On-Site Testing of 3,4-Methylenedioxymethamphetamine (Ecstasy) in Saliva with Drugwipe and Drugread: A Controlled Study in Recreational Users, Simona Pichini,1,2 Mónica Navarro,2 Magi Farré,2,3 Jordi Ortuño,2 Pere Nolasco Roset,2 Roberta Pacifici,1 Piergiorgio Zuccaro,1 Jordi Segura,2,4 and Rafael de la Torre.4* 1 Clinical Biochemistry Department, Istituto Superiore di Sanità, 00161 Rome, Italy; 2 Department of Pharmacology, Institut Municipal d’Investigació Mèdica (IMIM), E-08003 Barcelona, Spain; 3 Universitat Autònoma de Barcelona, E-08193 Barcelona, Spain; and 4 Universitat Pompeu Fabra, E-08003 Barcelona, Spain; * address correspondence to this author at: Drug Research Unit, Department of Pharmacology, Institut Municipal d’investigació Mèdica (IMIM), C/Doctor Aiguader 80, E-08003 Barcelona, Spain; fax 34-93-2213237, e-mail rtorre@imim.es

Saliva is an alternative biologic specimen for drugs-of-abuse testing with several advantages over conventional matrices such as blood and urine (1, 2), e.g., weak bases tend to concentrate in saliva because its pH is usually more acidic than the pH of plasma. The most important advantage of saliva is the ease of sample collection. Specimens can be obtained in a matter of minutes under direct observation and without embarrassment to the donor. Special devices have recently been introduced that allow analysis of saliva at the site of specimen collection for on-site screening tests. Commercially available on-site devices include two multistest electronic readers, the Cozart Rapidscan® (Abingdon) and the Avitar OralScreen® (Avitar Inc.), and one single-test visual device, the Drugwipe® (Securetec). Drugwipe is the only on-site test on saliva for which results of clinical studies have been published (3, 4). Briefly, Drugwipe is an immunochromatographic test strip, based on the Frontline urine test strip from Boehringer Mannheim (F. Hoffmann-La Roche) (5). A pink color in the test window indicates the presence of the analyte to which the test is specifically addressed, and different devices are needed for detection of each class of drugs of abuse. Although Drugwipe was designed to be read visually, evaluation of color intensity may be highly subjective, and easy readout of the resulting coloration may be hindered by poor light conditions. For this reason, a Drugread® hand photometer has recently been developed. Drugread measures, in a reflectometric mode through a photodiode, the absorbance of the monochromatic light produced by gold antibody conjugates in the read-out area of the Drugwipe. Drugread translates the color intensity in the read-out window into a numeric value (arbitrary units) in the range of 300–2500. To date, no definitive threshold has been established for differentiating samples containing an analyte under investigation from samples not containing the substance.

Recreational use of 3,4-methylenedioxymethamphetamine (MDMA; “ecstasy”), either alone or in combination with other drugs, such as alcohol and cannabis, has become increasingly popular among young people (6). Several cases of acute intoxication leading to death have been reported, and law enforcement agencies are increasingly interested in roadside on-site testing of potentially intoxicated drivers (4). We evaluated the suitability of saliva testing of MDMA with the Drugwipe “amphetamine” and Drugread in individuals administered a single oral dose of 100 mg of MDMA.

Eight healthy volunteers who were recreational MDMA users gave their written informed consent to participate in a randomized, double-blind, crossover balanced with placebo clinical trial. The volunteers participated as outpatients in two experimental sessions, separated by a 1-week washout period, held in a controlled indoor setting. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Institutional Review Board of our hospital and authorized by the Spanish Health Authorities (AEM 98/532).

Each participant was given a single dose of 100 mg of MDMA or placebo by the oral route, as white soft-gelatin capsules. Saliva drug testing with the Drugwipe analytic device was performed on site, in the clinical trials unit. The device was wiped for 10 s on the tongue of each participant at 0 time (predose) and at 1.5, 4, 6, 10, and 24 h after MDMA administration. At the same time points, participants provided a sample of saliva (1–2 mL) by spitting into a plastic tube; samples were stored immediately at −20 °C. Samples were analyzed for MDMA and its metabolites by gas chromatography–mass spectrometry (GC-MS) as described previously (7). Because a preliminary evaluation of results showed quite relevant discrepancies between the two analytic approaches, saliva samples were reanalyzed with the Drugwipe test in the laboratory by application of a volume of saliva on the test pad. At the time of testing in the laboratory, the first prototypes of the Drugread hand photometer were available. Thus, the Drugread was used contextually to read the detection field for the Drugwipe once the test was performed. Laboratory personnel who read tests with the Drugwipe were unaware of the GC-MS results.

