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ORIGINAL ARTICLE

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On-site testing of saliva and sweat with Drugwipe and determination of concentrations of drugs of abuse in saliva, plasma and urine of suspected users

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Abstract Potential drug users participated voluntarily in a Belgian study on the usefulness of the non-instrumental immunoassay Drugwipe (Securetec, Germany) for the screening of cocaine, opiates, amphetamine and cannabinoids in saliva and sweat. If one of the screening assays (urine, oral fluid, sweat) showed a positive result, blood and saliva were collected. The on-site Drugwipe results were correlated with the Drugwipe results for saliva in the laboratory and with the GC/MS results of the corresponding saliva, plasma and urine samples and pharmacological effects at the time of sampling. The Drugwipe assay proved to be sufficiently sensitive for the detection of recent cocaine (n = 6) and amphetamine (n = 15) abuse, whether the device was wiped on the tongue or on the surface of the body, or when a saliva sample was applied to the wiping part. In five of the six potential cocaine users, the saliva concentrations of cocaine exceeded 1000 ng/ml. In the amphetamine group, the saliva concentrations of amphetamine, MDMA or both were high (> 1000 ng/ml) in 13 subjects. For cocaine and amphetamine, the positive scores for Drugwipe matched the GC/MS results for the three body fluids. Recent heroin abuse (n = 5) could be demonstrated to some extent with Drugwipe on samples from the tongue but only the two subjects with the highest saliva concentrations of MAM (> 500 ng/ml) and morphine (> 500 ng/ml) were positive. If the legal cut-off value for driving under the influence of opiates in Belgium (20 ng/ml of free morphine in plasma) was taken into account, only three subjects would have been legally positive. For cannabinoids (n = 15), false negatives and even some false positives were observed. Saliva can be considered as a useful analytical matrix for the detection of drugs of abuse after recent abuse when analysed with GC/MS.

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Introduction

During the last decade, saliva has been increasingly used as an analytical tool in pharmacokinetic studies [1–2], therapeutic drug monitoring [3–7] and the detection of drugs of abuse [8–13]. Particular interest has been expressed by law enforcement agencies for roadside testing of potentially intoxicated drivers [14–16]. The presence of certain drugs of abuse or their metabolites in urine can be interpreted as evidence of relatively recent exposure, except for cannabis. However, this does not necessarily mean that the subject was under the influence at the time of sampling. When the drug is detected in blood, there is a higher probability that the subject is experiencing pharmacological effects than when the drug is detected in urine. Saliva is probably the only body fluid where drug levels would show a parallel to those in blood and therefore relate to behavioural performance [17–20]. Kidwell et al. [21] also consider sweat testing as a possible part of a roadside sobriety program to reduce driving under the influence of drugs (DUID). Although it has long been known that drugs are excreted in sweat [22], this has not been extensively used as a drug detection medium. Recently, due to the development of the sweat patch technology, sweat analysis has been proposed as a means for evaluating drug exposure e.g. in detoxification centers, rather than for roadside testing purposes [23, 23, 25].

When testing for drugs in subjects suspected of impaired driving, some practical aspects should be considered. As for alcohol, police officers must regularly carry out roadside tests that require immediate results. Saliva and sweat sampling is easy, non-invasive, without the intrusion of privacy and with very little chance of adulteration. Recently, Securetec (Ottobrunn, Germany) introduced Drugwipe, a non-instrumental immunodiagnostic assay for the detection of drugs on surfaces. The use of Drugwipe for saliva and sweat is currently being investigated in several countries [26, 27, 28].

We evaluated the suitability of the Drugwipe test for saliva and sweat on a limited number of subjects who admitted recent drug abuse. The on-site results of Drugwipe for cocaine, opiates, amphetamine and cannabinoids were correlated with the Drugwipe results applied to a saliva sample in the laboratory, with the GC/MS results on saliva, plasma and urine and with the pharmacological effects at the time of sampling.

Materials and methods

Drugwipe is a pen size, immunochemical-based test strip, used for the detection of drugs of abuse on surfaces. Separate tests are available for opiates, cocaine, cannabinoids and the amphetamine group. The wiping part enables the user to sample drug particles from any kind of surface. Possible applications could be the surface of the body and the tongue. The main part of the detection element is an immunochromatographic test strip, which is based on the Frontline urine test strip from Boehringer Mannheim [29]. The immunochromatographic reaction is initiated by dipping the absorbant pad into tap water for 10 s. The colouration of the detection field will change from light pink to red depending on the amount of drug collected. If no drugs are present, the read-out window remains cream-coloured. According to the manufacturer, the surface sensitivities for cocaine-HCl, heroin-HCl, Δ-9-tetrahydrocannabinol (THC), amphetamine sulphate, 3,4-methylenedioxy-*N*-methyl-amphetamine-HCl (MDMA.HCl) and methamphetamine-HCl are 10, 25, 50, 50, 10 and 10 ng, respectively, when measured with the appropriate Drugwipe device.

