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Sweat Testing for Benzodiazepines

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ABSTRACT: Thirteen subjects participated in a clinical study to determine the cumulative excretion, the time course, the dose-concentration relationship, and concentrations of diazepam in sweat following oral administration of a single dose of the drug. Nordiazepam and oxazepam, two metabolites of diazepam, were also investigated. Sweat was collected by means of Sudormed™ sweat patch. Patches were removed at specified times over one week and drug content was determined by gas chromatography/mass spectrometry (GC/MS) in negative chemical ionization mode using deuterated internal standards. Irrespective of the time of collection, diazepam and nordiazepam were present, but oxazepam was never detected. Drugs were detectable in the 2 to 4-h period following the administration. Peaks of diazepam were obtained during the 48 to 72-h period. After the peak, a decrease of drug concentration was observed. Concentrations were in the range from 0.1 to 6.0 ng/patch for both drugs. After single administration of diazepam (10, 20, or 30 mg), drugs monitored in three groups of three subjects were suggestive to be dose related. All these data suggest that the sweat patch technology can be useful to document drug use over a week-long period of surveillance.

KEYWORDS: forensic science, toxicology, sweat, benzodiazepines, substance abuse detection

Recent studies have shown that several commonly abused drugs enter the sweat in which their concentrations may readily be measured. Data are available for heroin (1), codeine (2), cocaine (1,3–4), phencyclidine (5), and tetrahydrocannabinol (6).

It is apparent that this excretion provides the basis for a useful test to determine whether or not a subject is taking a particular drug. If sweat can be collected over a long period of time and then assayed for drug content, this can provide a qualitative and possibly quantitative index of the pattern of drug use.

When sweat is to be tested for drugs, the first problem is to collect an adequate specimen. In the literature, thermal (7) or pharmacological (8) stimulations were proposed to secrete an unusually large amount of sweat.

Recently, PharmChem™ laboratories have proposed a sweat patch technology to be used to collect sweat for the detection of drugs of abuse (9). The patch that can best be described as a Band-Aid® is easily applied to the subject's skin and can be worn for up to two weeks. It consists of an adhesive layer on a 2 by 3-in. (51 by 76-mm) thin transparent film that adheres the patch to the body. In the center of the patch, there is a special absorbent pad that collects sweat components that are excreted from the body. The patch contains no chemicals that can harm the skin. It is non-occlusive and allows the skin to breathe during wear. It can be worn during most normal activities. There is no special precautions to be observed during patch wear except to avoid rubbing with a towel after bathing. A unique number is imprinted on each patch to aid with chain of custody and identification. The patch is tamper evident with respect to the fact that once applied to the skin, one can not remove and then reapply the patch without visual detection of having done so.

Over a period of several days, sweat would saturate the pad and slowly concentrate, and drugs present in sweat will be retained. Previous studies have shown that during the patch wear period, any single episode of a significant amount of drug use will be detected after 24 h of wear, once the patch is removed from the skin and analyzed using conventional drug testing technology (1–6).

Surprisingly, the detection in sweat of benzodiazepines, the most abused pharmaceutical drugs in the world, appears not to be documented. This report describes a series of sweat excretion experiments with subjects who were receiving single dose of diazepam.

Experimental Procedure

Chemicals

Methanol was HPLC grade (Merck, Darmstadt, Germany). Diazepam-d_5, nordiazepam-d_5, and oxazepam-d_5 were purchased from Radian (Austin, USA). *N*-0,-bis-(trimethylsilyl)trifluroacetamide (BSTFA) + 1% trimethyl chlorosilane (TMCS) was purchased from Interchim (Montluçon, France).

GC/MS Method

The GC/MS system consisted of a Hewlett Packard (5890 Series II) chromatograph with a mass selective detector 5989 B MS Engine operated in negative chemical ionization mode. Methane was used as reactant gas at an apparent pressure of 1.5 torr (200 Pa) in the ion source. Carrier gas (helium, purity N 55) flow through the column (HP-5 MS capillary column 5% phenyl-95% methylsiloxane, 30-m by 0.25-mm inside diameter) was 1.0 mL/min via an inlet electronic pressure controller. The column oven temperature was programmed from an initial temperature of 60° to 290°C at 30°C/min and maintained at 290°C for the final 5 min. Splitless injection was a split value off-time of 0.75 min was used. Injector temperature was 260°C. The source temperature was 200°C. The ions monitored and typical retention times (*RT*) for the different analytes and the deuterated internal standards were as follows: diazepam and diazepam-d_5, *m/z* 284, and 289, respectively (*RT* = 9.96 min); nordiazepam and nordiazepam-d_5, *m/z* 234 and 239, respectively (*RT* = 9.26); oxazepam and oxazepam-d_5, *m/z* 268 and 273, respectively (*RT* = 9.48). Drugs were identified and quantified based upon comparison of retention times

