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Sweat testing in addicts under methadone treatment: An Italian experience

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Abstract

In the last years the interest in monitoring drug exposure with human sweat as alternative biological fluid, is increasing. Sweat collection is convenient, less invasive and difficult to adulterate compared to traditional specimens. The objective of this study was to determine the excretion profile of methadone and other drugs into human sweat. Pharmscope sweat patches (Medical Europe Diagnostic, Madrid, Spain) were used on heroin abusers under methadone treatment.

Sweat patches were applied to 10 heroin addicts and 3 drug free volunteers admitted into the study. Sweat patches were worn for about 1 week; urine, saliva and hair samples were collected at the time of the removal of patches.

After the extraction, sweat eluates were directly analyzed by GC/MS for the presence of nicotine, cotinine, caffeine, methadone, EDDP and cocaine.

The extracts were subsequently derivatized to detect benzoylecgonine, ecgonine methyl ester, morphine, codeine and 6-acetylmorphine. No false positive results were obtained on the drug free samples. All the patches showed positive results for methadone. Cocaine was detected in two cases. Mainly the parent drug was identified rather than the metabolites.

The results obtained show the usefulness of sweat as complementary specimen to saliva and urine providing a longer detection window. Moreover, sweat testing offers the advantage of being a non-invasive means of obtaining information about drug exposure.

Keywords: Patches; Sweat; Drugs of abuse; Alternative matrices

1. Introduction

Although traditional drug testing is based on blood or urine analysis, interest in monitoring drug exposure using sweat as alternative biological fluid, is increasing [1,2]. Advances in the sensitivity of analytical instrumentation, along with the recent introduction of convenient collection devices [7], have made sweat an attractive alternative matrix for monitoring drug abuse [3,4].

Sweat secretion is an important homeostatic mechanism for maintaining a constant core body temperature. It is secreted from the eccrine and apocrine glands, which are located deep within the skin dermis. These glands terminate in secretory ducts that empty onto the skin surface. Several mechanisms of incorporation of drugs into sweat have been suggested, including the passive diffusion from blood into sweat glands and the trans-dermal passage of drugs across the skin dermis [1,5]. Non-ionised basic drugs diffuse into sweat and become ionised as a result of its lower pH (pH 5.8) as compared to blood (pH 7.4). Generally, it is the parent drugs which are detected in sweat, rather than their polar metabolites, which usually predominate in urine [6].

The aim of this study was to determine the excretion profile of methadone and other drugs of abuse into human sweat by analysing sweat specimens collected from known heroin addicts enrolled in a methadone maintenance program. In addition, urine and saliva specimens were also collected to provide a complete overview of the patient's drug use.

2. Methods

2.1. Materials

Sweat patches were obtained from Pharmscope (Medical Europe Diagnostic, Madrid, Spain) and were used in accordance with manufacturer's instructions.

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The patches are non-occlusive and consist of an adhesive plastic film that holds an absorption pad in place against the skin. The pad is identified by a unique number which must be verified at the removal.

Urine and saliva specimens were screened for cocaine metabolite, opiates, cannabinoids, benzodiazepines, barbiturates, methadone and amphetamines using the STC MICRO-PLATE assays (STC Technologies, Inc.). All assays were performed according to manufacturers instructions using the following cut-offs: urine samples 50 ng/ml for cannabinoids, 300 ng/ml for benzodiazepines, cocaine metabolite, methadone and opiates, 400 ng/ml for barbiturates, 1000 ng/ml for amphetamines; saliva samples 5 ng/ml for methadone, 10 ng/ml for opiates and cannabinoids, 20 ng/ml for cocaine metabolite and barbiturates, 50 ng/ml for amphetamine and benzodiazepines.

All positive results were confirmed by GC/MS using pre-validated in house methods.

2.2. Chemicals

Nicotine, cotinine, caffeine, methadone, 2-ethyl-1,5-dimethyl-3,3-diphenylpyrrolinium perchlorate (EDDP), cocaine, benzoylecgonine (BEG), ecgonine methyl ester (EME), morphine, codeine and 6-acetylmorphine (MAM) together with Methadone-*d*3, EDDP-*d*3, Cocaine-*d*3, EME-*d*3, BEG-*d*3, Codeine-*d*3, morphine-*d*3, and MAM-*d*3 were obtained from LGC Promochem.