Protocol of the study

The following protocol and the informed consent form were reviewed and approved by the appropriate national ethical review committee. Two actions were organized in cooperation with the State Police: one at the main railway station in Antwerp (Belgium), the second at the roadside near a discotheque on a Sunday morning. Subjects were selected based on external signs of intoxication or impaired behaviour, a questionnaire was filled out if they agreed to participate in the study on a voluntary basis and informed consent was obtained.

A member of the medical staff performed a limited physical examination. Blood pressure, heart rate, and temperature were measured and some visual tests were performed (e.g. evaluation of the pupil size, reaction to light, nystagmus, etc.). A urine sample was taken and immediately screened with Frontline for one or two drug classes depending on the information in the questionnaire. One Drugwipe device was wiped on the tongue, another one on the neck or the back for the same drug classes. A saliva sample was provided by spitting into a plastic tube to obtain 1–2 ml to reapply the Drugwipe test in the laboratory and for quantitative analysis by GC/MS. For amphetamine users, some water had to be provided to stimulate the salivary flow. A swab was wiped on the neck to obtain a sweat sample for a possible later confirmation by the sweat test. Finally, if one of the three screening tests showed a positive result, a blood sample was drawn into a Venoject glass tube with NaF/KOx as anticoagulant. Centrifugation was performed on-site and the corresponding plasma samples were frozen.

Analytical procedures

The solid phase extraction (SPE) procedures for opiates and for cocaine and its metabolites were based on the previously published methods of Wang et al. [30] and Cone et al. [31]. Benzoylecgonine was derivatized with pentafluoropropionic anhydride (PFPA) and pentafluoropropanol (Sigma). Since both cannabinoids and amphetamine-like substances are retained on the same C8-cation exchange columns (Bond Elut Certify, Varian, Belgium) at pH 6.0, they were extracted from the same saliva sample but eluted separately. Cannabinoids were eluted with hexane/ethyl acetate (8 : 2, v/v). Amphetamine and its derivatives were extracted from biological samples according to the manufacturers instructions and derivatized with heptafluorobutyric anhydride (HFBA). Cannabinoids were extracted from urine and plasma using 5 ml of hexane/ethyl acetate (9 : 1,v/v) after acidification of the sample. Derivatization was performed with *N,O*-bis(trimethylsilyl)trifluoroacetamide + trimethylchlorosilane (BSTFA + 1% TMCS) and silanized glassware was used for the extraction and derivatization procedures.

Quantitative analyses were performed on a Hewlett-Packard 6890 gas chromatograph equipped with an autosampler (HP7673 A) and interfaced with a Hewlett-Packard 5973 mass selective detector. Analytical conditions were optimized for detection of the following:

1. Cocaine, benzoylecgonine (BE), anhydroecgonine methylester (AEME), ecgonine methyl ester (EME), morphine (MORP), 6-acetylmorphine (MAM) and codeine
2. Amphetamine (AMP), 3,4-methylenedioxy-*N*-amphetamine (MDA), MDMA, 3,4-methylenedioxy-*N*-ethylamphetamine (MDEA), *N*-methyl-1-(3,4-methylenedioxy-phenyl)-2-butanamine (MBDB) and ephedrine
3. THC, cannabinol, cannabidiol, 11-hydroxy-ΔD-9-tetrahydrocannabinol (OH-THC) and 11-nor-ΔD-9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH)

The MS was operated in SIM mode. At least three ions were monitored for the analytes and two ions for the internal standards.

Results and discussion

Table 1 summarizes the information gathered in the questionnaires. Of the 27 subjects tested, 5 admitted recent use of both cocaine and heroin and 15 abuse of amphetamine

Table 1 Information gathered in the questionnaires on the use of cocaine (*COC*), opiates (*OPI*), the amphetamine group (*AMPH*) and cannabis (*CANN*)

	COC	OPI	AMPH		CANN
Number of subjects	6	5	15		15
Presumed abuse			Speed	XTC	
Smoking	3	3	0	0	15
Intranasally	3	2	7	0	0
Orally	0	0	1	9	0
Time elapsed after last drug abuse	2–4 h	2–4 h	0.5–12 h		0.5–12 h

Table 2 The Drugwipe (*DW*) results for saliva on-site and the concentrations (ng/ml) of cocaine (*COC*) and benzoylecgonine (*BE*) in saliva, plasma and urine of cocaine users, determined with GC/MS