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TABLE 1—*Study protocol.*

Subject	Number of Patches	Diazepam Dose, mg	Experiments	Time of Patch Application, h	Time of Patch Removal, h
1, 2	10	30	Cumulative excretion	All patches at T_0	2, 4, 12, 24, 36, 48, 72, 96, 120, 144
3, 4	7	30	Time course	T_0, 2, 4, 12, 24, 48, 72	2, 4, 12, 24, 48, 72, 96
5, 6, 7	1	10	Dose-concentration	T_0	72
8, 9, 10	1	20	Dose-concentration	T_0	72
11, 12, 13	1	30	Dose-concentration	T_0	72

and relative abundance of two confirming ions to the deuterated internal standards.

Subjects

Subjects were recruited from the laboratory personnel. All denied use of benzodiazepines during the three previous months. The week before enrollment, two urine tests were performed that were negative. Subjects agreed to abstain from pharmaceuticals and alcohol during the study. Eight men aged 24 to 33 years and five women aged 25 to 33 years participated in the protocol. They signed an informed consent agreement and were paid for participation. They were encouraged to continue their normal hygiene practices. Sweat patches were generously offered by Pharmchem Laboratories (Menlo Park, US).

Sweat Collection

Sweat patches were applied to the outer portion of the upper arm or back. The selected skin site for patch placement was gently cleaned with a 70% isopropanol swab before application. The study protocol, along with the number of applied patches and time of removal, are presented in Table 1.

Diazepam was orally administered by solubilization in 100 mL of water. After removal of the patch, the absorption pad was stored in plastic tubes at −20°C.

Analytical Procedure

The absorbent pads were placed in extraction tubes, and 5 mL of methanol was added in presence of 1 ng of the following deuterated internal standards: diazepam-d_5, nordiazepam-d_5, and oxazepam-d_5. The tubes were shaken for 30 min on an orbital shaker at 200 rpm. After shaking, the methanol was removed, filtered, and evaporated to dryness. The dry extract was derived by silylation using 20 μL BSFTA + 1% TMCS, for 20 min at 60°C. A 1.5-μL portion of the derived extract was injected through the column.

Results

Analytical Procedure

Because diazepam undergoes *N*-demethylation to nordiazepam, which is then converted to oxazepam, these three compounds were considered as the target drugs.

Standard curves were constructed for each drug by addition of known concentrations (0.1, 0.2, 0.5, 1.0, 2.0, 5.0, and 10.0 ng) of drug and 1 ng of deuterated internal standard to drug-free absorption pads. The assay was linear for concentrations in the range tested with correlation coefficients of 0.998, 0.994, and 0.995 for diazepam, nordiazepam, and oxazepam, respectively. The extraction recoveries were higher than 92% for all the drugs. Within-run and between-run coefficients of variation were less than 16% for all the drugs. The limits of detection (LOD) were 0.01, 0.01, and 0.005 ng/patch with a signal-to-noise ratio of three for diazepam, nordiazepam, and oxazepam, respectively. These remarkable LOD were due to the high sensitivity of the negative chemical ionization mode.

Under the chromatographic conditions used, there was no interference with the target compounds by any extractable endogenous materials present in sweat.

Cumulative Excretion of Diazepam

Patches were applied before 30-mg diazepam administration and removed periodically over a period of seven days. The analysis of patches is presented in Table 2. Diazepam and its major metabolite, nordiazepam, were detected in sweat after 4-h collection. Oxazepam was never identified irrespective of the time of application. In all cases, diazepam concentrations exceeded nordiazepam. Increase of diazepam concentration was constant during the first 48 to 72 h followed by a slow decrease of the drug for the next periods. It can be postulated that diazepam is reabsorbed by the skin. Although not identical in term of peak concentrations (5.91 versus 3.80 ng/patch), the pattern of diazepam excretion in the sweat patches was similar for the two subjects.

