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Sweat testing for the detection of atomoxetine from paediatric patients with attention deficit/hyperactivity disorder: application to clinical practice

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Atomoxetine (ATX) is a selective norepinephrine reuptake inhibitor approved since 2002 for the treatment of attention deficit hyperactivity disorder (ADHD) in children, adolescents, and adults as an alternative treatment to methylphenidate. Within the framework of a project evaluating the use of alternative biological matrices for therapeutic monitoring of psychoactive drugs in paediatric and non-paediatric individuals, the excretion of ATX and its principal metabolites has been recently studied in oral fluid and hair. The aim of this study was to describe the excretion profile of ATX and its metabolites 4-hydroxyatomoxetine (4-OH-ATX) and N-desmethylatomoxetine (N-des-ATX) in sweat following the administration of different dosage regimens (60, 40, 35, and 18 mg/day) of ATX to six paediatric patients. Sweat patches were applied to the back of each participant and removed at timed intervals. ATX and its metabolites were measured in patches using a previously validated liquid chromatography-tandem mass spectrometric (LC-MS/MS) method. Independently from the administered dose, ATX appeared in the sweat patches 1 h post administration and reached its maximum concentration generally at 24 h. Peak ATX concentrations ranged between 2.31 and 40.4 ng/patch and did not correlate with the administered drug dose, or with body surface area. Total ATX excreted in sweat ranged between 0.008 and 0.121 mg, corresponding to 0.02 and 0.3% of the administered drug. Neither 4-OH-ATX, nor N-des-ATX was detected in either of the collected sweat patches. Measuring ATX in sweat patches can provide information on cumulative drug use from patch application until removal. Copyright © 2012 John Wiley & Sons, Ltd.

Keywords: atomoxetine; sweat testing; ADHD

Introduction

Atomoxetine (ATX) is a selective norepinephrine reuptake inhibitor approved since 2002 for the treatment of attention deficit hyperactivity disorder (ADHD) especially in children and adolescents as alternative treatment to methylphenidate.[1–3]

Monitoring exposure to therapeutic drugs in the paediatric population is more difficult than in adults. Some children and young people do not co-operate, for example, with blood sampling, because they find it painful, traumatic, and distressing. Therefore, painless, simple, and less distressing alternatives for sampling body fluids are required for non-invasive assessment of drug use in paediatric patients.

Alternative matrices, such as hair, oral fluid, and sweat offer additional options to testing in conventional matrices.[4–6] Particularly, oral fluid and sweat are the main alternative biological substrates for non-invasive assessment of short- term drugs use.[7–11]

Recently, the excretion profile of ATX and its principal metabolites 4-hydroxyatomoxetine (4-OH-ATX)- and N-des-methylatomoxetine (N-*des*-ATX) (Figure 1) has been investigated in plasma and in oral fluid together with the oral fluid to plasma concentration ratios after administration of different dosage regimens.[12] Indeed, since ATX is a weak base (pKa = 9.2), and since oral fluid is more acidic than blood, this substance should be incorporated in oral fluid by passive diffusion of the free fraction of the drug in its ionized form, which cannot diffuse back into plasma, as happens in the case of other basic drugs such as 3,4 methylenedioxymethamphetamine or methylphenidate.[12,13] However, independently from the administered dose and co-medications, it was shown that ATX and its metabolites do not diffuse to a large extent into oral fluid, probably due to the high plasma protein binding of these compounds.[12]

Sweat is the other alternative matrix that may provide additional information on drug use and misuse since the detection window of drugs in sweat is the time the sweat patch is worn, supplying cumulative drug use data from patch application until removal.[14,15] The development of the sweat patch technology has been an important step forward in facilitating sample collection. Because the patches can be worn for up to 1 week, and no drug degradation seems to occur during this time interval, the window of drug detection can be longer than the one provided by urine testing.[15]

