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Evaluation of three rapid oral fluid test devices on the screening of multiple drugs of abuse including ketamine

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ABSTRACT

Rapid oral fluid testing (ROFT) devices have been extensively evaluated for their ability to detect common drugs of abuse; however, the performance of such devices on simultaneous screening for ketamine has been scarcely investigated. The present study evaluated three ROFT devices (DrugWipe® 6S, Ora-Check® and SalivaScreen®) on the detection of ketamine, opiates, methamphetamine, cannabis, cocaine and MDMA.

A liquid chromatography tandem mass spectrometry (LCMS) assay was firstly established and validated for confirmation analysis of the six types of drugs and/or their metabolites. In the field test, the three ROFT devices were tested on subjects recruited from substance abuse clinics/rehabilitation centre. Oral fluid was also collected using Quantisal® for confirmation analysis.

A total of 549 samples were collected in the study. LCMS analysis on 491 samples revealed the following drugs: codeine (55%), morphine (49%), heroin (40%), methamphetamine (35%), THC (8%), ketamine (4%) and cocaine (2%). No MDMA-positive cases were observed.

Results showed that the overall specificity and accuracy were satisfactory and met the DRUID standard of >80% for all 3 devices. Ora-Check® had poor sensitivities (ketamine 36%, methamphetamine 63%, opiates 53%, cocaine 60%, THC 0%). DrugWipe® 6S showed good sensitivities in the methamphetamine (83%) and opiates (93%) tests but performed relatively poorly for ketamine (41%), cocaine (43%) and THC (22%). SalivaScreen® also demonstrated good sensitivities in the methamphetamine (83%) and opiates (100%) tests, and had the highest sensitivity for ketamine (76%) and cocaine (71%); however, it failed to detect any of the 28 THC-positive cases. The test completion rate (proportion of tests completed with quality control passed) were: 52% (Ora-Check®), 78% (SalivaScreen®) and 99% (DrugWipe® 6S).

1. Introduction

Oral fluid is becoming a popular matrix for rapid screening of drugs of abuse. In contrast to blood and urine, collection of oral fluid is easy and non-invasive with minimal intrusion into personal privacy. Oral fluid can also be collected under direct observation, thus eliminating the possibility of sample substitution or adulteration as with urine. As such, oral fluid can be useful in various settings that require drug testing, for example workplace, corrections, probation or for treatment. Importantly, it is by far the most convenient biological matrix that facilitates roadside testing for driving under the influence of drugs (drugged driving) [1]. Compared with urine, oral fluid is a better reflection of blood concentrations of a drug. It indicates recent drug use and provides better correlation with pharmacological effects such as impaired driving performance [2].

Drugged driving is a major concern worldwide. In the large-scale European Union (EU) study, Driving under the Influence of Drugs, Alcohol and Medicines (DRUID), it has been reported that the detection rate of illicit drugs in the general driving population was

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1.9%. This detection rate was higher in seriously injured drivers (2.3–12.6%) [3]. In Hong Kong, a study on the prevalence of illicit drug use in non-fatal traffic accident casualties showed that 10% of the injured drivers tested positive for drugs (although it should be noted that urine rather than blood was tested). Ketamine was the most commonly detected substance found in 45% of the subjects [4].

Currently, many countries including Germany, France, Belgium, Italy, Finland and Australia routinely conduct roadside rapid oral fluid testing (ROFT) to tackle drugged driving [5]. Prior to usage, ROFT devices must undergo rigorous scientific evaluation to ensure acceptable performance in terms of their sensitivity, specificity and overall accuracy. In the early EU studies on Roadside Testing Assessment (ROSITA-1 and -2), the proposed acceptance criteria of sensitivity and specificity were >90% and accuracy >95% [6,7]. These criteria were later lowered to 80% in the subsequent DRUID study [8]. During the past two decades, ROFT devices have been extensively evaluated and the results widely published [9–14]. However, whilst the performance of ROFT devices for detecting amphetamines, opiates, cocaine and cannabis (THC) has been comprehensively investigated, there is currently minimal data for ketamine.

Although the abuse of ketamine is widespread in Hong Kong and Asia, it has not traditionally been a popular drug of abuse in Europe and North America [15]. As a result, detailed investigations of ROFT device performance on screening for ketamine have been scarce thus far. One study evaluated the performance of OratectXP solely on the detection of ketamine [13]. On the other hand, recent publications have reported an increase in the use of ketamine in Europe [9,16]. In view of this, the current study was conducted to evaluate ROFT devices suitable for simultaneous screening of ketamine as well as five other illicit substances (heroin, methamphetamine, cannabis, cocaine and MDMA). Three ROFT devices (DrugWipe® 6S, Ora-Check® and SalivaScreen®) were chosen for evaluation of their sensitivity, specificity and accuracy. Prior to conducting the ROFT field test, a liquid chromatography tandem mass spectrometry (LCMS) assay was established for confirmation analysis, the results of which will be used to assess the performance of the ROFT devices.

2. Methods

2.1. Materials

Reference standards and deuterium internal standards (I.S.) for each analyte were purchased from Cerilliant (Round Rock, TX) or Lipomed (Arlesheim, Switzerland), including ketamine (KET), norketamine (NORKET), methamphetamine (MET), amphetamine (AMP), methylenedioxymethamphetamine (MDMA), methylenedioxyamphetamine (MDA), 6-monoacetylmorphine (6-MAM), codeine (COD), morphine (MOR), cocaine (COC), benzoylecgonine (BEG), cannabis (THC), KET-D4, NORKET-D4, MET-D5, AMP-D5, MDMA-D5, MDA-D5, 6-MAM-D3, COD-D6, MOR-D6, COC-D3, BEG-D8 and THC-D3.

Isolute® SLE+ supported-liquid extraction (SLE) 400 μL columns were obtained from Biotage (Uppsala, Sweden). Quantisal® synthetic negative oral fluid (pre-diluted in extraction buffer) and Quantisal® oral fluid collection devices were purchased from Alere (Waltham, MA).