Time Course of Excretion of Diazepam in Sweat

The time course of appearance of diazepam and metabolites in sweat was evaluated by application of sweat patches in incremental

TABLE 2—*Cumulative excretion in sweat of diazepam and nordiazepam following single oral administration of 30-mg diazepam. Results are expressed in ng/patch.*

	Subject 1		Subject 2	
Time of Removal, h	Diazepam	Nordiazepam	Diazepam	Nordiazepam
2	ND*	ND	ND	ND
4	0.22	0.03	0.18	ND
12	0.50	0.09	0.70	0.16
24	1.83	0.24	0.67	0.19
36	1.82	0.57	1.74	0.66
48	2.82	1.39	3.80	1.75
72	5.91	2.55	2.88	1.69
96	5.51	3.45	1.69	0.67
120	5.64	3.45	1.47	0.94
144	4.81	3.81	1.68	0.92

*Not detected.

TABLE 3—*Time course of diazepam and nordiazepam excretion in sweat following a single oral dose of 30-mg diazepam. Concentrations are expressed in ng/patch.*

Time of Sweat Collection, hours	Subject 3		Subject 4	
	Diazepam	Nordiazepam	Diazepam	Nordiazepam
0–2	ND*	ND	0.13	ND
2–4	0.50	0.39	0.38	ND
4–12	1.11	0.33	0.65	0.61
12–24	1.48	0.49	1.62	2.16
24–48	0.40	1.76	1.11	3.64
18–72	0.12	0.92	1.00	2.55
72–96	ND	0.84	0.74	1.46

*Not detected.

TABLE 4—*Dose-response relationship between the amount of diazepam ingested and the concentration in sweat after 72 h.*

Quantity Ingested, mg	Subject	Diazepam, ng/patch	Nordiazepam, ng/patch
10	5	1.63	0.39
10	6	0.89	1.33
10	7	1.01	0.59
Mean ± SD		1.18 ± 0.40	0.77 ± 0.50
20	8	2.07	0.98
20	9	1.51	1.83
20	10	2.28	1.98
Mean ± SD		1.95 ± 0.40	1.60 ± 0.54
30	11	2.75	1.86
30	12	5.91	2.55
30	13	2.88	1.69
Mean ± SD		3.85 ± 1.79	2.03 ± 0.46

time sequences following 30-mg oral administration. Results are presented in Table 3. Diazepam and nordiazepam appeared in sweat 2 h after drug administration, which was consistent with the first experiments in this study. Nordiazepam concentrations were lower than diazepam concentrations and generally were present whenever diazepam was detected in the patch. Oxazepam was not detected. Diazepam concentrations peaked at 12 to 24 h and decreased after that period.

Dose Relationship of Excretion of Diazepam in Sweat

Groups of three subjects received either a single oral dose of 10, 20, or 30 mg of diazepam on the first day. Sweat patches were worn for 72 h following drug administration. Results are presented in Table 4. There were substantial differences in the amount of diazepam collected by the sweat patches among the subjects receiving the same dose. In two cases, the metabolite concentration exceeded the concentration of the parent drug.

Although the limited subject data (three subjects for three doses) in the present study preclude generalization, the results are suggestive that a dose-response relationship exists between drug levels in sweat and the administered dose.

Discussion

Results from this study demonstrate the ability of the sweat patch to collect and contain benzodiazepines from sweat and to retain evidence of use for up to seven days. Although at the initial development, testing individuals for drugs with sweat patches increases the window of drug detection at least to one week. Sweat appears to offer the advantage of being a noninvasive means of obtaining an estimate of drug exposure over a period of several days or of compliance to abstinence in rehabilitation programs.

Subjects can wear one patch with minimal discomfort during at least one week. Nevertheless, few individuals developed slight irritation of the skin from the patches, especially after exposure to the sun. In this controlled study, no subject accidentally abraded the patch.

When compared with other data, e.g., for opiates (1,2) or cocaine (1,3), the concentrations of benzodiazepines in sweat appear to be very low, in the 1-ng range, compared to 10 to 100 ng in the other cases. Several mechanisms have been proposed to account for drug excretion in human sweat like lipid partition coefficients (10). Excretion in sweat appears to be maximal with basic drugs having high partition coefficients and pKa values close to the value of sweat pH (less than 5.0). The sweat patch is operating like an ion trap for the group of drugs that are weak bases with pKas around 8.0 (3). The findings of low diazepam concentrations in sweat are consistent with a pKa of 3.4 for the drug. Oxazepam, which is more polar than diazepam, will probably not diffuse across skin membranes as well as the parent drug. This can be one of the reasons why oxazepam was not detected in sweat in spite of a very low LOD.

Based on the findings from this study in which a single drug was being investigated, the advantage of the patch as an alternative to urine testing are significant. The patch provides a long-term continous monitor for drug abuse. It is easily applied and contains no chemicals that can harm the skin.

In conclusion, the sweat patch appears to be suitable to extend the window of drug detection to diagnose a single episode of drug use as far back as seven days.

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