C. The detection was flame ionization. Calibration and results were calculated using PerkinElmer Total Chrom 6.2.1 software. The calibration of the ethanol method was performed using certified calibration standards in water. The results for ethanol were compared to an untreated sample in the same run. The untreated sample was repeated several times within the run to detect any variation in results.

**Validation**

The performance of the GC–MS method was tested by a brief validation procedure to ensure repeatability of the method. Six calibration lines (concentration levels 2000, 1000, 500, 250, and 125 ng/mL) prepared in neat OF were measured in a single run to determine the linearity. A calibration line and three samples at three levels (2000, 500, and 125 ng/mL) were measured to determine the intraday precision of the method. Also, the selectivity of the method was determined by measuring blank OF samples collected from six drug-free subjects. The coefficient of determination \(r^2\) was > 0.992 for all analytes and the intraday variation was less than 10% RSD. OF specimens collected from the drug-free persons showed no interference with the analytes.

**OF collection and quantification**

OF volume obtained was studied in samples collected both from OF added into a test tube (in vitro) and from six volunteers (three women and three men of different ages). Each device was handled according to a distinct protocol that was based on the instructions of the manufacturer and the properties of the device. Measurements were made by weighing under the assumption that 1 mL of OF weighs 1 g (mean weight of 1 mL of OF was 0.993 g, determined by weighing, \(n = 42\)). The accuracy of the volume of collected OF was studied with Quantisal, Statsure, and Cozart in samples collected both in vitro and from the volunteers because the manufacturers stated that these would always collect 1 mL of OF, as indicated by the volume adequacy indicator in the collection pad. The accuracy of the buffer solution in Quantisal, Statsure, Intercept, and Cozart was also quantified gravimetrically using six replicates of the devices.

Both the amount of OF collected with the devices and recov-
erred from the devices were studied in vitro. Measurements were
done using six replicates of the devices. Only the devices that
contained absorbing components were tested (Quantisal, Inter-
cept, Statsure, Cozart, Salivate, OraCol, and OraTube). Two
milliliters of OF was added into a test tube, and the tube was
weighed. The collection pad was placed into the test tube, and
the manufacturer instructions were followed. Collection devices
that had a volume adequacy indicator were kept in OF until the
indicator turned blue. For the others, the collection times were
180 s, Intercept; 60 s, OraCol; 180 s, OraTube; and 45 s, Salivate.
After collection, the pad was removed from the test tube, and
the tube containing the remaining OF was weighed. The amount of
OF collected was measured as reduction in weight of 2 mL of OF.
After the collection, the manufacturer instructions for handling
the sample were followed. For the devices that did not include
any instructions (Quantisal and Cozart), attempts were made to
find the best suitable means for recovering the collected OF.

Each volunteer tested all 10 devices, but the quantification
of OF was only done on devices that included a collection pad;
the others were tested only for performance. Instructions for
the devices supplied by the manufacturers were translated into
Finnish and handed out to the volunteers. They were not fa-
familiar with the devices and collected the OF samples by fol-
lowing the given instructions. The instruction forms also
included questionnaires for feedback on the general perfor-
mance of the devices. Two devices per day were usually tested,
and the time between testing devices was advised to be at least
half an hour. Also, consumption of food and beverages had to
be avoided for half an hour before testing. After the collection,
the devices were weighed and assessed against the initial
weight of all parts of the devices, and the amount of collected
OF was determined as the increase in the weight of the device/test
tube containing the collected OF (and buffer) and the device/test
tube (and buffer) before collection.

Drug recovery
As the different devices differed in function, separate proto-
cols were established for each device. Blank OF was fortified to
a concentration of 1000 ng/mL by using the 1 mg/mL stock
solutions of the drug analytes in methanol. A fresh OF solution
was prepared for each device. Six replicates of the devices were
used. Known amounts of the OF solution were added to the
collection devices either to the pad or, in devices without a col-
lection pad, to the device itself. Added amounts were based on
the OF collection experiments and information provided by the
manufacturer. One milliliter was added to all devices except the
Intercept and the OraTube device. A volume of 850 μL was
used for the Intercept based on the OF collection experiments,
and 1.2 mL was used for OraTube because it was impossible to
recover enough OF for the analysis from the device if only 1 mL
was added. One milliliter of OF and 1.5 mL of extraction solu-
tion were added to the Greiner device. After the collection the
devices were left at room temperature for 1 h. Procedures
mentioned earlier were used to recover the samples.