The ROFT device DrugWipe® 6S was purchased from Securetec (Neubiberg, Germany), Ora-Check® from Safecare Biotech (Hangzhou, China) and SalivaScreen® from Ulti med Products (Ahrensburg, Germany).

2.2. ROFT field test

Subjects were recruited from the Hospital Authority substance abuse clinics at Castle Peak Hospital (CPH), Kwai Chung Hospital (KCH) and Pamela Youde Nethersole Eastern Hospital (PYNEH), as well as the Society of Rehabilitation and Crime Prevention (SRACP) in Hong Kong. Written informed consent was obtained from all subjects, who were at least 18 years of age. Repeated sampling was allowed provided that each collection was at least one week apart. The protocol had been approved by the Hospital Authority Kowloon West Cluster Research Ethics Committee.

For each subject, a confirmation sample was firstly collected using the Quantisal® oral fluid collection device. The sampling sponge was placed in the subject's oral cavity for 10 min (or when the indicator turned blue, whichever was earlier). The sponge, which was supposed to have collected 1 mL of oral fluid, was then deposited into the designated tube containing 3 mL of buffer. This sample was subsequently transported back to the laboratory and the weight of the whole tube was recorded for adjusting the volume of oral fluid collected. The sample was then stored at 4 °C for 3 days, after which a plunger separator was used to harvest all the buffered oral fluid inside the tube. The oral fluid sample was then stored in a separate container at −80 °C until analysis. Those samples with weight corresponding to less than 0.5 mL oral fluid were not subjected to confirmation analysis; whilst samples with volume between 0.5 and 1 mL were analysed for all analytes except cocaine and THC.

The ROFT devices, as shown in Fig. 1, were evaluated sequentially on each subject. Some subjects did not have sufficient oral fluid to complete all three evaluations. DrugWipe® 6S required the least amount of oral fluid (approximately 0.1 mL), thus was tested last of the three. In order to have similar number of completed tests for Ora-Check® and SalivaScreen®, these two devices were tested first on alternate days. When at least four LCMS-positive cases (with completed ROFT testing) have been achieved for all analytes on a device, the data was considered meaningful for interpretation [12,17] and thus testing on this device would be terminated.

Ora-Check® and SalivaScreen® were capable of separately testing all 6 drug classes: ketamine, methamphetamine, cannabis, cocaine, MDMA and opiates (OPI). DrugWipe® 6S only detected 5 types of drugs: ketamine, cannabis, cocaine, opiates and the amphetamines. This device was unable to differentiate among amphetamine-type drugs; this class of drugs was tested collectively by one "AMP/MET" test.

The DrugWipe® 6S device consisted of a sample collector containing 3 small sampling pads, the test cassette and an integrated liquid ampoule. Oral fluid was collected by wiping the sampling pads on the tongue several times until the pads changed colour. The collector was then placed back onto the test cassette, with the pads in contact with the test strips. The device was held vertically; the liquid ampoule was broken by compression and the buffer flowed along the test strips. After 10 s, the device was placed on a horizontal surface and the results read after 8 min. Result interpretation was performed according to the manufacturer's instructions (i.e. a visible band indicated a positive result. Faint bands were regarded as positive).

The Ora-Check® device comprised a sampling sponge, a collection chamber and the test cassette. The sponge was placed in the subject's mouth for 3 min (with occasional sweeping motion), during which supposedly 0.5 mL oral fluid would have been collected. The sponge was then firmly pushed into the collection chamber to release the oral fluid. The chamber was inverted and the oral fluid was transferred through the dropper onto the sampling area of the test cassette. After 10 min, results were interpreted according to the manufacturer's instructions (i.e. a visible band indicated a negative result. Faint bands were regarded as negative).

The SalivaScreen® device consisted of a sampling sponge with volume indicator (1 mL) and a test cassette that extracted the oral

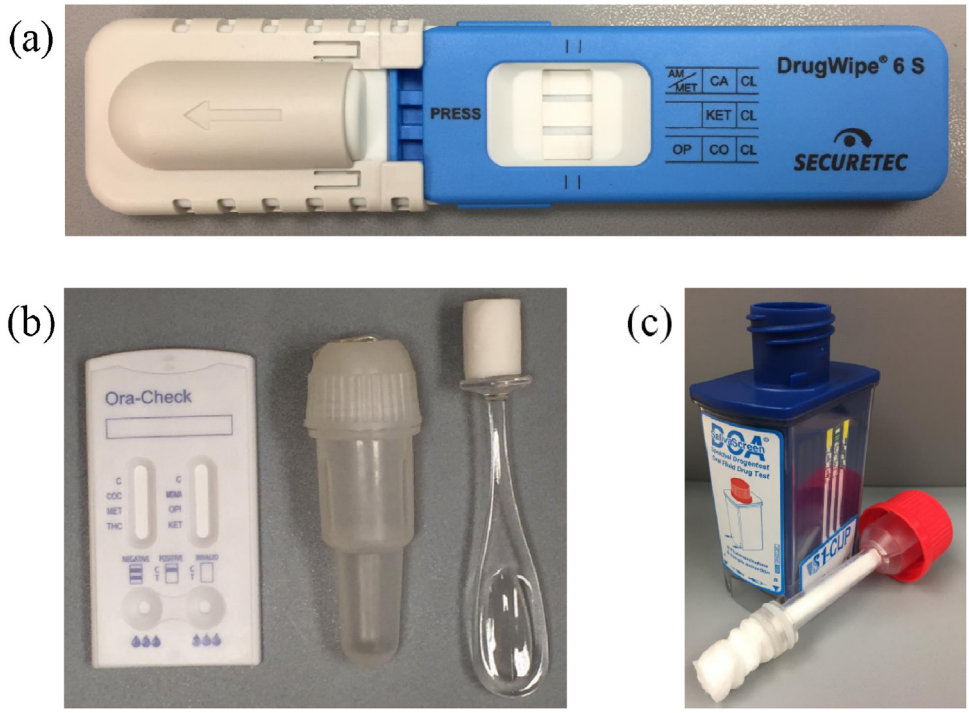

Fig. 1. ROFT devices included in the study: (a) DrugWipe® 6S; (b) Ora-Check®; (c) SalivaScreen®.

fluid and housed the test strips. The subject was first instructed to sweep the sampling sponge inside the oral cavity several times and leave the sponge inside for 7 min (or when the volume indicator turned red, whichever was earlier). The sponge was then pushed into the test cassette to release the oral fluid. The device was left on a flat surface for 10 min, after which results were read according to the manufacturer's instructions (i.e. a visible band indicated a negative result. Faint bands were regarded as negative).