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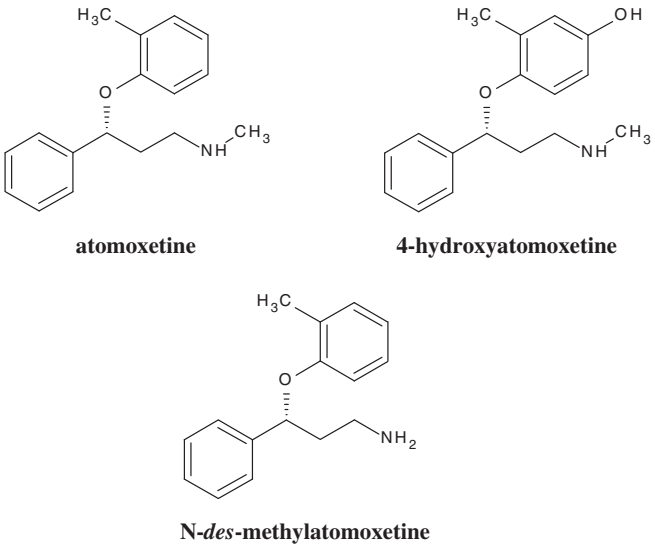

Figure 1. Chemical structure of the compounds under investigation.

Testing in sweat could be used as a non-invasive alternative to blood testing not only for purposes of drug monitoring,[16] and pharmacokinetic[8,17] studies, but also for monitoring recent use of ATX in children, adolescents, and young adults to evaluate drug compliance and associate with eventually observed clinical outcomes.

Whereas methylphenidate (MPH), an amphetamine derivative used in the treatment of ADHD, has been detected in sweat,[10,13] no information is available on the sweat excretion profile of ATX and its principal metabolites. For the first time, we evaluated the excretion of ATX and its metabolites in sweat. We aimed to investigate the presence and the time–course concentration of ATX and its metabolites in the sweat of a child and five adolescents chronically treated with different dosage regimens of ATX.

Materials and methods

Subjects and study design

Sweat samples were obtained from six subjects: one 7 year-old boy (weight 25.3 kg, height 123. 5 cm) and five 12–16 year-old adolescents (two girls and three boys, mean age: 14.0, mean weight 40.0 kg, and mean height 158.8 cm) all diagnosed with ADHD and receiving treatment for at least the last three months with different oral doses (18–60 mg/day) of ATX (Strattera®, Eli Lilly and Company, Indianapolis, IN, USA) at the Department of Pediatrics Hospital del Mar, Barcelona, Spain (Table 1). Moreover, three of them received concomitant psychotropic medication: 10 mg/day risperidone (subject 2), 900 mg/day carbamazepine (subject 4), and 75 mg/day sertraline and 1 mg/day risperidone (subject 5). These individuals were the same subjects recruited to investigate the presence and the time–course concentration of ATX and its metabolites (4-OH-ATX- and N-*des*- ATX) in oral fluid-plasma and hair after drug administration.[12,18,19] The study was conducted in accordance with the Declaration of Helsinki and was approved by the local Ethics Committee (CEIC-IMAS).

The parents or legal guardians of all subjects completed a written informed consent, and all subjects agreed to participate after the study protocol had been fully explained; they accepted to wear sweat patches on their back during the collection of oral fluid and blood up to 24 h after administration.

After the skin was cleaned with a 70% isopropyl alcohol swab, patches were applied to the back of each participant and removed at 1, 6, 12, and 24 h after administration. The PharmChek sweat patches (area of 4.6 cm^2) were provided by PharmChem Laboratories (Fort Worth, TX, USA). After removal, each patch was labelled and stored in plastic bags at -20 °C until analysis.

Analysis of ATX, 4-OH-ATX- and N-des-ATX in sweat patches

Sweat patches were allowed to thaw at room temperature, and the absorbent pad was removed with clean tweezers. Following spiking with 10 ng of duloxetine as internal standard (10 µl of 1 µg/ml methanolic solution) and 0.5 ml water (covering the whole pad), the patch was extracted twice with 2 ml tert-butyl methyl ether. After centrifugation at 1096 g-force for 3 min, the organic phase was evaporated to dryness under a stream of nitrogen and re-dissolved in 100 µl of mobile phase used for liquid chromatography–tandem mass spectrometry (LC-MS/MS) analysis (solvent A: water and solvent B: 5 mM ammonium acetate, 47.2 mM formic acid, 4 mM trifluoroacetic acid in acetonitrile–water (85:15, *v/v*). An LC-MS/MS validated method, described elsewhere, was used for the determination of ATX and its metabolites.[18] The limits of quantification (LOQs) of the reported assay were 0.5 ng per patch for ATX and its metabolites (4-OH-ATX- and N-*des*-ATX). The intra- and inter-assay imprecision (measured as % relative standard deviation) and inaccuracy (measured as % error) values for all analytes were always lower than 20%.

