Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography B, 852 (2007) 333–337
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Determination of the volume of sweat accumulated in a sweat-patch using sodium and potassium as internal reference

Brice M.R. Appenzeller*, Claude Schummer, Sophie Boura Rodrigues, Robert Wennig

Laboratoire de Toxicologie, Centre de Recherche Public – Santé/Laboratoire National de Santé; Université du Luxembourg, Campus Limpertsberg, 162a, avenue de la Faïencerie, L-1511 Luxembourg

Received 13 October 2006; accepted 22 January 2007
Available online 1 February 2007

Abstract

In the present work, we assessed the suitability of sodium and potassium physiologically present in sweat, as internal reference allowing to re-calculate the corresponding volume of sweat collected on a PharmChek™ Patch. A method using capillary electrophoresis with indirect ultraviolet detection was developed for the determination of sodium and potassium in sweat. The concentrations determined in specimens collected from 12 females and 10 males, using a home-made system composed of polypropylene copolymer bag, were 1039 ± 89 mg/L and 711 ± 45 mg/L for sodium, and 489 ± 293 mg/L and 474 ± 196 mg/L for potassium, respectively. In parallel, for seven females and eight males, the comparison of the volume of sweat collected in the same way to the re-calculated volume of sweat accumulated in a patch using sodium as internal standard, gave an average agreement of $98.4 \pm 15.0\%$. Results demonstrated the usefulness of sodium as internal standard to determine the volume of sweat accumulated in a patch, and confirm the suitability of PharmChek™ patch for the collection and determination of cations in sweat.

Keywords: Sweat patch; Sodium; Potassium; Internal reference; Capillary electrophoresis

1. Introduction

Sweat is produced by the sweat glands in order to regulate the temperature of the body and to hydrate and protect the skin. It is composed at 99% of water and ions such as chloride, sodium and potassium, and in lesser extent calcium, magnesium, lactate, and other minor electrolytes. Since the beginning of the past century, one knows that it may also contain xenobiotics excreted in the sweat following their consumption [1]. However, the use of sweat as a matrix for toxicological analyses was hampered by the lack of adapted way for its collection. In 1995, PharmChem™ laboratories proposed a normalized solution for sweat collection, by commercializing a sweat patch technology approved by the American "Food and Drug Administration". A patch is composed of a cellulose absorbent pad where non-volatile substances from sweat may accumulate, covered by an adhesive semi-permeable membrane, which prevents from external contamination and allows water to evaporate, maintaining the skin sound during the period of application.

From this time, many xenobiotics such as cocaine [2–4], benzodiazepines [5], opioids [6–8], phenobarbital [9], methamphetamine [10,11], nicotine [12], and cannabinoids [13] have been detected in sweat collected using such patch. The use of sweat for toxicological analyses presents the advantage of a non-invasive collection, allowing repeated sampling, avoiding discomfort for weaker people such as neonates or older patients, and severely decreases the risk of contamination in case of people suffering of infectious diseases. Moreover, a patch may reveal the consumption for an extended period, by staying applied on the skin up to 10 days. Nevertheless, a patch gives only access to partial information, since the amount of sweat corresponding to xenobiotics measured after extraction remains unknown. Results may then only be expressed as amount of xenobiotic per patch and not as concentration in sweat.

To go further and give access to quantitative response, we assessed the suitability of sodium and potassium physiologically present in sweat, as internal reference allowing to re-calculate the corresponding volume of sweat collected on a patch. We first developed a method for the determination of

* Corresponding author. Tel.: +352 46 66 44 6727; fax: +352 22 13 31.
E-mail address: brice.appenzeller@lns.etat.lu (B.M.R. Appenzeller).

1570-0232/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2007.01.037

sodium and potassium concentration in sweat, using capillary zone electrophoresis. Second, we evaluated their inter-individual variability by analyzing sweat from 22 volunteers. We finally tested the suitability of patch for the collection and analysis of sodium by comparing analyses performed on patch extracts to analyses performed on sweat gathered using a home-made system composed of polypropylene copolymer bag allowing to obtain native (not evaporated) sweat.