For all devices including a buffer, all calibration samples
were prepared in OF and buffer, with the concentration level set
to correspond to the concentration in neat OF. The results of
the reference samples and controls and samples without a
buffer were calculated using the calibrators in neat OF. The
concentration levels were 2000, 1000, 500, 250 and 125 ng/mL.
The sample volume used was 500 μL. A 500-μL aliquot of 0.5
mol/L Na2HPO4·2H2O adjusted to pH 10 was added to each
sample as a buffer. The buffer contained 125 ng/mL of deuter-
ated analogues of the analytes serving as internal standards.
The samples were extracted using 5 mL of ethyl acetate, and
the organic phase was evaporated under a stream of air at
45°C. The residue was reconstituted in 70 μL of acetonitrile/
MSTFA (5:2, v/v), transferred to vials, capped, and heated
for 0.5 h at 80°C.

A control sample containing 700 ng/mL of the analytes in OF
was prepared, stored in plastic centrifuge tubes (Eppendorf)
at −18°C, and analyzed with all runs as quality control. In addi-
tion, a neat OF sample and, when testing devices including
buffer solution, a neat OF-buffer sample was analyzed in each
run as blank. Six replicates of fortified OF taken from the same
pool as the samples were measured as reference samples. The
recovery of the analytes was calculated as the ratio of the mean
concentration of the samples and the mean concentration of the
reference samples.

Devices with absorbing components were also studied to
see if they would concentrate analytes from an excess amount
of OF. Two milliliters of fortified OF, taken from the same pool
as the quantification and reference samples, was added to six
preweighed test tubes. The same collection procedure was
used as in the in vitro OF collection experiments. For OraTube
and Salivette, larger amounts of OF were used, 3.5 mL for
Salivette and 3 mL for OraTube, because these could collect
more than 2 mL. (This was seen earlier in the study). After the
collection, the samples were handled and analyzed according to
the procedure described.

Ethanol recovery
Ethanol recovery for each device was determined using only
three replicates. Results were calculated from the average of
three analyses of each replicate sample. The samples were pre-
pared in the same way as in the drug analysis. The stock solution
used consisted of fortified OF with a target concentration of
0.2% (v/v). A 500-μL aliquot of a 0.06% (v/v) solution of 2-
methyl-2-propanol in water was dispensed into headspace vials and 100 μL of sample added. The vials were capped and analyzed using the described method. The results were calculated as the ratio of ethanol concentration in the sample collected with the collection device to the average results for untreated stock solution.

Sample stability
The stability of the drug analytes was studied after 0, 14, and 28 days storage. All the devices except Greiner were stored at −18°C. Greiner was stored at +4°C. The devices were stored in accordance with the instructions of the manufacturer or if instructions were not provided at −18°C. The day 0 samples were the same as the drug recovery samples. Six replicates were analyzed on days 14 and 28 using the same procedure as described in Drug recovery, but no reference samples were analyzed. All samples of each device were collected from the same pool of fortified OF as the drug recovery and reference samples. Drug stability was calculated against the theoretical value (1000 ng/mL) of the fortified sample to obtain comparable results.

Results and Discussion
The differences in function between the devices were acknowledged and considered when testing the devices. As far as possible, the different devices have been treated individually. Results should, even if shown in parallel for all parameters, be interpreted with the differences in the devices in mind.

Of collection and quantification
The OF collection and quantification results are presented in Table II. The variation in the volume of collected OF was quite small in the in vitro samples, except for Cozart and OraCol, which had an RSD of more than 10%. It was very difficult to determine the OF recovery reliably for Quantisal, Stature, and Cozart, all of which included a buffer solution, because it was next to impossible to find out the ratio of OF and buffer solution in the recovered samples. Although the manufacturers state that the devices always collect a certain amount of OF and that the volume of the buffer is constant in all individual devices, the amount of OF in the mixture is not necessarily the amount collected because some of the OF and perhaps some quantity of the drugs, may remain in the collection pad. Thus, the ratio of OF and the buffer solution in the recovered sample is not automatically the ratio of collected OF and the buffer in the tube. Therefore, the results for Quantisal, Stature, and Cozart are not included in Table II. However, because the samples collected with the Intercept device (also including a buffer) were centrifuged, almost all liquid was recovered from the collection pad, and knowing the amount of buffer solution, the volume of recovered OF could be determined quite accurately.