MDMA consumption could already be detected in saliva, by wiping the Drugwipe over the tongue, at 1.5 h and as long as 10 h after drug administration (Fig. 1A). However, although the GC-MS data showed that volunteers had salivary MDMA concentrations in the range of thousands of micrograms per liter in the first hours after drug administration (mean values, 3375.6 and 1762.7 µg/L at 1.5 and 4 h, respectively) (7), direct application of the device on the tongue produced one negative result and four negative results at 1.5 and 4 h after drug use, respectively. The positivity of the test decreased noticeably at 6 and 10 h after MDMA administration.

The most reasonable explanation for the high number of negative results was the insufficient amount of saliva collected from the tongues of volunteers by the device. To verify this hypothesis, we repeated the test in the laboratory, applying a preselected volume of 2 µL of saliva to the device. This volume was the largest volume that could...
be completely absorbed by the Drugwipe test pad. Once run, the test was read visually and then with the Drugread hand photometer. Results obtained with Drugwipe and Drugread in this phase of the study were compared with the MDMA concentrations measured by GC-MS in saliva samples (7).

The Drugwipe performed better when a preselected volume of saliva sample was applied to the pad than when the pad was wiped directly on the tongue (Fig. 1B): all participants gave a positive result at 1.5 and 4 h after drug administration, and at 6 h after treatment only one of eight participants gave a negative result. This individual had a salivary MDMA concentration of 414.4 µg/L by GC-MS, which was the lowest among all of the volunteers at that time.

At 10 h after administration of MDMA, it was still possible to detect consumption in five of the eight volunteers. The three individuals who had a negative result always had salivary MDMA concentrations <400 µg/L by GC-MS, whereas the other five always had concentrations >450 µg/L. At 24 h, no positive results were reported, as was the case with direct application of the device on the tongue. At that time, the mean salivary MDMA concentration in the eight volunteers was 126.2 µg/L (range, 27.7–318.0 µg/L).

On the other hand, when the Drugwipe was applied to salivary samples from the placebo group, a faint color change in the read-out window of the device was seen in some cases. The same problem had occurred in a previous use of the Drugwipe with sweat samples of individuals treated with MDMA or placebo (8) and was attributed to the previously described presence of endogenous amines that may interfere with the Drugwipe test for amphetamines (9).

On the basis of this previous experience, samples were classified as negative, but interpretation of some results remained difficult. The Drugread hand photometer was useful for solving problems of visual interpretation when reading the test window of Drugwipe. In fact, Drugread measurements in arbitrary units (mean ± SD; n = 8) were significantly different (Student t-test, P <0.001) when results obtained in the placebo group at 1.5, 4, and 6 h (519.1 ± 116.7, 497.1 ± 118.1, and 476.5 ± 65.7 arbitrary units, respectively) were compared with those obtained in individuals given MDMA at 1.5 h (1100.0 ± 177.4 units), 4 h (1083.7 ± 217.4 units), and 6 h (992.9 ± 205.1 units) after drug administration. Furthermore, mean Drugread readings presented a time course profile similar to the mean time–concentration curve for MDMA in saliva measured by GC-MS (Fig. 1C). The apparent slower disappearance rate in the Drugread signal was probably attributable more to a saturation effect in Drugwipe test pad coloration (ceiling effect) than to the contribution of MDMA metabolites. In fact, MDMA was reported as the principal analyte that could be detected in saliva, whereas its principal metabolites were found only in minute amounts (7).