For all devices, absence of the quality control (QC) band indicated a failed test, i.e. QC failure, and the results were regarded as invalid. The test completion rate (%) was calculated for each device by comparing the number of tests that were completed with QC pass against the total number of tests.

2.3. Calibrators and quality controls

Calibrators and QC were prepared by spiking synthetic negative oral fluid with the standards. Calibrators were spiked at the following concentrations: THC (0.5–200 ng/mL); 6-MAM, COC and BEG (1–200 ng/mL); AMP, MET, MOR, COD, MDMA and MDA (5–500 ng/mL); KET and NORKET (5–1500 ng/mL). Three levels of QC were prepared by spiking at the low and high ends as well as near the middle of the calibration range of each analyte.

2.4. Oral fluid analysis

All oral fluid samples were analysed within 8 weeks post-collection. To 400 μL of oral fluid sample (in Quantisal® buffer) was added 50 μL of I.S. mix. 400 μL of sample was loaded onto the SLE column, and eluted twice by 1 mL of elution solution (dichloromethane:isopropanol 70:30 v/v). The eluate was dried under nitrogen at 40 °C and reconstituted in 100 μL. For THC, MOR, 6-MAM and AMP, the reconstituted fraction was injected directly for LCMS analysis. For the other analytes, the fraction was diluted 25-fold with reconstitution solution prior to LCMS analysis. Should initial LCMS analysis reveal concentrations above the highest calibrator for certain analyte(s), such samples were repeated with a 50-fold dilution (20 μL samples diluted with 980 μL of blank oral fluid). A maximum of 500-fold dilution was required for some analytes in a few samples.

2.5. LCMS analysis

LCMS analysis was performed on a Sciex 5500 QTrap triple-quadrupole mass spectrometer (Framingham, MA, USA) equipped with Waters Acquity UPLC (Milford, MA, USA). Chromatographic separation was performed with a Waters Acquity HSS C18 SB column (1.8 μm, 2.1 × 100 mm) and gradient elution. Analytes were detected by mass spectrometry using scheduled multiple reaction monitoring (MRM) in positive electrospray ionization mode. Positive identification of an analyte was based upon retention time (RT) and MRM ratio. Compounds were quantified by comparing the analyte/I.S. peak area ratio against the calibration curve. In order to adjust for the actual volume of oral fluid collected by Quantisal® (which in reality might not be exactly 1 mL), the following formula was used:

$$C\,adjusted = \frac{C\,unadjusted \times (3 + w - w')}{4 \times (w - w')}$$

where C *adjusted* = analyte concentration with adjustment of oral fluid volume collected; C *unadjusted* = unadjusted analyte concentration; w = weight of sample and Quantisal® oral fluid collection tube; w′ = average weight of Quantisal® oral fluid collection tubes (n = 30) without sample.

2.6. Method validation

The analytical method was validated according to international guidelines and published protocols [18–22]. The protocol included evaluation of selectivity, linearity, limit of quantitation (LOQ), accuracy, precision, extraction efficiency, matrix effect, carryover, dilution integrity and stability.

The recovery of analytes from and the stability in the Quantisal™ oral fluid collection device was assessed by adding

oral fluid spiked with analytes (at low, mid and high concentrations, n = 3) to the collection pad. The collection pad was left in the buffer and stored at different temperatures for certain time points, including: room temperature for 1 day, 4 °C for 1 day, 4 °C for 2 days, 4 °C for 3 days and 4 °C for 4 days. This storage is to allow the analytes to desorb from the pad into the buffer for subsequent analysis, whilst maintaining analyte stability. The buffered oral fluid was then separated from the collection pad using a plunger and then analysed. To establish the reference value, oral fluid of equivalent concentration but without adding to the device was analysed. The recovery at each concentration was calculated by: Recovery (%) = average of samples using device/average of reference without using device. A recovery rate of >80% was considered desirable.

2.7. Data interpretation

In order to evaluate the sensitivity, specificity and overall accuracy of the ROFT devices, the analyte concentrations measured by LCMS (and adjusted for volume of oral fluid collected) were interpreted against the DRUID cut-off [17]; for ketamine and norketamine, no DRUID cut-off was available and the LCMS cut-off (LOQ of the method) was used. If any drug or its cross-reacting compound is quantitated at or above the respective cut-off, the result is considered to be positive. A summary of the DRUID and LCMS cut-offs, as well as the manufacturer-claimed device cut-offs, is shown in Table 1.

In this way, the ROFT field test data could be classified into the following categories: true positive (TP) where a positive ROFT device result matches a positive LCMS result; true negative (TN) where a negative ROFT device result matches a negative LCMS result; false positive (FP) where the ROFT device result was positive but with a negative LCMS result; and false negative (FN) where the ROFT device result was negative but the LCMS result was positive.

Taking into consideration the above classification, the following parameters could be calculated: Sensitivity (%) = TP/(TP + FN) × 100, Specificity (%) = TN/(TN + FP) × 100, Accuracy (%) = (TP + TN)/(TP + TN + FP + FN) × 100, Prevalence (%) = (TP + FN)/total no. of results × 100, Positive predictive value (PPV) (%) = TP/(TP + FP) × 100, Negative predictive value (NPV) (%) = TN/(TN + FN) × 100. Evaluation of the above parameters was only conducted for analytes with at least four positive cases [12,17].