Subject ID	DW	GC/MS saliva		GC/MS plasma		GC/MS urine	
		COC	BE	COC	BE	COC	BE
COC 1	+	1964	409	54	366	2430	12720
COC 2	+	22	0	0	0	Urine not available	
COC 3	+	3505	2395	162	2257	> 20000	> 20000
COC 4	+	1450	1415	90	1061	2016	> 20000
COC 5	+	2560	830	130	1409	8250	> 20000
COC 6	+	1136	486	72	661	Urine not available	

Table 3 The Drugwipe (*DW*) results for saliva on-site and the concentrations (ng/ml) of free morphine (*MOR*), 6-acetylmorphine (*MAM*) and codeine (*COD*) in saliva, plasma and urine of heroin users, determined with GC/MS

Subject ID	DW	GC/MS saliva			GC/MS plasma			GC/MS urine		
		MOR	MAM	COD	MOR	MAM	COD	MOR	MAM	COD
OPI 1	+	8096	3080	481	54	20	11	6820	3100	930
OPI 2	+	987	735	166	37	6	9	Urine not available		
OPI 3	–	94	285	26	12	3	–	1648	1150	160
OPI 4	–	925	350	60	6	–	3	1269	131	375
OPI 5	–	283	157	113	70	9	15	Urine not available		

Table 4 The Drugwipe (DW) results for saliva on-site and the concentrations (ng/ml) of amphetamine (AMP) and MDMA in saliva, plasma and urine of amphetamine users, determined with GC/MS

Subject ID	DW	GC/MS saliva		GC/MS plasma		GC/MS urine	
		AMP	MDMA	AMP	MDMA	AMP	MDMA
AMP 1	+	2724	–	232	–	> 20000	–
AMP 2	+	–	218	–	218	Urine not available	
AMP 3	+	1279	6280	161	1063	Urine not available	
AMP 4	+	12355	–	700	–	> 20000	–
AMP 5	+	2766	–	416	–	> 20000	–
AMP 6	+	363	3838	28	314	3600	> 20000
AMP 7	+	1657	4229	82	407	> 20000	> 20000
AMP 8	+	12585	–	937	–	> 20000	–
AMP 9	+	1792	–	232	–	3183	–
AMP 10	+	2582	446	299	53	> 20000	19410
AMP 11	+	3660	–	Blood not available		Urine not available	
AMP 12	+	–	361	–	67	–	1836
AMP 13	+	–	1816	–	270	–	> 20000
AMP 14	+	15	1200	–	247	–	7400
AMP 15	+	597	5495	44	334	3532	19700

Table 5 The Drugwipe (DW) results for saliva on-site and the concentrations (ng/ml) of THC and its metabolites OH-THC and THC-COOH in saliva, plasma and urine of cannabis users, determined with GC/MS

Subject ID	DW	GC/MS saliva	GC/MS plasma			GC/MS urine
		THC	THC	OH-THC	THC-COOH	THC-COOH
CAN 1	–	5.6	–	0.4	26.5	184
CAN 2	–	10.0	7.5	2.6	22.2	64
CAN 3	–	10.6	3.4	1.1	13.0	268
CAN 4	–	36.6	12.6	5.4	61.0	339
CAN 5	–	9.1	5.8	2.0	41.0	625
CAN 6	–	4.5	8.2	2.3	44.5	Urine not available
CAN 7	–	1.4	7.0	4.0	41.5	233
CAN 8	–	1.5	–	–	10.8	Urine not available
CAN 9	+	42.1	–	0.8	10.6	Urine not available
CAN 10	–	10.6	–	0.8	16.7	215
CAN 11	–	–	–	–	5.6	–
CAN 12	–	–	Blood not available			Urine not available
CAN 13	+	–	–	–	8.3	41
CAN 14	+	–	–	–	8.7	–
CAN 15	+	–	–	–	1.9	15

or its derivatives. Out of the 15 marihuana users, 6 were tested for cannabis alone and 9 were also tested for amphetamine.

A comparison of the on-site Drugwipe results for saliva and the GC/MS results for the three body fluids is given for every class of drugs in Tables 2, 3, 4, 5.