Pentafluoropropanol and pentafluoropropionic anhydride (PFPA) were obtained from Sigma–Aldrich.

2.3. Samples

Ten heroin addicts, receiving methadone treatment, and three drug free volunteers were voluntarily admitted into the study. The study was conducted under the guidelines for protection of human subjects and all subjects provided informed consent. Details of the participants weight, age, sex, years of therapy and current methadone dose are reported in Table 1.

A sweat patch was applied to the upper arm of each subject for 1 week. At the end of the 1 week study period the sweat patches were removed, and urine, saliva and hair samples collected.

All samples, with the exception of hair sample, which were kept at room temperature, were frozen at −30 °C until analysis.

2.4. Extraction procedure

Half a sweat patch was cut into four pieces, incubated with 2 ml HCl 0.1 M for about 2 h at room temperature, and then spiked with 25 μl of a mixture of 10 ng/μl deuterated internal standards. The remaining half of the patch was reserved so that the analysis could be repeated to confirm the initial result.

After separation of the aqueous phase, a liquid/liquid extraction with chloroform/isopropanol (9:1) at alkaline pH was performed. The extracts were evaporated to dryness and reconstituted in 20 μl. A 1 μl aliquot was injected directly on to the column to determine the presence/absence of nicotine, cotinine, caffeine, methadone, EDDP and cocaine. Subsequently the extracts were derivatised using 50 μl PFPA and 30 μl pentafluoropropanol. A 1 μl aliquot was injected directly on to the column to determine the presence/absence of BEG, EME, morphine, codeine and MAM.

Table 1
Details of the participants to the study

Patient	Weight	Age	Years of therapy	Sex	Dosage (mg/day)
1	65	30	7	M	30
2	47	34	14	F	25
3	85	38	15	M	80
4	96	32	10	M	80
5	56	34	12	F	20
6	70	27	6	M	90
7	75	30	8	M	80
8	52	27	6	F	30
9	75	35	10	M	60
10	68	33	12	M	30

Table 2
m/z monitored for the compounds just after the extraction procedure

Compound	R.T.	Target ion	Qualifiers
Nicotine	5.37	84	133–162
Cotinine	8.26	98	176–119
Caffeine	9.23	194	195–109
EDDP	10.68	277	276–262
EDDP-*d*3	10.66	280	279–265
Methadone	11.38	294	223–309
Methadone-*d*3	11.36	297	226–312
Cocaine	11.74	303	182–198
Cocaine-*d*3	11.73	306	185–201

Hair samples were examined for cocaine, methadone and opiate positivity with GC/MS analysis after acid hydrolysis, liquid/liquid extraction and derivatisation.

Only qualitative analyses were performed for urine, saliva and hair specimens.

2.5. Instrumentation

A Focus GC coupled with a DSQ mass spectrometer (Thermo Electron Corporation) operating in electron impact mode at 70 eV was used. The separation was performed with a Equity-5 capillary column 30 m × 0.25 mm × 0.25 μm film thickness, employing a temperature program from 70 °C (1 min initial isotherm) to 280 °C with 15 °C/min linear increments (5 min final isotherm). In Tables 2 and 3 the *m/z* monitored for the different drugs are reported.

2.6. Calibration

Calibration curves for urine, saliva and hair were used (10 ng/ml limit of detection (LOD) and 20 ng/ml limit of quantitation (LOQ) for each compound).

Sweat calibration curves for each analyte (methadone, EDDP, cocaine, BEG, EME, morphine, codeine, MAM) were prepared adding known amounts of analytes (between 50 and 1000 ng/patch) and deuterated analogs (250 ng/patch) to blank worn patches. Linear regression lines were obtained and LOD and LOQ calculated by measurement of spiked samples were 20 and 50 ng/patch, respectively.

3. Results

3.1. Patch results

No false positive results were obtained on drug free samples. All the patches obtained from heroin abusers showed positive

Table 3
m/z monitored after PFPA derivatization

Compound	R.T.	Target ion	Qualifiers
EME	2.96	345	182–314
EME-*d*3	2.95	348	185–317
BEG	6.37	300	421–316
BEG-*d*3	6.36	303	424–319
Morphine	8.73	414	577–558
Morphine-*d*3	8.72	417	580–561
Codeine	9.15	282	445–446
Codeine-*d*3	9.14	285	448–449
MAM	10.63	414	473–361
MAM-*d*3	10.61	417	476–364