3. Results

3.1. Validation results

According to international guidelines and published protocols, the method was validated with satisfactory performance in all evaluation criteria (refer to Supplementary data for details). In brief, the method was found to be selective, accurate and precise, with acceptable extraction efficiency, matrix effect and stability.

In order to investigate the optimal storage conditions for maximum recovery of analytes from the Quantisal® device, analytes were spiked onto the device and stored at different temperatures and for different durations prior to analysis. Results showed that THC was poorly recovered from the collection device on the first 2 days (recovery: 48.7–67.5%); other analytes like norketamine, 6-MAM, codeine, BEG and MDMA also had marginal recovery (77.6–79.7%). Upon storage at 4 °C for 3 days, all analytes had >80% recovery; this storage condition was chosen for all subsequent analysis. On day 4, the recovery of THC and cocaine decreased again (53.4% and 76% respectively).

3.2. LCMS analysis

In total, 549 samples were collected in the study – 207 (38%) from SRACP, 173 (32%) from CPH, 100 (18%) from PYNEH and 69 (13%) from KCH. Among the 549 samples, 491 (89%) could be subjected to LCMS analysis whilst the remainder did not have sufficient oral fluid for confirmation analysis.

Opiates were the most commonly encountered drugs with prevalence of 55% (codeine), 49% (morphine) and 40% (heroin). This was followed by methamphetamine (35%). Ketamine, THC and cocaine were detected at relatively low prevalence rates (2–8%). MDMA was not detected in any samples. The LCMS analysis results of individual analytes are summarized in Table 2.

3.3. General performance of ROFT devices

The number of tests performed on each device was as follows: SalivaScreen® (n = 549), Ora-Check® (n = 547), DrugWipe® (n = 515). Many problems were encountered while using the Ora-Check® device. Despite strict adherence to the manufacturer's protocol, in nearly half of the cases the volume of oral fluid collected was insufficient for the testing to continue. Specifically, after placing the collection sponge in the subject's mouth for the designated duration (3 min), the sponge was still too hard and no oral fluid could be squeezed out of the sponge; as such, the testing could not proceed further since no oral fluid was available for adding to the test cassette. This problem was communicated to the manufacturer, whose advice was to increase the collection time to 5 min. However, this was to no avail and the success rate was not found to increase. As such, the test completion rate of Ora-Check® was very low (52%) due to the large number of cases with insufficient oral fluid (n = 255) and 5 cases of QC failure. There was 1 case with completed ROFT testing but missing LCMS analysis due to insufficient oral fluid volume. The overall number of valid samples (successful ROFT and LCMS testing) was 286 (52%).

Table 1
Summary of cut-off values.

	Cut-off value (ng/mL)				
	DrugWipe® 6S	Ora-Check®	SalivaScreen®	DRUID	LCMS
KET	5	50	25	–	5
NORKET	75	50	30	–	5
MET	80	50	50	25	5
AMP	80	–	–	25	5
MDMA	25	50	50	25	5
MDA	10	250	250	25	5
6-MAM	5	25	10	5	1
COD	5	10	8	20	5
MOR	10	40	10	20	5
COC	10	20	20	10	1
BEG	75	20	200	10	1
THC	20	50	50	1	0.5

Table 2
The number of positive samples and concentrations detected by LCMS analysis.

	No. of positive samples	Concentration (ng/mL)		
		Mean	Median	Range
KET	18 (4%)	4887	210	6–55,136
NORKET	18 (4%)	406	165	7.4–2270
MET	174 (35%)	1917	602	5.1–23,612
AMP	157 (32%)	310	96	5.3–16,713
6-MAM	197 (40%)	587	28	1.1–25,436
COD	269 (55%)	1515	93	5–40,776
MOR	239 (49%)	553	132	5–16,337
COC	9 (2%)	123	10	1.2–753
BEG	8 (2%)	24	19	1.4–59
THC	39 (8%)	95	6	0.5–1958

In contrast to Ora-Check®, the test completion rate of DrugWipe® 6S was very high (99%). Due to 5 cases of QC failure and 55 cases missing LCMS analysis due to insufficient oral fluid volume, the overall proportion of valid samples was 88%.

The test completion rate of SalivaScreen® was 78%. All failed tests (n = 123) were attributed to QC failure, whilst 6 samples were missing LCMS analysis due to insufficient oral fluid volume. The overall proportion of valid samples was 77%.

3.4. Evaluation of ROFT device performance

The specificity and accuracy were in general >80% (the DRUID recommended "satisfactory" level) across all devices, except for the accuracy of Ora-Check® in detecting OPI (76%). In contrast, wide variability was observed in the sensitivities of the devices. A summary of the ROFT evaluation data is presented in Table 3. No MDMA-positive case was observed across the entire study; hence, it was not possible to assess the sensitivity of the devices.

The sensitivity plot is shown in Fig. 2. The 95% confidence intervals (CI) were calculated using the modified Wald method [23]. SalivaScreen® had the highest sensitivity on average across the analytes. The sensitivities for methamphetamine (MET) and opiates (OPI) were >80% for both DrugWipe® 6S and SalivaScreen®; however, the sensitivities for ketamine (KET) and cocaine (COC) were generally higher with SalivaScreen®. On the other hand, the sensitivity for THC was better with DrugWipe® 6S (Ora-Check® and SalivaScreen® had sensitivities of 0%). None of the tests achieved ≥80% sensitivity with the Ora-Check® device.

