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Codeine testing in sweat and saliva with the Drugwipe

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Abstract With the growing interest in drug testing within different sectors of society, there has become a need for drug assays that can be performed immediately at the site of specimen collection. Recently, Securetec (Ottobrunn, Germany) has introduced the Drugwipe, a non instrument-based, on-site immunodiagnostic assay for the detection of drugs on surfaces. Different tests are available for opiates, cocaine and cannabis. To document the applications of the Drugwipe "opiate" on human biological fluids, 60 mg codeine phosphate were orally administered to 6 subjects. First, sweat testing with the Drugwipe was studied. The wiping section of the kit was used to swab the forehead of the subjects for 10 s, at 1, 4, 9 and 24 h after codeine administration. At the same time, for each period, a sweat patch (Pharmchek, USA) was applied to the outer portion of the upper arm. Codeine was then quantified in the patch by GC/MS and the measured concentrations used as reference. In all subjects except one the Drugwipe tested positive for opiates, however with few false negative results. In the second part of the study, results of the Drugwipe were compared with those obtained by GC/MS for saliva. The tongue of the subjects was carefully wiped over a period 24 h, and at the same time a specimen of saliva collected. Although codeine could be detected using the Drugwipe, numerous false negative results were observed. Codeine tested positive by GC/MS but remained negative using the Drugwipe in several cases. This can be explained by a codeine concentration which was too low to show positive with the Drugwipe, interfering substances may be present in saliva or the sampling procedure is inadequate.

Key words Drugwipe · Codeine · Saliva · Sweat

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Introduction

It is generally accepted that chemical testing of biological fluids is the most objective means of diagnosis of drug use. The presence of a drug analyte in a biological specimen can be used as evidence of recent exposure. The standard in drug testing is the immunoassay screening, followed by the gas chromatographic-mass spectrometric (GC/MS) confirmation conducted on a urine sample. More recently, a variety of body fluids other than urine, such as saliva [1, 2] and sweat [3, 4] have been proposed to document recent drug exposure.

The advantages of saliva or sweat over traditional fluids are that collection is almost non-invasive, relatively easy to perform and in forensic situations can be achieved under close supervision of law enforcement officiers to prevent adulteration or substitution of the samples. In addition, it has been claimed by some authors that the concentrations of many drugs in saliva correlate well with blood concentrations, which suggests that quantitative measurements in saliva may be a valuable technique to determine the current degree of exposure to a definite drug at the time of sampling.

Among various possible applications in the field of clinical or forensic toxicology, particular attention has been recently paid to the use of saliva [5] or sweat [6] for roadside testing of subjects suspected of impaired driving, as a consequence of the increasing interest expressed by the authorities of several countries towards the problem of driving under the influence (DUI) of psychoactive drugs.

In cooperation with the French Ministry of Transportation, our laboratory has developed a research program on the applicability of saliva or sweat testing for DUI control. Considering that a conditio sine qua non for such drug testing is that they can be organized in the same way as alcohol testing (i.e. systematic controls directly carried out on the roadside by police officers with minimal scientific background, and obtention of immediate results allowing, if positive, the immobilization of the vehicle and the suspension of the driver's license until eventual con-

firmation tests), it has been especially emphasized that they should fulfill the following requirements: 1) acceptability by the population, which compels the sampling procedure to be simple, fast and non-invasive and 2) simplicity and rapidity of sample handling and analysis, which practically restricts the choice of techniques to non-instrumental immunoassay.

Recently [7], the Drugwipe (Securetec, Ottobrunn, Germany), a non-instrumental immunoassay designed for use on site to obtain indications of the presence of illegal drugs (opiates, cocaine or cannabis) on surfaces was used to document fatal drug overdoses when used as a skin swab. By wiping the skin of the arm-pit it was demonstrated that the opiate Drugwipe tests matched the results of blood and urine examinations in more than 80% of the cases.

This report describes the use of the Drugwipe opiates for saliva and sweat testing after single oral administration of 60 mg of codeine to 6 subjects.