The Drugwipe was initially developed for detecting drugs of abuse on surfaces and was subsequently applied to sweat and only recently to saliva (3, 4). Nevertheless, the procedure for sample collection on the surface of the tongue does not appear to be adequate for the performance of on-site saliva testing of amphetamines and may need to be redefined. In addition to potential technical problems in the design of the device, the pharmacologic properties of amphetamines may hinder the test. MDMA impairs salivary flow, producing a sympathetic constriction of the salivary bed (7) and making saliva procurement difficult after a surface wiping procedure.
On the basis of observations made in the present study, we recommend that direct wiping be avoided, preferring application of an established volume of saliva that could allow delivery of a sufficient quantity to the test pad, which can be easily done “on site” by collecting saliva and applying it to the test pad. In the future, manufacturers of Drugwipe may want to standardize batch-to-batch devices to preselected concentrations for amphetamine-related drugs, taking into account the differences in doses and routes of administration currently in use as well as the time window to be covered by the analytic device in relation to peak effects of the drug. In fact, although a limited number of individuals participated in this study, the present results show that the Drugwipe in combination with the Drugread adequately detected MDMA in saliva in the first 6 h after administration. On the other hand, the analytic device gives a negative response in a range of salivary concentrations down to ~450 μg/L (0.9 ng of MDMA in 2 μL of saliva applied to the test pad). These concentrations can be found in individuals ~6–10 h after the administration of 100 mg of MDMA, corresponding to a mean range of 80–120 μg/L in plasma and 3–12 mg/L in urine (10, 11). Conversely, the 0–6 h time window is the period of maximal pharmacologic effects of MDMA. In this time interval, an individual is at highest risk of psychomotor impairment that may have consequences in some demanding tasks, such as driving. Six hours after MDMA ingestion, although the drug is still present in several biologic fluids, most subjective and physiologic effects (i.e., cardiovascular function) return to basal conditions (10). Hence, if the objective of on-site saliva testing is not only to detect the consumption of a given drug but also to determine whether an individual is under the effects of the drug, on-site saliva testing with the Drugwipe coupled with the Drugread might be suitable. Ultimately, appropriate confirmation with a reference chromatographic method for saliva samples should be performed.

This investigation was supported by the Department of Social Affair (Italy), FIS 97/1198 and 98/0181, CIRIT 99-SGR-242, and PNSD (Spain). We thank Esther Menoyo and Isabel Sanchez for assistance in the experimental sessions and laboratory tests, Dr. Marta Pulido for editing the manuscript, and Securetec (Ottobrunn, Germany) for technical support.

References


Addition of Quantitative 3-Hydroxy-Octadecanoic Acid to the Stable Isotope Gas Chromatography–Mass Spectrometry Method for Measuring 3-Hydroxy Fatty Acids, Patricia M. Jones,1* Susan Tjoa,2 Paul V. Fennessey,2 Stephen I. Goodman,2 and Michael J. Bennett1 (1 University of Texas Southwestern Medical Center, Department of Pathology, and Children’s Medical Center of Dallas, Dallas, TX 75235; 2 University of Colorado Health Sciences Center, Department of Pediatrics, Denver, CO 80262; * address correspondence to this author at: Children’s Medical Center, Department of Pathology, 1935 Motor St., Dallas, TX 75235; fax 214-456-6199, e-mail Patricia.Jones@email.swmed.edu or pjones@childmed.dallas.tx.us)

Mitochondrial fatty acid oxidation (FAO) is a catabolic pathway that supplies energy for the normal physiologic functioning of many tissues when glucose is unavailable, and it also supplies energy for some tissues even when glucose is available (1, 2). The FAO pathway is complex and not fully understood. Quantitative measurement of the concentrations of 3-hydroxy-fatty acids (3-OHFA) in plasma or serum samples from individuals who are suspected of having a deficiency in FAO, especially in the enzyme step involving the l-3-hydroxyacyl-CoA-dehydrogenases, is a useful tool to aid in diagnosis (3, 4). This study adds the quantitative measurement of 3-hydroxy-octadecanoic acid (3-OH-C18) to the previously reported assay (4) that measures the six shorter chain-length FAO intermediates, 3-hydroxy-hexanoic acid (3-OH-C6), 3-hydroxy-octanoic acid (3-OH-C8), 3-hydroxy-decanoic acid (3-OH-C10), 3-hydroxy-dodecanoic (3-OH-C12), 3-hydroxy-tetradecanoic acid (3-OH-C14), and 3-hydroxy-hexadecanoic acid (3-OH-C16).

3-OH-C18 was synthesized by the method of Jones et al. (4), with the following changes. The precursor for 3-OH-C18 was not commercially available; thus the 3-OH-C18 precursor, hexadecanal, was synthesized first by the method of Landini et al. (5). A saturated solution of potassium chromate (0.55 mol/L) in 300 mL/L aqueous sulfuric acid was reacted with 0.01 mol of 1-hexadecanal dissolved in 60 mL of methylene chloride in the presence of 0.001 mol of tetrabutylammonium hydrogen sulfate as