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Short communication

Nicotine monitoring in sweat with a sweat patch

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Abstract

In recent years, remarkable advances in sensitive analytical techniques have enabled the analysis of drugs in unconventional samples, such as sweat. In a study conducted with cigarettes smokers and nonsmokers, PharmChek sweat patches were applied to 29 subjects for 72 h. Nicotine was extracted in 5 ml methanol in the presence of 200 ng nicotine-d_4, used as internal standard. After 20 min agitation, the methanolic solution was evaporated to dryness in the presence of 10 μl octanol to ensure nonvolatility of nicotine. Nicotine was determined using gas chromatography coupled to mass spectrometry after separation on a 30-m capillary HP5 MS column. The assay was linear in the range 50–2500 ng/patch, with an extraction recovery of 76±5%. Limit of detection was 10 ng/patch. Nicotine concentrations in sweat were not detected for the nonexposed nonsmokers (n=8), 87 to 266 ng/patch for the passive smokers (n=6) and 150 to 2498 ng/patch for the smokers (n=15). This study demonstrated a useful application of the sweat patch for monitoring tobacco exposure.

Keywords: Sweat patch; Nicotine

1. Introduction

To document drug exposure in the body, a variety of well-established procedures has been used, and blood and/or urine have been the specimens of choice. Usually, a large volume of urine can be obtained, and analytical procedures are generally easier than are those required for blood. However, one limitation of these analyses is their inability to determine a history of exposure unless frequent analyses are performed, depending on the half-life of the target substances. The plasma half-life of nicotine in smokers has been found to range from 24 to 84 min, with an average of 40 min [1]. The mean biological half-life of cotinine, the major metabolite, in urine, collected from smokers was 16.5±1.2 h [2].

Nicotine testing in hair [3] has been proposed to complement blood and urine analyses. However, hair does not appear to be a suitable specimen for monitoring exposure on a weekly basis.

First observations on outward transcutaneous nicotine delivery date from 1990 [4], rapidly followed by two other papers [5,6]. At this time, the concentrations were determined by radioimmunoassay, after collection with filter paper and stimulation with pilocarpine. Nicotine tested positive in both smokers and passive smokers, with largely higher concentrations in the former population.

Nowadays, a commercial transcutaneous drug collection device is available. The patch consists of an acrylate adhesive layer on a thin transparent

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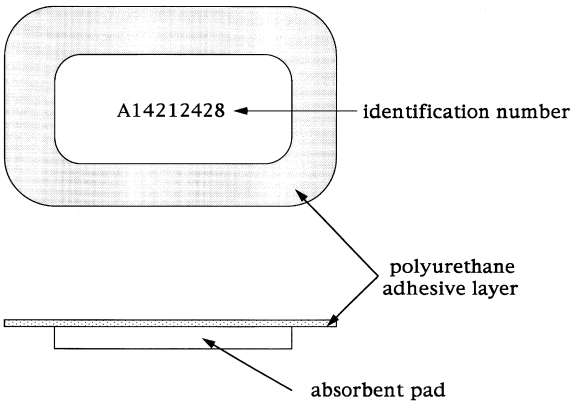

Fig. 1. Presentation of the sweat patch.

polyurethane film. Sweat components are collected on a special absorbent pad, located in the center of the patch (Fig. 1). Over a period of several days, sweat saturates the pad and slowly concentrates; drugs present in sweat are retained. A unique number is imprinted on each patch to ensure chain of custody and identification.

To date, a few applications of the sweat patch have been published; these applications include tests for cocaine [7–10], opiates [8,10–12], benzodiazepines [10,13], amphetamines [10,14,15], methadone [16,17], phenobarbital [12], cannabis and buprenorphine [10].

The aim of the present study was to evaluate excretion of nicotine in sweat obtained from smokers and nonsmokers.

2. Experimental

2.1. Chemicals

Methanol was HPLC grade (Merck, Darmstadt, Germany). Nicotine and nicotine-d_4 were purchased from Isotec (Miamisburg, OH, USA).