It was also suggested by the manufacturer that OraCol could be centrifuged to recover the collected OF, but this was not possible because the collection pad was pressed to the bottom of the collection tube in the centrifuge, and after the centrifugation the sample was quickly reabsorbed. The pad had to be squeezed against the side of the tube to get as much OF as possible for the analysis. This, however, proved to be quite messy and time-consuming. The recovery was fairly low, and the variation in the results quite large. The same procedure was applied to Cozart. The collection pad of the Quantisal device disintegrated easily, so the procedure used with OraCol and Cozart could not be applied here. The pad was simply lifted from the solution.

The variation in the volume of OF collected was greater in the samples collected from volunteers than in the in vitro samples. The large variation between samples is problematic for the devices with buffer solution, as it is important to know the exact ratio of buffer and OF in the sample in order to get quantitative results for the drug recoveries. If the amount of OF collected differs considerably between samples taken from dif-

<table>
<thead>
<tr>
<th>Table II. The Results of the OF Collection of Samples Collected Both In Vitro and from Volunteers and the Accuracy of the Collected OF Volume for Devices with a Volume Adequacy Indicator (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>In Vitro</strong></td>
</tr>
<tr>
<td><strong>OF collected</strong></td>
</tr>
<tr>
<td>Mean (mL)</td>
</tr>
<tr>
<td>Quantisal</td>
</tr>
<tr>
<td>Stature</td>
</tr>
<tr>
<td>Intercept</td>
</tr>
<tr>
<td>Cozart</td>
</tr>
<tr>
<td>OraCol</td>
</tr>
<tr>
<td>OraTube</td>
</tr>
<tr>
<td>Salvette</td>
</tr>
<tr>
<td>Greiner</td>
</tr>
</tbody>
</table>

* Only for devices with a volume adequacy indicator.

† One result (included in the mean) differed substantially from the others; it was much smaller, 0.092 mL.

‡ The result for the mean collected OF is as a percentage of the total OF-buffer solution.
different individuals and the ratio of buffer and OF cannot be determined separately for each sample, the reliability of the results is compromised. With Greiner this problem does not exist because the quantification of the OF volume in the sample is done spectrophotometrically. The OF recoveries of Quantisal, Statsure, and Cozart are not included in Table II because, as mentioned earlier, calculating the recoveries for them would probably give unreliable results. Although the amount of recovered OF could not be reliably determined for Quantisal, Statsure, and Cozart, it is important that the amount of the OF recoveries of Quantisal, Statsure, and Cozart are not included in Table II because, as mentioned earlier, calculating the recoveries for them would probably give unreliable results. Although the amount of recovered OF could not be reliably determined for Quantisal, Statsure, and Cozart, it is important that the amount of the OF volumes collected from these devices was always more than 500 µL, which is the volume needed for the analysis. One volume of recovered OF for OraTube was substantially lower than the others, 0.092 mL, and this would not be enough to be used in the analysis, which is alarming. Although the volume of collected OF-buffer solution was not measured for Greiner, it was always at least 3 mL, so there were no problems getting enough sample for the analysis.

### Table III. Buffer Solution Volume Quantification (n = 6)

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Volume Stated by the Manufacturer (mL)</th>
<th>Mean Volume Measured (mL)</th>
<th>RSD (%)</th>
<th>Bias (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quantisal</td>
<td>3</td>
<td>3.015</td>
<td>0.40</td>
<td>0.50</td>
</tr>
<tr>
<td>Stature</td>
<td>1</td>
<td>0.970</td>
<td>2.27</td>
<td>-3.00</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.8</td>
<td>0.771</td>
<td>2.46</td>
<td>3.63</td>
</tr>
<tr>
<td>Cozart</td>
<td>2</td>
<td>2.171</td>
<td>1.57</td>
<td>8.55</td>
</tr>
</tbody>
</table>