2. Experimental

2.1. Reagents and solutions

All chemicals used were of analytical grade, and purchased by Merck (Darmstadt, Germany). All solutions, electrolytes and standards were prepared using water purified with a Vivendi Water System 8000 E/14, with conductivity below 0.04 μS/cm and sodium and potassium concentrations below the limit of detection. In order to avoid cations exchange with glass material, all solutions were prepared in vessel made of polypropylene (BRAND, PLASTIBRAND, Germany). Alkaline solutions used for capillary washing were 1 M and 0.1 M LiOH in water. Sodium and potassium stock solutions were prepared by dissolving KCl and NaCl in water. Calibration standards were then prepared by diluting stock solutions to the following concentrations: 1, 2, 8, 10, 20, 50, 80 and 100 mg/L. The background electrolyte was constituted of imidazole, added with citric acid to adjust pH. Various conditions were tested in order to optimize resolution and migration time: pH of 3.6, 4.4, 5.3, 6.3, 9.4, and imidazole concentration of 0.5 mM, 2.5 mM, 5 mM, 10 mM and 50 mM.

2.2. Instrumentation and procedure

Cations measurements were carried out using capillary zone electrophoresis (Beckman Coulter™, P/ACE MDQ) with PDA detector set for indirect detection at 214 nm, and cartridge temperature adjusted to 25 °C. Fused-silica capillaries were purchased from Composite Metal Services LTD., UK, had a total length of 60.2 cm (50 cm to the detector) and an inner diameter of 50 μm. Prior to the first use, capillary was washed with 1 M LiOH (20 min, 20 psi), and rinsed with water (20 min, 20 psi). Afterwards, capillary was washed with LiOH (10 min, 20 psi), rinsed with water (10 min, 20 psi), and conditioned with the background electrolyte (10 min, 20 psi) at the beginning of each day. Run consisted of washing with 0.1 M LiOH (2.5 min, 20 psi), rinsing with water (0.5 min, 20 psi) and conditioning with background electrolyte (3 min, 20 psi). Injection was in hydrodynamic mode (0.5 psi, 5 s), and consumed 5 nl of sample (determined by the Beckman CE Expert software). The voltage applied for separation was 30 kV.

2.3. Specimen collection

Sweat patch (PharmChek™, PharmChem Inc.) consists of a transparent, water-resistant adhesive backing securing a cellulose absorption pad to the surface of the skin. Two different versions of the sweat patch were used in this study. "Old patches" had a pad covering the skin surface 14.1 cm^2, and were used for subjects W, X and Y. "New patches" had a pad covering the skin surface 14.8 cm^2 and were used for the all other subjects. Patches were applied to the lower back of subjects after gently washing the skin once with de-ionized water and once with isopropanol. Patches were removed according to patch manufacturer's instructions. After collection, pads from patches were enclosed in polyethylene tubes and 7 mL of de-ionized water was added. Extraction was then performed under agitation (30 min, 150 rpm), and the extract was collected for analysis.

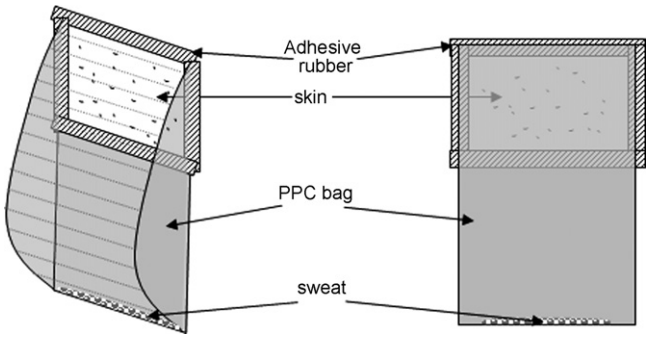

Fig. 1. Schematic representation of the native sweat collecting system (NSCS).

In order to obtain sweat of composition which was not modified by evaporation, a "native" sweat collecting system (NSCS) was brought up from polypropylene copolymer (PPC) bag and adhesive rubber (Leukoplast®, $BSN_{MEDICAL}$) as described in Fig. 1. The NSCS included a window covering a skin surface of 150 cm^2, and a "bag part" allowing sweat to accumulate. The NSCS was airtight and watertight and all components of the NSCS had no influence on sweat composition for the period of use. After gently washing the skin with de-ionized water and isopropanol, the NSCS was applied to the lower back of subjects before physical activity. Afterwards, sweat was collected from NSCS and stored in polyethylene tubes at 4 °C before analysis. During this study, the volume of sweat collected with NSCS varied from 0.45 to 3.80 mL. Before analysis, sweat was diluted with water to reach a concentration compatible with the calibration curve, 1/10 for men's sweat and 1/20 for women's sweat, respectively.