Among the analytes, a larger variability in the sensitivities of the KET, COC and THC tests was observed across the three devices. The mean analyte concentrations in the false-negative samples according to the device cut-off are summarized in Table 4. For KET, the median concentration of DrugWipe® 6S and Ora-Check® false-negative samples were 5–6 fold higher than the claimed cut-off; in particular, the max concentrations were remarkably high. In contrast, the concentrations of SalivaScreen® false-negative samples were indeed close to the claimed cut-off. For COC, again the max concentration in DrugWipe® 6S false-negative samples was up to 19 times higher than the claimed cut-off. In the case of THC, the median concentrations of Ora-Check® and SalivaScreen® false-negative samples were 2–3 fold higher than the claimed cut-off, and both devices failed to identify THC concentrations as high as 1958 ng/mL.

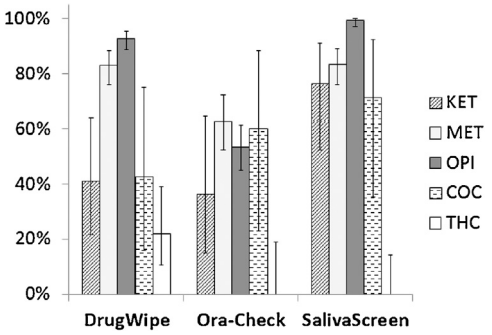

Fig. 2. Sensitivity plots (±95% CI) of individual tests of each ROFT device.

4. Discussion

In the past, ROFT devices have been extensively evaluated on screening for amphetamines, opiates, cocaine, THC and benzodiazepines [9–14,17]. Ketamine has not traditionally been a commonly abused drug except in Asia; hence, there has thus far been a lack of evaluation of ROFT devices that include screening for ketamine among other drugs. However, an increasing trend in the abuse of this drug has been reported [9,16], and ketamine has been

Table 3
Summary of ROFT device performance.

	Ketamine				Methamphetamine[a]				Opiates		
	DrugWipe 6S	Ora-Check	Saliva Screen		DrugWipe 6S	Ora-Check	Saliva Screen		DrugWipe 6S	Ora-Check	Saliva Screen
TP	7	4	13	TP	122	54	106	TP	218	73	212
TN	411	271	398	TN	274	185	241	TN	200	145	171
FP	27	4	5	FP	34	15	52	FP	20	4	36
FN	10	7	4	FN	25	32	21	FN	17	64	1
Total	455	286	420	Total	455	286	420	Total	455	286	420
Sensitivity	41%	36%	76%	Sensitivity	83%	63%	83%	Sensitivity	93%	53%	100%
Specificity	94%	99%	99%	Specificity	89%	93%	82%	Specificity	91%	97%	83%
Accuracy	92%	96%	98%	Accuracy	87%	84%	83%	Accuracy	92%	76%	91%
Prevalence	3.7%	3.8%	4.0%	Prevalence	32%	30%	30%	Prevalence	52%	48%	51%
PPV	21%	50%	72%	PPV	78%	78%	67%	PPV	92%	95%	85%
NPV	98%	97%	99%	NPV	92%	85%	92%	NPV	92%	69%	99%
	Cocaine				THC				MDMA[b]		
	DrugWipe 6S	Ora-Check	Saliva Screen		DrugWipe 6S	Ora-Check	Saliva Screen		DrugWipe 6S	Ora-Check	Saliva Screen
TP	3	3	5	TP	7	0	0	TP	–	0	0
TN	422	280	404	TN	397	265	384	TN	–	275	405
FP	0	0	1	FP	0	0	0	FP	–	11	15
FN	4	2	2	FN	25	20	28	FN	–	0	0
Total	429	285	412	Total	429	285	412	Total	–	286	420
Sensitivity	43%	60%	71%	Sensitivity	22%	0%	0%	Sensitivity	–	n.a.[c]	n.a.[c]
Specificity	100%	100%	100%	Specificity	100%	100%	100%	Specificity	–	96%	96%
Accuracy	99%	99%	99%	Accuracy	94%	93%	93%	Accuracy	–	96%	96%
Prevalence	1.6%	1.8%	1.7%	Prevalence	7.5%	7.0%	6.8%	Prevalence	–	0%	0%
PPV	100%	100%	83%	PPV	100%	n.a.	n.a.	PPV	–	n.a.[c]	n.a.[c]
NPV	99%	99%	100%	NPV	94%	93%	93%	NPV	–	n.a.[c]	n.a.[c]

n.a. – not applicable.
[a] Includes the whole amphetamine group for DrugWipe 6S.
[b] MDMA included in the "Methamphetamine" test for DrugWipe 6S.
[c] These parameters were not calculated since the number of positive samples was <4.

Table 4
Concentrations of KET, COC and THC in false-negative samples according to the device cut-off.

Test	Device	Device cut-off (ng/mL)[a]	No. of false-negatives	Concentration (ng/mL)		
				Median	Min	Max
KET	DW	5	10	29	6.5	2122
	OC	50	5	259	100	1023
	SS	25	2	n/a	31	36
COC	DW	10	3	13	10	188
	OC	20	1	n/a	60	60
	SS	20	0	n/a	n/a	n/a
THC	DW	20	9	93	20	208
	OC	50	4	126	51	1958
	SS	50	5	154	51	1958

DW: DrugWipe® 6S, OC: Ora-Check®, SS: SalivaScreen®.
[a] The device cut-off takes into account the cross-reactivity of metabolites according to the manufacturer's insert. The concentration results shown here also take into account the cross-reactivity.

associated with driving impairment resulting in road accidents [4]. In view of the rising need of ketamine-including ROFT devices, the present study was conducted to assess the performance of three such devices in the detection of heroin, methamphetamine, cannabis, cocaine, MDMA and importantly, ketamine.

A liquid chromatography tandem mass spectrometry method was firstly established for the simultaneous quantitation of the aforementioned six drugs as well as their metabolites in oral fluid. The method was fully validated and deemed to be fit for use according to international standards [18–22].