Material and methods

The Drugwipe test kit is designed for use on site in order to obtain indications of the presence of illegal drugs on surfaces. Basically all smooth surfaces are suitable for testing, but moderately rough surfaces (suede, textiles, surface of the body, etc) should be wiped carefully. The Drugwipe elements are presented in a closed protective foil, stored at ambiant temperature. Different tests are available for opiates, cocaine and cannabis. According to the manufacturer, a positive test result for the Drugwipe "opiate" is obtained with 5 ng heroin, morphine or codeine.

The test function is based on an immunological detection method. The drug particles captured by the wiping section are transferred to the chromatographic strip. During immersion in tap water the test absorbs an adequate volume for chromatography. The water passes through the zone with specific binding to various opiates. Excess conjugate is held back by a retention zone with immobilized morphine deviate, so that only the conjugate with the wiped off drug particles reaches the detection field. Depending on the quantity wiped off, a coloration ranging from cream white to red will result (Drugwipe test. Drug detection kit. Securetec instructions. Ottobrunn, Germany).

Subjects

Six subjects, aged 25–36 years old were recruited from laboratory personnel. All denied use of codeine during the 3 previous months. The week before enrollment, two urine tests were performed and the results were negative. Four males and 2 females participated in the protocol. They signed an informed consent agreement and were not paid for participation.

The subjects received 60 mg codeine phosphate orally in 100 ml water.

Sweat collection and analysis

The wiping section of the Drugwipe was used to swab the forehead of the subjects 10 s, after 1, 4, 9 and 24 h after codeine administration. The results were obtained as described previously for surfaces. At the same time, for each period, a Pharm-Chek sweat patch (PharmChem Laboratories, Menlo Park, California) was applied to the outer portion of the upper arm. Codeine was then quantified in the patch by GC/MS according to our procedure [8] and the measured concentrations used as reference.

Saliva collection and analysis

Over a period of 24 h, the tongue of the subjects was carefully wiped for 10 s, and at the same time a specimen (about 1 ml) of saliva was collected without stimulation in a dry tube. The results of the Drugwipe tests were obtained as for surfaces. The saliva samples were centrifuged and the supernatant stored at –20° C until GC/MS analysis.

Briefly, to 1 ml of saliva were added 100 ng of codeine-d_3 (Promochem, Molsheim, France) as internal standard, 1 ml of 1 M phosphate buffer (pH 8.4) and 10 ml chloroform-isopropanol-n-heptane (50 : 17 : 33, v/v/v). After agitation and centrifugation, the organic phase was evaporated to dryness and the residue was silylated with BSTFA +1% TMCS and analyzed by GC/MS [8]. The assay was linear for concentrations in the range 10–600 ng/ml, with an extraction recovery (at 100 ng/ml) of 81.2%. The limit of detection and limit of quantification were 1 and 5 ng/ml, respectively. The within-run coefficient of variation (at 100 ng/ml, $n = 8$) was 10.8%.

Results

According to the manufacturer, it is not necessary for the read-out field of the Drugwipe to show an even coloration. The coloration of part sections of the read-out field (on the edge, for example) according to reference coloration also qualifies as a positive result. Coloration remains stable at least for 1 h.

Results of the Drugwipe tests are obtained in less than 5 min. The analyses for sweat are presented in Table 1. As demonstrated by the results, codeine can be easily detected in forehead sweat using the Drugwipe. Concentrations in the patch were in the range of previously reported values [8]. It was not possible to detect codeine exposure with the Drugwipe in the case of subject 2. This was also observed with saliva. Results of saliva testing are presented in Table 2. Numerous false negative results were observed with the Drugwipe taking GC/MS as the reference method. To date, these findings are not explained, nor the specimen collection protocol definitively proposed. From the literature, codeine has been detected in saliva following 30 mg oral [9] and 60 and 120 mg intramuscular [10] administration. Peak concentrations in saliva occurred at 1 and 0.5–0.75 h after oral or intramuscular