2.4. Subjects

Subjects were volunteers recruited from the laboratory personnel. They declared to be healthy and denied to take any medical treatment at the time of experiment. Thirteen females (A to L, plus W) (age 28 ± 9 years; body mass 64 ± 9 kg; BMI 21.92 ± 3.16 kg/m^2) and 12 males (M to V, plus X and Y) (28 ± 9 years; body mass 70 ± 11 kg; BMI 23.98 ± 2.52 kg/m^2) were included in the study. Description according to age, size and weight is detailed in Table 1. After applying NSCS or NSCS and patch, subjects were submitted to physical activity which consisted of 0.5 h jogging at a speed of 8–12 km/h, according to subject's ability, at temperature of 28 ± 3 °C. From applying time to collection, patches were in place for less than 3 h. The study neither required consumption of any substance for par-

Table 1
Sodium and potassium concentration in sweat of males and females

	Subjects			Concentration in sweat (mg/L)	
	Age (years)	High (cm)	Weight (kg)	Sodium	Potassium
Females					
A	26	168	54	1089	856
B	23	172	73	1040	874
C	23	183	70	1051	321
D	51	165	83	1125	795
E	26	168	58	1095	752
F	23	165	61	1058	273
G	29	166	65	1076	797
H	32	173	62	880	344
I	24	178	65	1153	272
J	23	177	65	944	168
K	24	169	55	882	258
L	23	164	54	1069	162
Total females	27	171	64	1039 ± 89	489 ± 293
Males					
M	24	173	73	629	551
N	56	169	70	671	498
O	23	178	72	724	902
P	23	182	95	669	304
Q	23	180	70	690	691
R	32	186	82	770	344
S	32	188	77	728	324
T	19	180	80	762	457
U	27	178	86	733	310
V	24	182	68	734	358
Total males	28	180	77	711 ± 45	474 ± 196

ticipants nor invasive sampling. All participants were informed about the procedure and gave an informed consent agreement.

3. Results and discussion

3.1. Capillary electrophoresis dosage of cations in sweat

Optimal conditions according to resolution and migration time were obtained with background electrolyte concentration of 5 mM and pH set at 4.4 (Fig. 2). Sodium and potassium determination was performed within a 13 min run. For the following concentrations: 1, 2, 8, 10, 20, 50, 80 and 100 mg/L, the calibration curves equations were $y = 0.00755x$ ($r^2 = 0.9949$) and $y = 0.00288x$ ($r^2 = 0.9978$) for potassium and sodium, respectively. The inter-day ($n = 6$) and intra-day ($n = 6$) repeatability, expressed as coefficient of variation, and determined for two different concentrations (15 mg/L and 70 mg/L) were 9.26% and 4.53% for potassium and 8.27% and 3.75% for sodium, respectively. The limit of detection (LOD) and lower limit of quantification (LLOQ) were 0.54 mg/L and 1.78 mg/L, respectively for potassium, and 0.25 mg/L and 0.83 mg/L, respectively for sodium. No interference due to matrix effect was observed.

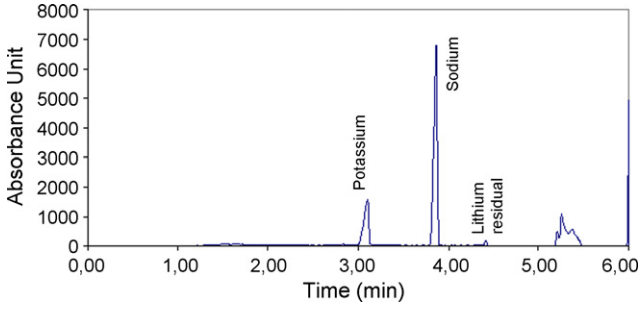

Fig. 2. Electrophoregram of sweat analysis, displaying peaks of potassium and sodium present in sweat, and lithium residual due to capillary washing with LiOH.

The method of sodium and potassium determination in sweat/patch extract using capillary zone electrophoresis described here appears to be well adapted for the determination of a reference substance in sweat. The analyses displayed good repeatability, sufficient sensitivity, and required a short time. Moreover, the reduced volume necessary for one analysis (5 nL), makes merely the whole sample available for further analysis of xenobiotics.