2.2. Specimen collection

Subjects were recruited from the laboratory personnel. Smokers agreed to abstain from smoking 24 h before enrolment. To be eligible as a nonsmoker, the subject had to attest to the absence of cigarette smoking for 1 year. Twenty-nine subjects, aged 20 to 56 years participated in the study. The population was as follows: nonsmokers, 14 subjects, including 6 passive smokers; smokers, 15 subjects, smoking 5 to 20 cigarettes/day.

Conditions of personal exposure levels in passive smokers were as follows: subjects I, J, and M were exposed at workplace (8 h/day) from smokers in their office, subject K was twice exposed for about 3 h during dinner in a restaurant, and finally, subjects L and N were exposed in the evening, at home, by a smoking partner.

All volunteers were encouraged to continue their normal hygiene practices. Sweat patches were generously donated by PharmChem Laboratories (Menlo Park, CA, USA).

Sweat patches were applied to the outer portion of the upper arm, on the left side. The selected skin site for patch placement was gently cleaned with a 70% isopropanol swab before application. The subjects were asked to have the same smoking habits as usual during the 3 days of the protocol. The patch was removed 72 h after placement, by pulling an edge of the adhesive backing, taking care not to touch the absorbent pad. After removal of the patch, the pads were stored separately in sealed plastic tubes at −20°C until analysis within 3 weeks.

2.3. Analysis of the sweat patch

Nicotine was extracted from the absorbent pad in 5 ml of methanol in presence of 200 ng of nicotine-d_4, used as internal standard (I.S.). The samples were shaken for 20 min on an orbital shaker at 200 rpm. After centrifugation, the methanol was removed and a 10-μl aliquot of octanol was added to ensure nonvolatility of nicotine. After evaporation of the methanol, the residue was dissolved in 20 μl methanol, and 1.5 μl of the solution was injected through a HP5-MS capillary column (5% phenyl–95% methylsiloxane, 30 m×0.25 mm I.D., 0.25 μm film thickness) into a model HP 5890 Serie II gas chromatograph coupled with a HP 5972 mass selective detector (all from Hewlett-Packard, Les Ulis, France). Injector temperature was 240°C, and splitless injection was used with a split-value off-time of 0.75 min. The flow of helium through the column was 1 ml/min. Column temperature was pro-

grammed to rise from an initial temperature of 60°C (held 1 min) to 260°C at 30°C/min, and held at 260°C for the final 2 min.

The ions monitored and typical retention times (t_R) for nicotine and the deuterated I.S. were as follows: nicotine, m/z 84, 133, and 162 (t_R: 5.68 min); nicotine-d_4, m/z 84, 136, and 166 (t_R: 5.68 min). The ions m/z 133 and 136 were used for quantification.

3. Results and discussion

Under the chromatographic conditions used, there was no interference with nicotine or the I.S. by any extractable endogenous material present in sweat.

A standard curve was constructed for nicotine by addition of known concentrations (50, 100, 500, 750, 1000, 1500 and 2500 ng) of nicotine in methanol and 200 ng of deuterated I.S. to drug-free absorbent pads. The assay was linear for the concentrations in the range tested, with a correlation coefficient of 0.994. Within-run and between-run precision ($n=8$), studied after addition of 200, 500 and 1000 ng of nicotine to drug-free absorbent pads, were 13 and 16% (200 ng/patch), 13 and 15% (500 ng/patch), and 9 and 11% (1000 ng/patch), respectively.

At the initial stage of this work, various phases were tested to extract nicotine, added to drug-free absorbent pads at 200 ng. Apparent extraction recoveries ($n=4$) were 53±8, 48±6, 23±10, 42±7 and 76±5%, for diethyl ether, 1 *M* NaOH–diethyl ether (1:5, v/v), 0.2 *M* sodium acetate buffer (pH 5.0)–methanol (1:3, v/v), concentrated ammonia solution–dichloromethane (1:5, v/v) and methanol, respectively. As it is the case for other compounds, methanol extraction gave the best recovery.

The limit of detection for extracted nicotine added to the pad was 10 ng/patch, with a signal-to-noise ratio>3.