Results

Figure 2 shows the time course of ATX concentrations in sweat patches for a 24-h period for the six subjects as a function of the administered ATX dose (40, 60 and 18 mg/day, respectively).

Considering the three subjects treated with 40 mg/day ATX, the body surface area of the first two adolescents – the 14-year-old female (subject 1) and the 12-year-old male (subject 2) – was

Table 1. Samples information from one child and five adolescents treated with ATX and carbamazepine, risperidone and sertraline as co-medication

Subject	Sex	Age (years)	Body surface (m2)	ATX mg/day (mg/kg/day)	Concomitant medication
1	female	14	1.39	40 (0.89)	No
2	male	12	1.55	40 (0.69)	Risperidone 10 mg/day
3	male	7	0.93	40 (1.38)	No
4	male	16	1.72	60 (1.06)	No
5	female	12	1.14	60 (1.79)	Carbamazepine 900 mg/day
6	male	16	1.80	18 (0.26)	Sertraline 75 mg/day + Risperidone 1 mg/day

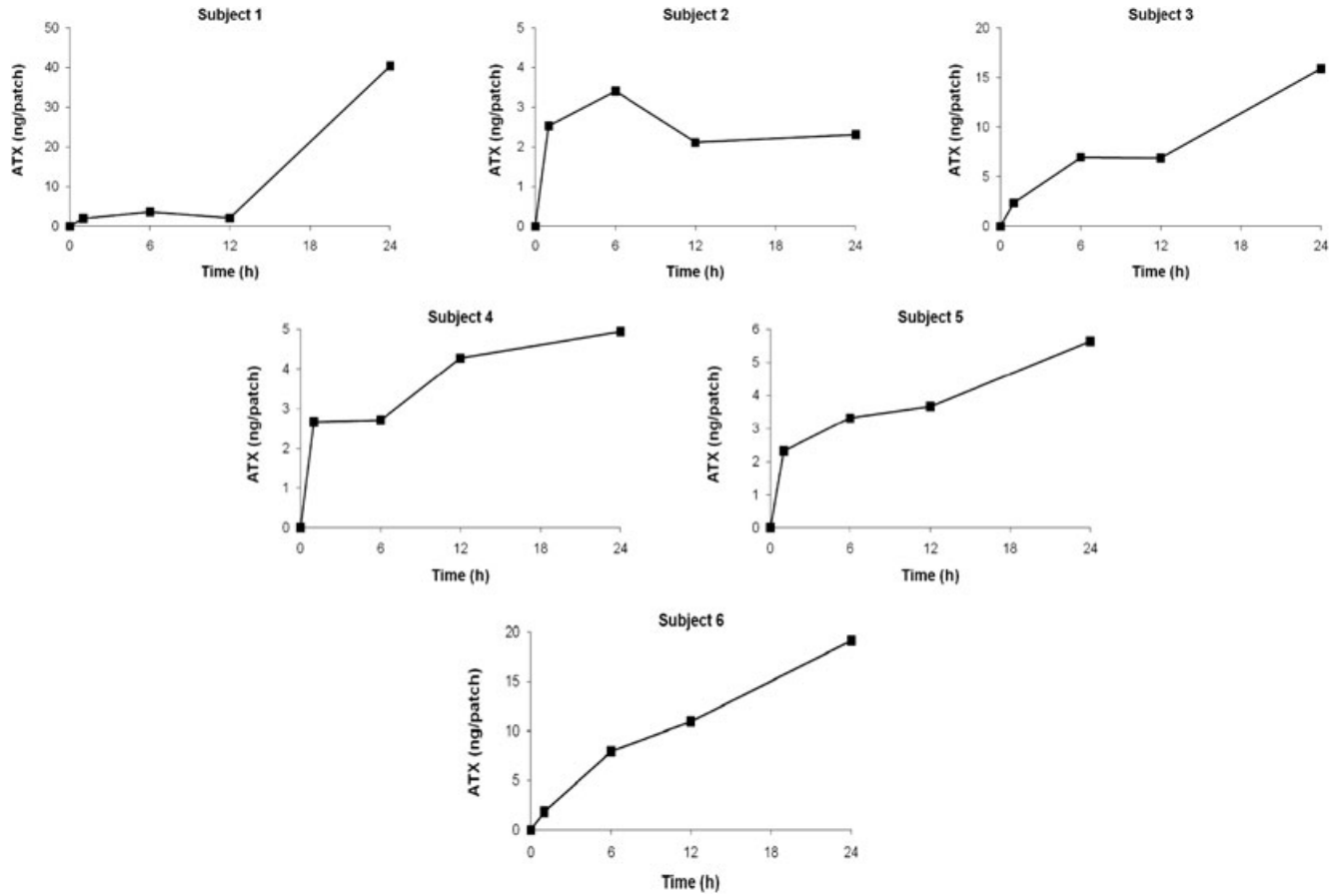

Figure 2. Concentration–time profile of ATX in sweat patches applied to the back of subjects 1–3 treated with 40 mg/day ATX, of subjects 4 and 5 treated with 60 mg/day ATX and of subject 6 treated with 18 mg/day ATX and removed at different time intervals.

comparable (1.39 m2 and 1.55 m2) as well as their mg per kg administered dose (0.89 and 0.69 mg/kg). In both cases, ATX appeared in sweat 1 h after administration and concentration in the patches (ranged between 2 and 3 ng/patch) remained constant up to 12 h after administration. At 24 h, ATX concentration in sweat patch of subject 1 peaked at 40.4 ng/patch, while in case of subject 2 (co-treated with 10 mg/day risperidone) it remained at 2 ng/patch (Figure 2).

In the case of the 7-year-old boy (subject 3) treated with the same ATX dose (40 mg) but mg per kg dose doubled (1.38 mg/kg) due to a lower body surface area (0.93 m2) with respect to the previous two teens, significantly higher ATX concentrations were found in patches removed at 6 and 12 h after drug administration (6.9 and 6.8 ng/patch, respectively), but 24 h peak of 15.9 ng/patch was lower than that of subject 1 (Figure 2).