Table 1 Sweat analysis using the Drugwipe and the sweat patch for the 6 subjects

Subjects	Time after codeine administration (hours)							
	1		4		9		24	
	Wipe	Patch	Wipe	Patch	Wipe	Patch	Wipe	Patch
1	–	12	+	34	+	17	+	29
2	–	8	–	15	–	59	–	17
3	+	27	+	124	+	18	+	100
4	+	20	+	24	+	18	+	31
5	–	3	+	15	+	17	+	12
6	+	24	+	37	+	56	+	28

For the Drugwipe: (+): positive result, (–): negative result
For the patch: all concentrations in ng/patch

Table 2 Saliva analysis using the Drugwipe and GC/MS for the 6 subjects

Time (hours)	Subject 1		Subject 2		Subject 3		Subject 4		Subject 5		Subject 6	
	Wipe	GC/MS	Wipe	GC/MS	Wipe	GC/MS	Wipe	GC/MS	Wipe	GC/MS	Wipe	GC/MS
0.5	+	113	–	26	–	310	+	82	+	99	+	259
1.0	–	67	–	46	–	402	+	207	+	137	+	288
1.5	+	165	–	166	+	353	+	301	+	89	+	489
2.0	+	208	–	390	+	215	+	351	+	71	+	359
3.0	+	546	–	342	+	212	+	366	+	56	+	206
4.0	+	186	–	158	+	72	+	158	–	40	+	138
6.0	+	108	–	108	–	43	–	69	–	21	–	40
9.0	–	16	–	10	–	17	–	24	–	12	–	24
12.0	–	25	–	< 5	–	7	–	13	–	< 5	–	11
24.0	–	< 5	–	< 5	–	< 5	–	< 5	–	< 5	–	< 5

For the Drugwipe: (+): positive result, (–): negative result
For GC/MS: all concentrations in ng/ml

administration, respectively. In our experiments, variation between individuals was obvious, with peak concentrations observed at 1–3 h. Morphine was never detected in saliva (LOQ: 5 ng/ml).

Discussion

The Drugwipe method may provide a rapid way to gather data about drug use without the embarrassment often felt when obtaining urine samples. Clearly, saliva and sweat drug tests can reveal the presence of a pharmacologically active drug in an individual at the time of testing. Reference methods, like the sweat patch method, or saliva testing with GC/MS can provide valuable information for diagnostics, treatment and forensic investigations of individuals suspected of drug abuse. Results with the Drugwipe are more controversial. With the exception of one female, all the subjects tested positive with the Drugwipe in sweat. No explanation was found to document the absence of positivity observed with subject 2 who was not under medical treatment and no drugs were detected after urine analysis. For the same subject, saliva testing with the Drugwipe remained negative, although codeine GC/MS concentrations were in the range 10–390 ng/ml. If results of the Drugwipe seem satisfactory with sweat, this is not the case for saliva. Too many false negative results were observed that can prevent the use of the test kit for this medium. Several factors can anormalous results. The codeine concentration was too low to show positive by the Drugwipe, some interfering substances may be present in saliva or the wiping procedure of the tongue during sampling is inadequate, or sampling time (10 s) was too short. This can happen due to an inability to obtain a large enough volume of saliva or when a too large sample of saliva is wiped and excess moisture damages the wiping section. However, a definitive decision about the use of the Drugwipe to test saliva cannot be taken, and the conclusion of this preliminary study must be softened. Perhaps, it would have been better to apply a small sample of the saliva that was used for GC/MS analysis onto the wiping section rather than wipe the tongue. Furthermore, it is difficult to understand if the negative Drugwipe tests were due to the inadequate sample collection or failure of the test. For example, if one assumes that 50 µl of saliva is collected during the wiping and the test sensitivity is 5 ng, then any codeine levels below 100 ng/ml in saliva should be negative.

Before using the Drugwipe in routine for saliva, standardization of the collection of the specimens must be established. At present, the Drugwipe will constitute an excellent non-instrumental immunoassay that will be rapid sensitive and applicable on noninvasive biological samples such as sweat or saliva.

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