The Quantisal® oral fluid collection device has been shown to have good analyte recovery in previous evaluations [24–26] and was chosen for the present study. Satisfactory performance was also observed presently with >80% recovery of the analytes. Since the storage duration and temperature may also affect the extraction of analytes from the collection sponge into the buffer, these parameters were evaluated. The optimal conditions were found to be storage at 4 °C for 3 days. Prior to 3 days, certain analytes might not have sufficient time to extract into the buffer; alternatively, after 3 days susceptible analytes (e.g. THC and cocaine are known to be relatively unstable [22]) could be prone to degradation.

The current study population included predominantly patients undergoing drug rehabilitation or persons known to be active drug users, hence a higher prevalence compared with the normal population is expected. This choice is justified since a larger number of positive samples will yield more accurate and precise findings [27]. Indeed, as with previous studies adopting a similar approach, results would not be interpreted for a particular analyte if the number of positive specimens was less than four [12,17].

In the present study, a total of 549 oral fluid samples were collected from participants, among which confirmation analysis was performed on 491 samples. Analysis revealed that the most prevalent drugs detected were opiates (codeine 55%, morphine 49%, 6-MAM 40%), followed by methamphetamine (35%) and THC (8%). Despite a lack of local prevalence data on drugs of abuse detected in oral fluid, the Central Registry of Drug Abuse in Hong Kong reported similar statistics with heroin and methamphetamine being the most commonly abused substances [28]. A recent local study also reported opiates and methamphetamine as the most prevalent drugs detected in the urine of 964 drug abusers [29]. On the contrary, perhaps owing to the difference in sample matrix and study population, THC was detected at a higher rate (8% versus 3%) and ketamine at a lower rate (4% versus 20%) in the present evaluation. In both studies, cocaine was detected at a relatively low frequency and MDMA was not detected at all.

The oral fluid concentrations of ketamine and norketamine detected in the current evaluation were similar to previously reported studies (6–14431 ng/mL and 7.4–2270 ng/mL respectively) [13,30], except for the grossly elevated ketamine level in one sample (55136 ng/mL), which might be due to oral contamination by recent drug use. Comparison with a previous study conducted in Belgium [10] of the median drug concentrations of cocaine and BEG showed lower levels in the current study (cocaine 52.2 versus 10 ng/mL; BEG 81.5 versus 19 ng/mL). This could partly be explained by the lower prevalence of cocaine use or variation in the dosage across different regions. Apart from cocaine, considerable (>3-fold) difference in the median drug concentrations of amphetamine (685.1 ng/mL) and THC (31.4 ng/mL) was also observed in the Belgium study. Oral fluid concentrations of other analytes in the current study were broadly similar to those in previous reports [10,17].

In the present study, three ROFT devices – DrugWipe® 6S, Ora-Check® and SalivaScreen® – were chosen for evaluation. These three devices were commercially available locally at the time of the study and included all the six illicit drugs (these six drugs were chosen since they were specified in the Hong Kong Road Traffic Ordinance [31]). Previous versions of DrugWipe® (mainly for detecting 5 drugs) have been extensively studied [9,11,12,17], whilst Ora-Check® and SalivaScreen® have thus far not been tested on authentic oral fluid samples before.

DRUID cut-offs were used to evaluate the devices. Unlike adopting manufacturer-claimed cut-offs, this approach does not take into account the cross-reactivity of related compounds. However, in comparison to the manufacturer-claimed cut-off (which vary widely among devices) or LCMS cut-off (which vary among different methods), the DRUID cut-off is universal and allows direct comparison across devices and across different studies [12,17]. Additionally, this set of cut-off evaluates the performance of devices in detecting the presence of drugs at clinically relevant levels, rather than in adhering to the cut-offs claimed by the manufacturer.

In addition to analytical performance, the test completion rate is another important factor in determining the usefulness of a ROFT device. In this regard, DrugWipe® 6S has excellent performance with a completion rate of 99%. On the contrary, nearly half of the tests performed on Ora-Check® did not reach completion. In the majority of these cases, the testing could not proceed beyond the collection step since the sponge failed to yield any oral fluid for the testing to continue, despite strict adherence to the manufacturer's protocol. In the author's opinion, the sponge was too hard such that even after the designated collection duration, it still could not soften enough to yield any oral fluid. Indeed, the sponge of the SalivaScreen® device is much softer and no uncompleted tests have been observed due to failure in harvesting oral fluid from the sponge.

On the other hand, compared with DrugWipe® 6S, the QC failure rate of SalivaScreen® was considerably higher (22% versus 1%). This may again possibly be due to not having sufficient oral fluid collected. SalivaScreen® was designed to collect 1 mL of oral fluid, while DrugWipe® 6S only required approximately 0.1 mL. In drug users who often have reduced salivation [2] (and from whom 1 mL of oral fluid had already been collected for confirmation analysis), the likelihood of having sufficient oral fluid to complete DrugWipe® 6S testing is understandably much higher than that of SalivaScreen®.

Similar to previous reports, the specificity and accuracy of the ROFT devices were in general satisfactory and met the DRUID recommendation of >80% (except for the 76% accuracy of Ora-Check® in detecting opiates). So far, the problem encountered with most ROFT devices has been the sensitivity. In most studies, none of the devices could reach 80% sensitivity for all the detected analytes; in particular, the cocaine and THC tests have always been problematic [10,12,17]. In the present study, as shown in Fig. 2, only the methamphetamine and opiates tests reached 80% sensitivity and only for DrugWipe® 6S and SalivaScreen®.

Similar to published data [10,17], the sensitivity of the cocaine and THC tests was considerably lower in comparison and was <80% across all presently studied devices. Indeed, Ora-Check® and SalivaScreen® failed to identify any of the 20+ THC-positive cases; whilst DrugWipe® 6S, albeit far from satisfactory, achieved the highest sensitivity at 22%. In terms of cocaine, SalivaScreen® achieved the highest sensitivity (71%), followed by Ora-Check® (60%) and lastly DrugWipe® 6S (43%). Previous studies adopting the DRUID cut-off reported THC and cocaine sensitivities of 43–47% and 90%, respectively, for DrugWipe® 5+ [12,17]. Due to the low prevalence of cocaine and THC in the present study, the 95% CI of the sensitivity were relatively wide for these two analytes (Fig. 2).