Before starting with nicotine measurements in sweat obtained from smokers, the possibility of environmental contamination was of major concern, particularly because nicotine is present in smoke. Therefore, sweat patches were challenged in a passive contamination study. Smoke, from an entire cigarette was puffed against four patches, placed on the arm of one subject. Analyses of these patches produced negative results in all cases, indicating that environmental contamination is not a critical issue for the application of the transcutaneous collection device tested. The tightness of the polyurethane film to externally applied substances was also confirmed by Cone et al. [8] and Skopp et al. [16].

Subjects wore the patch with minimal discomfort for 3 days. No subject accidentally abraded the patch.

The results of nicotine monitoring of the 29 subjects are shown in Table 1. No nicotine was detected in sweat collected from eight nonexposed nonsmokers (subjects A to H), with a limit of detection of 10 ng/patch. However, six passive smokers (subjects I, J, K, L, M, and N) were confirmed with nicotine concentrations in the range 87–266 ng/patch, clearly indicating that the sweat patch technology is a sensitive method to document environmental smoke exposure.

Table 1
Concentrations of nicotine in the sweat patch

Subject	Smoker status	Nicotine (ng/patch)
A	Nonsmoker	<10
B	Nonsmoker	<10
C	Nonsmoker	<10
D	Nonsmoker	<10
E	Nonsmoker	<10
F	Nonsmoker	<10
G	Nonsmoker	<10
H	Nonsmoker	<10
I	Passive smoker	203
J	Passive smoker	217
K	Passive smoker	116
L	Passive smoker	87
M	Passive smoker	266
N	Passive smoker	134
O	Smoker	360
P	Smoker	2498
Q	Smoker	1390
R	Smoker	1517
S	Smoker	191
T	Smoker	1813
U	Smoker	965
V	Smoker	1146
W	Smoker	488
X	Smoker	583
Y	Smoker	1170
Z	Smoker	607
AA	Smoker	570
AB	Smoker	1013
AC	Smoker	150

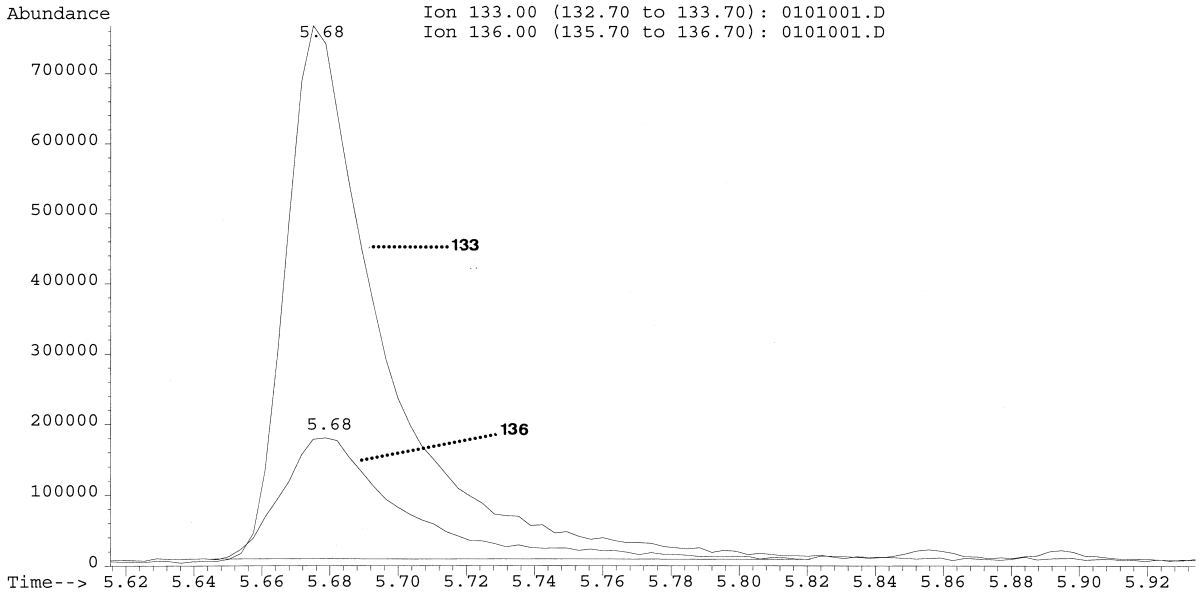

Fig. 2. Typical chromatogram in SIM mode for nicotine (m/z 133) and nicotine-d_4 (m/z 136). Nicotine concentration was 583 ng/patch.