Looking at ATX excretion in sweat patches of two adolescents (one 16-year-old male, subject 4, and one 12-year-old female, subject 5) treated with 60 mg ATX per day, it can be observed that in both cases and similarly to subjects 1 and 2, ATX concentration in sweat patches removed up to 6 h after drug administration ranged between 2 and 4 ng/patch. Subsequently, although the girl (subject 5) took a higher ATX mg/kg/day than the boy (subject 6 (1.79 *vs* 1.06 for the boy, body surface area 1.14 *vs* 1.72 m^2) and also carbamazepine (900 mg/day), her ATX peak value in sweat patch at 24 h was similar to that of the boy (4.94 *vs* 5.63 ng/patch) (Figure 2).

Finally, the 16-year-old boy (subject 6) treated with the lowest ATX daily dose (18 mg/day) and the lowest mg per kg of weight (0.26 mg/kg/day, body surface area of 1.80 m^2) concomitantly with risperidone (1 mg/day) and sertraline (75 mg/day) presented an increasing excretion of ATX in sweat with a maximum concentration of 19.3 ng/patch at 24 h post administration (Figure 2). The total amount of drug excreted in the first 24 h was estimated using the concentrations of ATX in the 24-h post-administration patches and the ratio between the area of the patch and total body surface area of each subject (Figure 3). Total ATX excreted in sweat after the administration of different drug doses of ATX ranged between 0.01 and 0.12 mg, which is equivalent to about 0.02 and 0.41% of the administered doses.

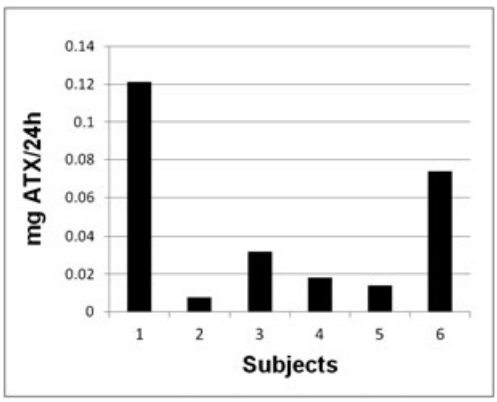

Figure 3. Total amount of ATX excreted in sweat during 24 h post administration for six subjects.

Generally speaking, no correlation was found between administered ATX dose and ATX excreted in sweat (Figure 4), and no correlation was found between concentrations of ATX in sweat patches and those reported in the previous study for plasma at the same time intervals (Figure 5).[12]

Neither of the two principal ATX metabolites (4-OH-ATX- and N-*des*-ATX) was detected in any of the collected patches.