The results for the cocaine group are shown in Table 2. All subjects tested positive with Drugwipe on-site and cocaine was detected in all saliva samples with GC/MS. Only the sample with the lowest cocaine concentration gave a negative Drugwipe result in the laboratory test. The corresponding urine sample was not available but the blood cocaine was negative. AEME was detected in the saliva of subjects who smoked cocaine. This is in agreement with previously published results [17, 32]. The saliva/plasma (S/P) ratio varied between 15 and 36 for cocaine and 0.6–1.3 for BE. The higher saliva concentrations of cocaine were probably due to a contamination of the buccal

Table 6 Comparison of the on-site Drugwipe results for saliva and the results of the medical evaluation of the subjects at the time of sampling

[a] Only the subjects that were tested for cannabis alone were considered

	Cocaine		Opiates		Amphetamine		Cannabis[a]	
	Effect +	Effect –	Effect +	Effect –	Effect +	Effect –	Effect +	Effect –
Drugwipe +	5	1	1	1	15	0	0	0
Drugwipe –	0	0	2	1	0	0	5	1

cavity during the first hours after smoking or sniffing cocaine [17, 33].

Table 3 shows that morphine and MAM were easily detected in the saliva of all five heroin users by GC/MS. Previous studies also showed that substantial saliva concentrations of opiates were obtained after recent smoking or sniffing of heroin [8, 33]. Only the two subjects with the highest concentrations of analytes in saliva tested clearly positive with Drugwipe, both on-site and in the laboratory. They also showed plasma concentrations of free morphine above the legal analytical cut-off value for DUID in Belgium (20 ng/ml). The S/P ratios for MAM and morphine varied widely but were always larger than 1, sometimes even larger than 100. The sweat test failed to detect recent heroin abuse which contradicts the results of the German Drugwipe study [34] and the codeine study by Kintz et al. [35] where the device was applied to the armpit and the forehead, respectively. It is well known that sweat concentrations of several drugs differ according to the collection site [36].

The results for the amphetamine group (Table 4) are comparable to those for cocaine. The one negative Drugwipe result in the laboratory corresponded to a saliva sample with a low MDMA concentration. In MDMA positive samples, the metabolite MDA was also detected but in lower concentrations. MDEA was found in the saliva of four subjects (> 100 ng/ml) in addition to MDMA and one sample contained MBDB in addition to amphetamine. This was in agreement with the results of the corresponding urine and plasma samples and the S/P ratios for amphetamine and for MDMA exceeded 5. Kintz [37] also found high concentrations of MBDB in the saliva of one subject more than 12 h after a single oral administration of 100 mg of MBDB.

The four positive Drugwipe results for cannabis on-site were not confirmed by the laboratory test or by the GC/MS result for saliva (Table 5). Only 10 subjects provided a saliva sample in which THC was detected with GC/MS and the concentrations measured were lower than those reported in the literature [38, 39]. We observed that the stability of THC in saliva samples stored at –18 °C in a plastic tube and not centrifuged before storage, is poor (unpublished results). In addition to THC, cannabinol and cannabidiol were detected in saliva but OH-THC and THC-COOH were never identified. Also the Drugwipe test for sweat failed to provide valid results for cannabinoids.

In Table 6 an attempt was made to correlate the presence of a pharmacological effect at the time of sampling with the results obtained for saliva and plasma. For subjects who abused both cocaine and heroin, it was difficult to observe a physiological effect because of some antagonistic actions of the drugs. The evaluation of the effects due to cannabis alone was difficult because the effect of marihuana on blood pressure and heart rate is similar to the effect of amphetamine and a number of subjects had abused both.

Conclusions

In conclusion, the Drugwipe device is simple to use and results can be obtained after 2 min. As it is sometimes difficult to classify a result as negative or weakly positive, the manufacturer is currently developing a hand photometer to read the colouration. There were no distinct differences between the Drugwipe results on-site and in the laboratory, except for cannabinoids. Obviously, the interpretation of the result is more difficult on-site. To increase the reliability of the screening result, a good training of the police officers who are going to use the on-site test is of the greatest importance.

The use of the Drugwipe assay on saliva and sweat offers promising results for the detection of recent abuse of cocaine and the amphetamine group. To demonstrate the abuse of heroin, however, the test lacks sensitivity. Nonetheless, if the results for saliva and the proposed legal analytical cut-off value for DUID are considered, a drastic improvement in the sensitivity could produce too many legally false positives. The Drugwipe for cannabinoids failed to produce any valuable results for saliva and sweat. The antibody in the cannabis test is much more sensitive to THC-COOH than to THC (by a factor of 20). Since THC is the major analyte in saliva and sweat and its concentration is in the low nanogram range [25, 38], the development of a new antibody with a higher sensitivity for THC is probably the only option to improve the Drugwipe test for cannabis.

For cocaine and the amphetamine group, a good correlation was observed between the number of positive results for saliva and plasma and the presence of a pharmacological effect. In the future, a more complete field sobriety test should provide more information.

Although the number of subjects participating in this study was limited, the results indicate that saliva is a useful matrix for the detection of recent drug abuse. In view of DUID, the results need to be confirmed in a larger study where important parameters such as sampling, storage and on-site screening require further evaluation.

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