For ketamine, it is interesting to note first of all that there is considerable difference across the cut-offs claimed by the manufacturer (up to 10-fold difference between DrugWipe® 6S and Ora-Check®). Although DrugWipe® 6S has the lowest claimed cut-off (5 ng/mL), this was not reflected in the actual results, in that the device only had 41% sensitivity (compared with 76% for SalivaScreen®) and indeed failed to detect ketamine at extremely high concentrations in oral fluid (Table 4).

All three devices had satisfactory specificities (≥94%) for ketamine. On the other hand, the sensitivity had a wide variation – SalivaScreen® correctly identified 13 out of 17 ketamine-positive samples and achieved the highest sensitivity (76%) close to the DRUID satisfactory score of 80%, while the performance of the other two devices was far below that (Ora-Check® 36%, DrugWipe® 6S 41%). There was also considerable difference in the PPV between DrugWipe® 6S and SalivaScreen® (21% versus 72%) due to the high number of false-positive results (n = 27) in comparison to true-positives (n = 7) for the former. These results indicate that whilst DrugWipe® 6S is a reliable device in correctly detecting the negative cases, it lacks sensitivity in identifying the positive cases; and where the DrugWipe result is positive, the majority (79%) will be false signals. This has important implications for real-life use on the field, where a positive result may have serious legal consequences, such as in drugged driving. Overall, SalivaScreen® has the best performance in ketamine detection according to the DRUID criteria (sensitivity 76%, specificity 99% and accuracy 98%).

A previous study evaluated the performance of another device, OratectXP – this device was used for detecting ketamine only and not the other analytes [13]. In this study, the manufacturer's device cut-off (15 ng/mL) was employed in the interpretation of results, with the calculated sensitivity, specificity and accuracy being 88%, 98% and 94% respectively. SalivaScreen® in the present study achieved similar results. When the LCMS cut-off (5 ng/mL) was used, the sensitivity was 76%; however, when the manufacturer's device cut-off of 25 ng/mL was used, the sensitivity was higher at 87% (data not shown).

In the present study, variation across the devices was observed in their sensitivities of the ketamine, cocaine and THC tests. In an attempt to explain in part this variability, the concentrations detected in the false-negative samples were studied in order to investigate whether such cases were due to drug concentrations being close to the device cut-off. As shown in Table 4, the concentrations detected in the SalivaScreen® false-negative samples for ketamine (31–36 ng/mL) were indeed close to the device cut-off (25 ng/mL). On the other hand, KET concentrations as high as 2122 and 1023 ng/mL were observed in the DrugWipe® 6S and Ora-Check® false-negative cases respectively; these concentrations were remarkably higher than the device cut-offs. For cocaine, the maximum concentration observed in the DrugWipe® 6S false-negative cases (188 ng/mL) was again considerably higher than the device cut-off (10 ng/mL). In the case of THC, concentrations as high as 1958 ng/mL were missed by both Ora-Check® and SalivaScreen®; the DrugWipe® 6S false-negative cases had comparatively lower concentrations (max 208 ng/mL), despite still being much higher than the device cut-off (20 ng/mL). To conclude, these results indicate that DrugWipe® 6S may be unable to identify ketamine and cocaine even at extremely high concentrations in oral fluid. Conversely, the same is also true for Ora-Check® and SalivaScreen® in detecting THC.

5. Conclusion

Overall, the specificity and accuracy of the devices were satisfactory and met the DRUID recommendation of >80%, but the sensitivity varied. All devices performed poorly for THC. Ora-Check® had the poorest sensitivity among the 3 devices and did not achieve 80% in any of the tests. DrugWipe® 6S achieved >80% sensitivity in the methamphetamine and opiates tests but performed relatively poorly for ketamine and cocaine. Among the three devices, SalivaScreen® achieved >80% sensitivity in the methamphetamine and opiates tests, and was found to have the highest sensitivity for ketamine, cocaine and opiates.

Conflicts of interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.forsciint.2018.03.004.

References

[1] M.A. Huestis, A. Verstraete, T.C. Kwong, J. Morland, M.J. Vincent, R. de la Torre, Oral fluid testing: promises and pitfalls, Clin. Chem. 57 (2011) 805–810.
[2] W.M. Bosker, M.A. Huestis, Oral fluid testing for drugs of abuse, Clin. Chem. 55 (2009) 1910–1931.
[3] A.G. Verstraete, S. Legrand, Drug Use, Impaired Driving and Traffic Accidents, 2nd ed., European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), Lisbon, 2014.