Discussion

The present results show for the first time that ATX is excreted in sweat in the first 24 h after administration of different oral doses (18–60 mg/day). Drugs are generally incorporated into sweat by passive diffusion because of a concentration gradient in which only the free fraction of drug (unbound to proteins) diffuses through lipid membranes from plasma to sweat.[7] ATX is a basic drug with a pKa around 9.2, and because in normal conditions, sweat is more acidic than blood (mean pH value 6.3), ATX is converted to its ionized form and should tend to accumulate in sweat.[7] In the case of ATX, the theoretical sweat to plasma ATX ratio, calculated by a modification of the Henderson–Hasselbalch equation[7] (Concentration in sweat/Concentration in plasma = 1+10(pKa-pHsweat)/1 +10(pKa-pHplasma) is around 12, indicating a significant accumulation of drug in sweat. Unfortunately, this hypothesis could not be verified in our study because the sweat patch, which loses water content during wear, did not allow the calculation of volume of sweat collected or accurate sweat pH. A great inter-individual variability was observed even within the individuals administered with the same daily dosage.

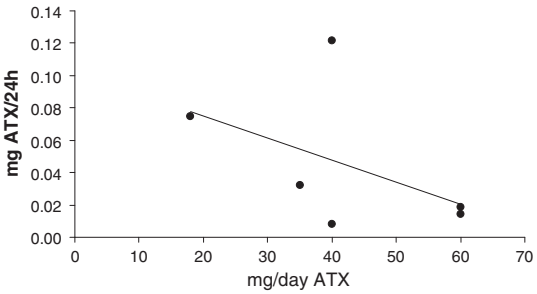

Figure 4. Correlation between ATX administered dose and ATX excreted in sweat during 24 h post administration.

However, it should be noted that the two subjects (3 and 6) with the greatest ATX excretion in sweat also showed the highest ATX concentrations in plasma and oral fluid after being administered with 40 and 18 mg ATX, respectively.[12] The reasons for these higher concentrations in the three biological matrices are different.

Subject 3 was the unique child (7 years old and 40 mg/day or 1.38 mg/kg/day ATX) of the study and presented of the highest values ATX concentrations in sweat patch such as in plasma and oral fluid not only with respect to the other two adolescents