Fifteen subjects, smoking 5–20 cigarettes/day exhibited nicotine in sweat in all cases, with concentrations in the range 150–2498 ng/patch. Fig. 2 is the chromatogram obtained from subject X, with a nicotine concentration of 583 ng/patch.

Although nicotine concentrations were present in greater amounts in the smoking population, it was not possible to establish a cut-off value between the passive smokers and the smokers, due to an overlap between the drug concentration in both populations.

The few data of this pilot study did not legitimate an attempt to correlate the amount of drug detected in the patch with the daily cigarette consumption. However, the observed results are suggestive of a lack of a relationship between dose and concentration. For example, subjects AA and AB reported, on the basis of a self-questionnaire, that they smoked the same number and type of cigarettes (about 15 per day) during the 3-day period, while nicotine concentrations in sweat were in a magnitude of 1 to 2. The same observations can be made with subjects S and P, who smoked the same number of cigarettes (10 cigarettes/day), but the smoke was inhaled by subject P (2498 ng/patch), and not by subject S (191 ng/patch). Therefore, sweat testing seems to be a qualitative rather than a quantitative test to estimate the amount of drug used. This new technology may find useful applications in the treatment of tobacco dependant subjects and monitoring of nicotine abusers.

In spite of its identification, in concentrations ranging from not detected to 86 ng/patch, no particular attention was given to cotinine, the major metabolite, since it has been definitively demonstrated that the main analyte excreted in sweat is the parent drug [18].

In conclusion, the sweat patch has demonstrated useful application for monitoring nicotine exposure. Although the quantitative interpretation of the results of perspiration is rather difficult, these analyses may provide a probative value for establishing environmental smoke exposure. The patch can be worn continuously and sweat analysis may be an useful adjunct to conventional drug testing.

References

[1] A.K. Armitage, C.T. Dollery, C.F. George, Br. Med. J. 4 (1975) 313.
[2] N.J. Haley, D.W. Sepkovic, D. Hoffmann, Am. J. Public Health 79 (1989) 1046.

[3] P. Kintz, J. Chromatogr. 580 (1992) 347.
[4] S. Balabanova, E. Schneider, Beitr. Gerchtl. Med. 48 (1990) 45.
[5] E. Schneider, S. Balabanova, Arch. Kriminol. 188 (1991) 97.
[6] S. Balabanova, G. Bühler, E. Schneider, H.J. Boschek, H. Schneitler, Hautarzt 43 (1992) 73.
[7] M. Burns, R.C. Baselt, J. Anal. Toxicol. 19 (1995) 41.
[8] E.J. Cone, M.J. Hillsgrove, A.J. Jenkins, R.M. Keenam, W.D. Darwin, J. Anal. Toxicol. 19 (1995) 298.
[9] V. Spiehler, J. Fay, R. Fogerson, D. Schoendorfer, R.S. Niedbala, Clin. Chem. 42 (1996) 34.
[10] P. Kintz, A. Tracqui, P. Mangin, Y. Edel, J. Anal. Toxicol. 20 (1996) 393.
[11] P. Kintz, R. Brenneisen, P. Bundeli, P. Mangin, Clin. Chem. 43 (1997) 736.
[12] P. Kintz, A. Tracqui, C. Jamey, P. Mangin, J. Anal. Toxicol. 20 (1996) 197.
[13] P. Kintz, A. Tracqui, P. Mangin, J. Forensic Sci. 41 (1996) 851.
[14] J. Fay, R. Fogerson, D. Schoendorfer, R.S. Niedbala, V. Spiehler, J. Anal. Toxicol. 20 (1996) 398.
[15] P. Kintz, J. Anal. Toxicol. 21 (1997) 570.
[16] G. Skopp, L. Pötsch, H.P. Eser, M.R. Möller, J. Forensic Sci. 41 (1996) 933.
[17] P. Kintz, A. Tracqui, C. Marzullo, A. Darreye, F. Tremeau, P. Greth, B. Ludes, Ther. Drug Monit., in press.
[18] P. Kintz, Ther. Drug Monit. 18 (1996) 450.