### Table IV. Accuracy of the Spectrophotometric Determination of the OF-Buffer Solution Ratio (OF/BS) in Greiner (n = 3) Measured at Two OF/BS Levels

<table>
<thead>
<tr>
<th>OF/BS (%)</th>
<th>Set Value</th>
<th>Measured Value OF/BS (%)</th>
<th>RSD (%)</th>
<th>Bias (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>33.33 (level 1)</td>
<td>33.96*</td>
<td>3.4</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td>16.67 (level 2)</td>
<td>16.48</td>
<td>2.4</td>
<td>-1.1</td>
<td></td>
</tr>
</tbody>
</table>

* n = 2 because of one unsuccessful measurement.

### Table V. Drug and Ethanol (EtOH) Recoveries as Percentages of the Reference (n = 6)

<table>
<thead>
<tr>
<th></th>
<th>% Reference [Mean (RSD)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphetamine</td>
<td>MDMA</td>
</tr>
<tr>
<td>Quantisal</td>
<td>89.7 (9.7)</td>
</tr>
<tr>
<td>Stature</td>
<td>86.7 (5.7)</td>
</tr>
<tr>
<td>Cozart</td>
<td>75.4 (5.8)</td>
</tr>
<tr>
<td>Intercept</td>
<td>103.1 (2.7)</td>
</tr>
<tr>
<td>Greiner</td>
<td>86.4 (3.5)</td>
</tr>
<tr>
<td>OraCol</td>
<td>69.1 (1.2)</td>
</tr>
<tr>
<td>Salivette</td>
<td>51.8 (9.3)</td>
</tr>
<tr>
<td>OraTube</td>
<td>76.1 (4.4)</td>
</tr>
<tr>
<td>Salicule</td>
<td>98.1 (3.9)</td>
</tr>
<tr>
<td>Plastic tube</td>
<td>102.2 (2.1)</td>
</tr>
</tbody>
</table>

* Below cut-off (< 12.5% recovery).

### Accuracy of the volume adequacy indicator

The results for the accuracy of the volume of OF collected with Quantisal, Statsure, and Cozart are presented in Table II as biases (differing from the value of 1 mL). All devices had biases less than 10% (Statsure and Cozart less than 5%) in the samples collected from the volunteers, which suggests that the volume adequacy indicators are quite reliable. In the samples collected in vitro, Quantisal had a low bias but the others collected more OF than stated by the manufacturer. However, the devices are not designed for collecting samples in vitro so the results from the volunteers are more important. It should be noted, however, that the OF volumes collected from the volunteers had quite a large variation, as mentioned. The bias for the Cozart samples collected in the laboratory is calculated using all six samples, including the one from the device with the differently shaped collection pad, and if this value is excluded the bias is even higher.

### Quantification of the buffer solution

The quantification results of the amount of buffer solution for Quantisal, Statsure, Cozart, and Intercept are presented in Table III. As shown in the Table, the amounts stated by the suppliers are quite accurate, except for Cozart, in which the measured volume of the buffer is slightly greater than stated by the manufacturer. An important observation is that the variation in the results is quite small. This means that the buffer volume can be trusted to be fairly equal between individual devices, which may eliminate one problem in getting quantitative results for the drug recoveries. The results for the OF-buffer ratio in Greiner in Table IV show that the spectrophotometrical measurement is highly accurate.

### General performance of the devices

All the devices had collection times under 5 min, and the times for Quantisal, Statsure, OraCol, and Salivette were all less than 2 min. However, it should be remembered that all volunteers were healthy and not using drugs, and the collection times would possibly be much longer in situations where the samples are collected from suspected drug users. Greiner has an effective means of increasing salivation, which would...
be useful when the subject has difficulties producing enough sample, but the degree of stimulation caused by the citrate buffer and its effect on drug concentrations in OF are not known. This might lead to false-negative results. Also, the rather complicated collection procedure and the 2-min rinsing time would probably cause problems in sampling. All the devices, except Greiner and Salicule, were mainly assessed as easy to use. Intercept, Salivette, and OraTube got negative feedback on the taste of the collection pads, and the rinsing and extraction solutions in Greiner were considered to taste unpleasant. The most negative comments were given on the taste and feel of the pad of Salivette, which was said to taste awful and to feel like cardboard. Overall, the most positive evaluations were given for Quantisal and the plastic tube.