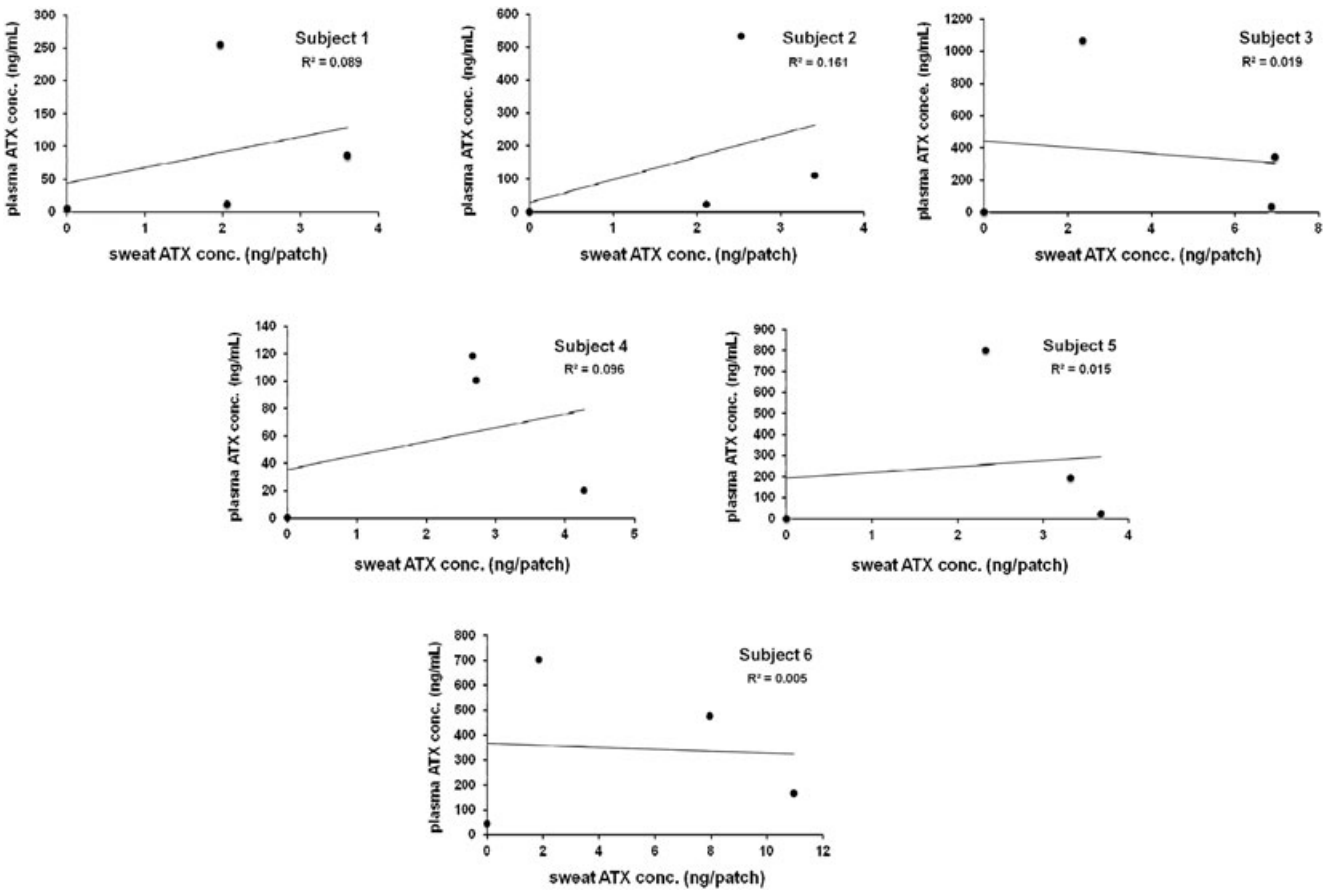

Figure 5. Correlation between sweat and plasma concentrations of ATX in subjects 1–a3 treated with 40 mg/day ATX, in subjects 4 and 5 treated with 60 mg/day ATX and in subject 6 treated with 18 mg/day ATX.

(subjects 1 and 2) treated with the same total drug dosage day but lower mg/kg dose, but also with respect to the two other adolescents (subjects 4 and 5) treated with higher daily dosages and higher mg/kg dose.

In the case of subject 6, treated with the lowest ATX dose (18 mg/day) and co-treated with risperidone (1 mg/day) and sertraline (75 mg/day), two psychotropic drugs which are both major substrates and inhibitors of CYP2D6 isoenzyme and thus of ATX metabolism, the increasing ATX concentration in sweat patches likely reflected the considerably high ATX plasma and oral fluid concentrations.

Indeed, inter-individual variations of sweat concentrations observed also in the other study subjects may be due not only to different perspiration rates and volumes of sweat but also to variability in plasma drug concentrations, since drugs getting into sweat have to first get into the systemic circulation. As previously shown, fluctuations in ATX plasma concentration of these study subjects may be also explained by inter-individual differences in metabolism including those due to genetic polymorphism of CYP2D6 (poor versus extensive metabolizer) or by the use of concomitant psychotropic medications metabolized by the same isoenzymes.[20] Furthermore, it has to be kept in mind that ATX is extensively plasma protein bound (98%) and has a low volume of distribution (Vd): 0.85 L/kg suggesting little tissue sequestration[21] and this is ascertained in the relatively low sweat concentrations measured in the majority of sweat patches collected from the study subjects.

Although this is a preliminary study involving a few subjects, the obtained results are clear evidence that even a single administration of ATX formulation can be detected in sweat only as the parent drug in a concentration range of a few nanograms per sweat patch. This is in accordance with the findings we have already reported for amphetamine-type stimulants such as MDMA and methylphenidate.[10,13] ATX tends to accumulate in sweat in the first 24 h after administration with a certain inter-subject variability in the amount of ATX excreted. This information was used to calculate total amount of ATX excreted in sweat. This calculation was most probably an overestimation of the real figures as perspiration is not homogeneous in the whole body surface but is a first approach for estimating the amount of drug excreted through this biological fluid. Estimations suggest that a non-negligible fraction of the dose is excreted through sweat, providing a sensitive basis for sweat testing of ATX exposure.

In spite of high inter-subjective variability in the amount of excreted ATX, sweat can be used for non-invasive semi-quantitative monitoring of ATX use.

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