3.2. Sodium and potassium concentration in sweat

The average sodium concentration in sweat was 1039 ± 89 mg/L ($CI_{0.95}$ = [982; 1095]) for females and 711 ± 45 mg/L ($CI_{0.95}$ = [679; 743]) for males (Table 1). The concentration was statistically different between males and females (Mann–Whitney test: $p < 0.0001$) but displayed few variability within groups since variation coefficients were 8.6% and 6.3% for females and males, respectively. The average

potassium concentration was 489 ± 293 mg/L ($CI_{0.95} = [303; 676]$) for females and 474 ± 196 mg/L ($CI_{0.95} = [334; 614]$) for males (Table 1). The concentration was not statistically different between males and females (Mann–Whitney test: $p = 0.5529$) but was highly variable within both females and males (variation coefficients: 60% and 41%, respectively). The sweat rate (calculated as volume of sweat produced per skin surface unit per time unit) ranged from 8.0 to 50.7 $\mu L\,cm^{-2}\,h^{-1}$ in females and was not correlated to the sodium ($r = 0.021$, $p = 0.9644$) and to the potassium ($r = 0.1464$, $p = 0.7541$) concentration. The sweat rate ranged from 6.0 to 38.6 $\mu L\,cm^{-2}\,h^{-1}$ in males, was slightly correlated to the potassium concentration in sweat ($r = 0.7280$, $p = 0.0406$), but not to the sodium concentration ($r = 0.2944$, $p = 0.4090$). In both females and males, age was neither correlated to the sodium concentration ($r = 0.1904$, $p = 0.5533$ and $r = -0.1903$, $p = 0.5984$, respectively) nor to the potassium concentration in sweat ($r = 0.4080$, $p = 0.1880$ and $r = -0.1261$, $p = 0.7285$, respectively).

Although the average $[K^+]$ in sweat was quite similar for males and females, the high inter-individual variability observed here makes potassium unsuitable as a reference substance. At the opposite, although $[Na^+]$ was different between males and females, it displayed very low variability within groups. To our knowledge, the gender difference in sweat sodium concentration observed in the current investigation was not previously documented. This difference was not due to sweat rate, since this parameter was not correlated to the cations concentration. Comparing cations concentrations observed here to the values from other studies must take into account the skin region where sweat was collected (lower back in this study), since this parameter was reported to influence the sweat composition [14]. The $[Na^+]$ observed in males of our study (30.9 ± 1.9 mM) was close to values previously reported for lower back sweat (26.2 ± 19.4 mM) in healthy men [14], but with lower inter-individual variability. Like in the study from Patterson et al. [14], the $[Na^+]$ did not seem to be correlated to the sweat rate, unlike $[K^+]$, which is a further argument in favor of sodium as a reference substance. In view of these observations, sodium was definitely more suitable than potassium as a reference substance to determine the volume of sweat collected by a patch. This implies taking into account the gender of subjects, since sodium concentration was quite different between males and females.

3.3. Suitability of PharmCheck™ patches for sodium collection and determination

The recovery of cations from patch was tested in triplicate with six different cations amount ranging from 30 to 360 μg per patch. The apparent recovery percent was $116 \pm 11\%$ and $101 \pm 11\%$ for sodium and potassium, respectively for new patches, and $72 \pm 12\%$ and $55 \pm 26\%$ for sodium and potassium, respectively for old patches. In view of the highly variable concentration of potassium in sweat between subjects, whatever being the gender, only sodium was taken into account in the assessment of patch suitability for sweat collection. Depending on subjects, the volume of sweat collected by NSCS ranged from 450 to 3800 μL. Divided by the skin surface covered by the NSCS, this corresponded to a volume of sweat excreted ranging from 3 to 25 μL per cm^2 of skin. The analyses of patches displayed that the amount of sodium accumulated was ranging from 16 to 484 μg per patch. Divided by the skin surface covered by a patch and adjusted with the recovery percent, this corresponded to a sodium excretion ranging from 1.57 to 28 μg per cm^2 of skin. Using the average sodium concentration determined above in sweat from males and females, respectively (Table 1), the calculated volume of sweat excreted ranged from 3.1 to 27.0 μL per cm^2 of skin. The average agreement between NSCS and patch according to the volume of sweat produced was 98.4% (Table 2).

Table 2
Comparison of sodium and sweat collected with NSCS vs. PharmChek™ patches