**Analyte recovery**

The drug recovery results are presented in Table V as percentages of the reference sample. As shown, Statsure was the only device with recoveries of more than 80% for all the analytes. The recoveries for Quantisal, Intercept, Greiner, Salicule, and the plastic tube were above 80% for all drugs except Δ⁹-THC, and the recovery of Δ⁹-THC for the plastic tube and Greiner was just below this mark. It was anticipated that the recovery of Δ⁹-THC would not be as good as that of the other analytes due to the absorption of Δ⁹-THC to the plastic and the absorptive components in the devices (17). Using organic solvents to recover the Δ⁹-THC from the collection pad has been shown to enhance the recovery (17,22). Moore et al. (12) studied the recovery and stability of Δ⁹-THC in OF samples collected with the Quantisal device. The recoveries obtained were 80–90%. Also, Quintela et al. (9) gained similar recoveries for Δ⁹-THC with the Quantisal device. In both studies the collection pads were kept in the buffer for a longer period of time (>12 h) than in this study (1 h), which might explain the better recoveries. The concentration of 1000 ng/mL is, for some analytes (e.g., Δ⁹-THC), much higher than the concentrations usually found in OF. This concentration was, however, chosen because the aim was to clearly see the differences in the recoveries between the different devices.

Ethanol was analyzed with fewer replicate samples than the other investigated substances. The deviation between samples was however small so more replicates would probably not have been of significance for this study. The recovery was good for all devices. For Salivette, the recovery was above 100%, indicating that the sample recovered after centrifugation was more concentrated. For the plastic tube, the recovery results were almost the same as the untreated samples. Cozart, Greiner, and Salicule were also close to the untreated sample. Other devices, all with absorptive components, had lower recoveries but still above 80%.

**Drug recovery from excess amount of OF**

Using excess amounts of OF did not change the recoveries in Quantisal, Statsure, Intercept, and OraTube, which suggests that the analytes do not concentrate on the collection pads of these devices. Salivette, on the other hand, had considerably higher results when the samples were collected from an amount of 3.5 mL, instead of 1 mL, of fortified OF. Although the results seemed better than for those samples with a lower volume of OF, the recoveries should not vary along with the sample volume because the concentration of the analytes in a device without a buffer solution should still be the same regardless of the volume of the sample. As Table II shows, the variation in volumes was quite notable for Salivette.

**Sample stability**

Losses of the analytes after 14 and 28 days of storage are presented in Table VI as percentages of day 0 recoveries (calculated per 1000 ng/mL). The concentrations of amphetamine and alprazolam in the Quantisal and Statsure samples decreased markedly during the 28-day testing period, but the other analytes were quite stable. For Cozart, the day 28 result for alpra-

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**Table VI. Stability of the Analytes Between Days 0 and 14 and 0 and 28 (n = 6)**