[4] O.F. Wong, K.L. Tsui, T.S. Lam, N.N. Sze, S.C. Wong, F.L. Lau, S.H. Liu, Prevalence of drugged drivers among non-fatal driver casualties presenting to a trauma centre in Hong Kong, Hong Kong Med. J. 16 (2010) 246–251.
[5] A.S. Christophersen, J. Morland, K. Stewart, H. Gjerde, International trends in alcohol and drug use among vehicle drivers, Forensic Sci. Rev. 28 (2016) 37–66.
[6] Roadside Testing Assessment (ROSITA) Final Report. http://www.transport-research.info/project/roadsite-testing-assessment. (Accessed 26 April 2017).
[7] Rosita-2 Final Report. http://rosita.org/. (Accessed 27 April 2017).
[8] Analytical evaluation of oral fluid screening devices and preceding selection procedures. http://www.druid-project.eu/Druid/EN/deliverales-list/downloads/Deliverable_3_2_2.html?nn=613800. (Accessed 27 April 2017).
[9] S. Gentili, R. Solimini, R. Tittarelli, G. Mannocchi, F.P. Busardo, A study on the reliability of an on-site oral fluid drug test in a recreational context, J. Anal. Methods Chem. 2016 (2016) 1234581.
[10] A.S. Goessaert, K. Pil, J. Veramme, A. Verstraete, Analytical evaluation of a rapid on-site oral fluid drug test, Anal. Bioanal. Chem. 396 (2010) 2461–2468.
[11] A. Pehrsson, T. Blencowe, K. Vimpari, A. Impinen, T. Gunnar, P. Lillsunde, Performance evaluation of the DrugWipe(R) 5/5+ on-site oral fluid screening device, Int. J. Legal Med. 125 (2011) 675–683.
[12] S. Strano-Rossi, E. Castrignano, L. Anzillotti, G. Serpelloni, R. Mollica, F. Tagliaro, J.P. Pascali, D. di Stefano, R. Sgalla, M. Chiarotti, Evaluation of four oral fluid devices (DDS(R), Drugtest 5000(R) Drugwipe 5+(R) and RapidSTAT(R)) for on-site monitoring drugged driving in comparison with UHPLC-MS/MS analysis, Forensic Sci. Int. 221 (2012) 70–76.
[13] T.K. Tsui, A.S. Chan, C.W. Lo, A. Wong, R.C. Wong, C.S. Ho, Performance of a point-of-care device for oral fluid ketamine evaluated by a liquid chromatography–tandem mass spectrometry method, J. Anal. Toxicol. 36 (2012) 210–216.
[14] S.M. Wille, N. Samyn, M. Ramirez-Fernandez Mdel, G. De Boeck, Evaluation of on-site oral fluid screening using Drugwipe-5(+), RapidSTAT and Drug Test 5000 for the detection of drugs of abuse in drivers, Forensic Sci. Int. 198 (2010) 2–6.
[15] S.S. Rao, D.M. Wood, P.I. Dargan, Ketamine – epidemiology of misuse and patterns of acute and chronic toxicity, in: D.T. Yew (Ed.), Ketamine: Use and Abuse, CRC Press, Boca Raton, FL, 2015, pp. 104–108.
[16] A.L. van Nuijs, A. Gheorghe, P.G. Jorens, K. Maudens, H. Neels, A. Covaci, Optimization, validation, and the application of liquid chromatography–tandem mass spectrometry for the analysis of new drugs of abuse in wastewater, Drug Test. Anal. 6 (2014) 861–867.
[17] T. Blencowe, A. Pehrsson, P. Lillsunde, K. Vimpari, S. Houwing, B. Smink, R. Mathijssen, T. Van der Linden, S.A. Legrand, K. Pil, A. Verstraete, An analytical evaluation of eight on-site oral fluid drug screening devices using laboratory confirmation results from oral fluid, Forensic Sci. Int. 208 (2011) 173–179.
[18] Clinical and Laboratory Standards Institute (CLSI), Liquid chromatography–mass spectrometry methods; approved guideline. C62-A, 2014.
[19] M. Concheiro, T.R. Gray, D.M. Shakleya, M.A. Huestis, High-throughput simultaneous analysis of buprenorphine, methadone, cocaine, opiates, nicotine, and metabolites in oral fluid by liquid chromatography tandem mass spectrometry, Anal. Bioanal. Chem. 398 (2010) 915–924.
[20] European Medicines Agency Committee for Medicinal Products for Human Use (CHMP), Guideline on bioanalytical method validation, 2012.
[21] F.T. Peters, O.H. Drummer, F. Musshoff, Validation of new methods, Forensic Sci. Int. 165 (2007) 216–224.
[22] S. Strano-Rossi, L. Anzillotti, E. Castrignano, M. Felli, G. Serpelloni, R. Mollica, M. Chiarotti, UHPLC-ESI-MS/MS method for direct analysis of drugs of abuse in oral fluid for DUID assessment, Anal. Bioanal. Chem. 401 (2011) 609–624.
[23] A. Agresti, B.A. Coull, Approximate is better than "exact" for interval estimation of binomial proportions, Am. Stat. 52 (1998) 119–126.
[24] H. Choi, S. Baeck, M. Jang, S. Lee, H. Chung, Simultaneous analysis of psychotropic phenylalkylamines in oral fluid by GC–MS with automated SPE and its application to legal cases, Forensic Sci. Int. 215 (2012) 81–87.
[25] K. Langel, C. Engblom, A. Pehrsson, T. Gunnar, K. Ariniemi, P. Lillsunde, Drug testing in oral fluid-evaluation of sample collection devices, J. Anal. Toxicol. 32 (2008) 393–401.
[26] O. Quintela, D.J. Crouch, D.M. Andrenyak, Recovery of drugs of abuse from the Immunalysis Quantisal oral fluid collection device, J. Anal. Toxicol. 30 (2006) 614–616.
[27] S. Vanstechelman, C. Isalberti, T. Van der Linden, K. Pil, S.A. Legrand, A.G. Verstraete, Analytical evaluation of four on-site oral fluid drug testing devices, J. Anal. Toxicol. 36 (2012) 136––140.
[28] Central Registry of Drug Abuse, http://www.nd.gov.hk/en/statistics_list.htm. (Accessed 2 May 2017).
[29] M. Tang, C.K. Ching, M.L. Tse, C. Ng, C. Lee, Y.K. Chong, W. Wong, T.W. Mak, Surveillance of emerging drugs of abuse in Hong Kong: validation of an analytical tool, Hong Kong Med. J. 21 (2015) 114–123.
[30] W.C. Cheng, K.M. Ng, K.K. Chan, V.K. Mok, B.K. Cheung, Roadside detection of impairment under the influence of ketamine—evaluation of ketamine impairment symptoms with reference to its concentration in oral fluid and urine, Forensic Sci. Int. 170 (2007) 51–58.
[31] Hong Kong Special Administrative Region Road Traffic Ordinance Chapter 374. https://www.elegislation.gov.hk/. (Accessed 23 March 2017).