Subjects	NSCS			Patches			Sweat NSCS to sweat patch match (%)
	Sweat volume collected (μL)	Sweat per skin surface (μL/cm^2)	Surface Na^+ conc. (μg/cm^2)	Na^+ per patch (μg)	Surface Na^+ conc. (μg/cm^2)[a]	Calculated sweat per skin surface (μL/cm^2)[b]	
F	3750	25.0	26.5	484	28.0	27.0	92.6
H	3300	22.0	19.4	323	18.7	18.0	122.3
I	600	4.0	4.6	84	4.9	4.7	85.0
J	1500	10.0	9.4	169	9.8	9.4	106.2
K	1400	9.3	8.2	213	12.4	11.9	78.4
L	3800	25.3	27.1	408	23.7	22.8	111.2
M	2200	14.7	9.2	159	9.2	12.9	113.4
N	2895	19.3	12.9	238	13.8	19.4	99.6
P	600	4.0	2.7	46	2.7	3.8	105.9
T	2100	14.0	10.7	219	12.7	17.9	78.4
U	1300	8.7	6.3	134	7.8	10.9	79.2
V	450	3.0	2.2	30	1.7	2.4	123.6
W	3480	23.2	24.6	264	26.1	25.1	92.4
X	435	2.9	1.7	16	1.57	3.1	94.6
Y	430	2.9	1.8	16	1.57	3.1	93.8
Total							98.4 ± 15.0

[a] Calculated from "Na^+ per patch" divided by the patch surface, and adjusted with sodium apparent recovery (116% for F to V, and 72% for W, X and Y).
[b] Calculated from "surface Na^+ concentration" divided by the average sodium concentration in sweat (Table 1).

The good recovery from patch confirmed that patch material does not interfere with cations measurement. This parameter was previously tested for several xenobiotics in different studies, which also reported satisfactory recovery, ranging from 76% for nicotine [12] to values above 90% for cocaine, opiods, cannabinoids, benzodiazepines and phenobarbital [3–6,9,11,13]. The good match between the volume of sweat collected with NSCS and the re-calculated volume of sweat accumulated on patch (Table 2) clearly demonstrated that it was possible to determine the volume of sweat produced, by determining the amount of sodium accumulated on a patch. These observations confirm the suitability of patches for cations gathering and determination in sweat.

4. Conclusions

The method of cations quantification by capillary zone electrophoresis developed in this study displayed satisfactory sensitivity and repeatability, consumes a reduced volume of sample and is hence well adapted to the analysis of sweat.

The low inter-individual variability in sodium concentration observed here makes sodium a suitable internal standard in order to determine the volume of sweat accumulated on a patch, the only restrictions being the subject gender and the skin region where sweat is collected. The volume of sweat accumulated in a patch may then be assessed by determining the amount of sodium present in the patch. For this purpose, the PharmCheck^{TM} Patch appears to be well adapted, displaying satisfactory collection and recovery properties.

By combining analysis of xenobiotics classically performed in sweat using patches to the sodium analysis developed in this study, it will be possible to determine the concentration of xenobiotics in sweat using patch as collecting way.

References

[1] H. Tachau, Arch. Exp. Pathol. Pharmacol. 66 (1911) 224.
[2] M. Burns, R.C. Baselt, J. Anal. Toxicol. 19 (1995) 41.
[3] M.J. Damas Follador, M. Yonamine, R.L. de Moraes Moreau, O. Alves Silva, J. Chromatogr. B 811 (2004) 37.
[4] D.E. Moody, A.C. Spanbauer, J.L. Taccogno, E.K. Smith, J. Anal. Toxicol. 28 (2004) 86.
[5] P. Kintz, A. Tracqui, P. Mangin, J. Forensic Sci. 41 (1996) 851.
[6] P. Kintz, A. Tracqui, P. Mangin, J. Anal. Toxicol. 20 (1996) 393.
[7] P. Kintz, R. Brenneisen, P. Bundel, P. Mangin, Clin. Chem. 43 (1997) 736.
[8] E.W. Schwilke, A.J. Barnes, S.L. Kacinko, E.J. Cone, E.T. Moolchan, M.A. Huestis, Clin. Chem. 52 (2006) 1539.
[9] P. Kinz, A. Tracqui, C. Jamey, P. Mangin, J. Anal. Toxicol. 20 (1996) 197.
[10] J. Fay, R. Fogerson, D. Schoendorfer, R.S. Niedbala, V. Spiehler, J. Anal. Toxicol. 20 (1996) 398.
[11] S. Pichini, M. Navarro, R. Pacifici, P. Zuccaro, J. Ortuño, M. Farré, P. Nolasc Roset, J. Segura, R. De la Torre, J. Anal. Toxicol. 27 (2003) 294.
[12] P. Kintz, A. Henrich, V. Cirimele, B. Ludes, J. Chromatogr. B 705 (1998) 357.
[13] T. Saito, A. Wtsadik, K.B. Scheidweiler, N. Fortner, S. Takeichi, M.A. Huestis, Clin. Chem. 50 (2004) 2083.
[14] M.J. Patterson, S.D.R. Galloway, M.A. Nimmo, Exp. Physiol. 85 (2000) 869.