<table>
<thead>
<tr>
<th></th>
<th>Amphetamine</th>
<th>MDMA</th>
<th>Δ⁹-THC</th>
<th>Cocaine</th>
<th>Morphine</th>
<th>Codeine</th>
<th>Diazepam</th>
<th>Alprazolam</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quantisal</td>
<td>100.3</td>
<td>69.9</td>
<td>92.1</td>
<td>90.2</td>
<td>91.3</td>
<td>90.8</td>
<td>82.8</td>
<td>73.2</td>
</tr>
<tr>
<td>Statsure</td>
<td>75.1</td>
<td>69.4</td>
<td>90.9</td>
<td>87.2</td>
<td>95.2</td>
<td>78.4</td>
<td>86.1</td>
<td>82.6</td>
</tr>
<tr>
<td>Cozart</td>
<td>98.1</td>
<td>93.4</td>
<td>97.8</td>
<td>105.1</td>
<td>93.1</td>
<td>118.2</td>
<td>96.5</td>
<td>89.4</td>
</tr>
<tr>
<td>Intercept</td>
<td>97.9</td>
<td>75.2</td>
<td>91.3</td>
<td>82.5</td>
<td>94.6</td>
<td>108.5</td>
<td>75.7</td>
<td>72.3</td>
</tr>
<tr>
<td>Greiner</td>
<td>88.9</td>
<td>89.7</td>
<td>93.8</td>
<td>103.2</td>
<td>39.2</td>
<td>37.2</td>
<td>103.6</td>
<td>108.1</td>
</tr>
<tr>
<td>Oratube</td>
<td>84.7</td>
<td>76.0</td>
<td>73.4</td>
<td>59.2</td>
<td>B.C.</td>
<td>B.C.</td>
<td>90.5</td>
<td>78.4</td>
</tr>
<tr>
<td>Oracon</td>
<td>85.2</td>
<td>86.9</td>
<td>87.2</td>
<td>93.2</td>
<td>77.8</td>
<td>84.6</td>
<td>85.9</td>
<td>93.2</td>
</tr>
<tr>
<td>Salivette</td>
<td>91.6</td>
<td>83.9</td>
<td>115.0</td>
<td>109.5</td>
<td>B.C.</td>
<td>B.C.</td>
<td>97.7</td>
<td>95.9</td>
</tr>
<tr>
<td>Salicule</td>
<td>88.7</td>
<td>115.2</td>
<td>93.9</td>
<td>111.0</td>
<td>45.4</td>
<td>61.6</td>
<td>79.2</td>
<td>82.4</td>
</tr>
<tr>
<td>Plastic</td>
<td>86.9</td>
<td>87.8</td>
<td>91.4</td>
<td>93.6</td>
<td>86.4</td>
<td>82.0</td>
<td>81.1</td>
<td>74.6</td>
</tr>
</tbody>
</table>

* Recovery on day 0 is set to 100%.
* The day 28 result was excluded because of problems in calibration and variation in the results.
* Below cut-off (< 12.5% drug recovery).
zolam was excluded because the calibration curve had a low $r^2$, and there was considerable variation in the results of the individual samples. Otherwise, however, the analytes were quite stable in the Cozart samples. The amphetamine and cocaine concentrations decreased 25% and 28%, respectively, in the Intercept samples after 28 days of storage. The decreases in the analyte concentrations were otherwise quite small in the Greiner samples, but the concentration of $\Delta^9$-THC had decreased more than 60% after 14 days of storage. OraCol had decreases of about 0 to 25% in the results, but, because of the almost nonexistent concentration of diazepam and $\Delta^9$-THC, nothing can be said about their stability. Regarding $\Delta^9$-THC and diazepam, the same is true of Salivette. The decreases in the OraTube samples were between 0 and 20%. The analyte concentrations in Salicule seem to have decreased between days 14 and 28, but this can be explained by the control samples, which had slightly lower values in the measurements for day 14. The stability was quite good for the plastic tube with decreases of only 0 to 20% in the analyte concentrations, except for cocaine at 25%. Contrary to expectation, the stability was also quite good for the devices without a buffer solution, and the plastic tube did quite well in comparison with the ‘actual’ devices.

Contamination of the GC–MS equipment and problems with the buffer solutions

The baseline started rising soon after the beginning of the tests, and the shape of the peaks deteriorated during the six-week testing period, which indicates that the column had been contaminated by the non-volatile compounds of the buffers. In particular the amphetamines and alprazolam had very poor peaks at the end. Also, the liner in the GC had to be changed much more often than in the routine analysis of urine and blood samples. Unfortunately, it is not possible to tell, on the basis of these measurements, which of the devices specifically caused the baseline to rise during the testing.

Conclusions

The study shows that there are substantial differences between the OF collection devices on the market. Some devices are well suited for toxicological analysis but some give very poor recoveries for the analytes. The buffer solutions used in some devices may help to increase the recovery of the drugs and improve the stability of the samples. However, the buffers cause some problems in getting quantitative results for the drug recoveries and may also contaminate the analysis equipment. The recovery of $\Delta^9$-THC from the devices with absorbing components is a problem. If a collection device is to be used to collect samples for a toxicological analysis the recovery of the analytes and the overall reliability of the device has to be sufficiently tested before using it to collect actual samples. The described approach in testing the performance of the devices can easily be applied to test new devices on the market. The results of the study emphasize the impact of the selection of the OF collection device on the whole toxicological procedure.

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