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A B S T R A C T

A male driver was checked during a traffic stop. A blood sample was collected 35 min later and contained 7.3 ng/mL THC, 3.5 ng/mL 11-hydroxy-THC and 44.6 ng/mL 11-nor-9-carboxy-THC. The subject claimed to have used two commercially produced products topically that contained 1.7 ng and 102 ng THC per mg, respectively. In an experiment, three volunteers (25, 26 and 34 years) applied both types of salves over a period of 3 days every 2–4 h. The application was extensive (50–100 cm²). Each volunteer applied the products to different parts of the body (neck, arm/leg and trunk, respectively). After the first application blood and urine samples of the participants were taken every 2–4 h until 15 h after the last application (overall n = 10 urine and n = 10 blood samples, respectively, for each participant). All of these blood and urine samples were tested negative for THC, 11-hydroxy-THC and 11-nor-9-carboxy-THC by a GC–MS method (LoD (THC) = 0.40 ng/mL; LoD (11-hydroxy-THC) = 0.28 ng/mL; LoD (THC-COOH) = 1.6 ng/mL; LoD (THC-COOH in urine) = 1.2 ng/mL). According to our studies and further literature research on in vitro testing of transdermal uptake of THC, the exclusive application of these two topically applied products did not produce cannabinoid findings in blood or urine.

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1. Introduction

There exists only little information about the transcutaneous absorption of tetrahydrocannabinol (THC) by the application of hemp containing creams. Hemp oil is used in diverse personal hygiene products like in shampoos, body lotions, creams, salves, massage oil, lip balm. Vendors claim that hemp oil has preventive and therapeutic benefits for the skin. The content of poly-unsaturated fatty acids may alleviate dry-skin defects, improve the smoothness of dry and scaly skin and slow down skin aging [1–3].

Hemp seeds being used for the production of hemp oil themselves do not contain THC. However, the precursor of THC, tetrahydrocannabinolic acid A, and THC itself are produced in resin glands whose excretions adhere to the seed hulls [4,5]. Therefore, traces of cannabinoids can be found on processed hemp seeds and in oil pressed from them depending on the cleaning process. Typical THC levels in hulled seeds and oil are 2 and 5 μg/g [5]. The oral consumption of hemp food can therefore cause positive cannabinoid findings. However, these THC concentrations would be too low to cause any psychoactive effects assuming an application of normal doses of oil. The range of hemp oil content in cosmetics is mostly low (<10% (w/w)), however, there are also hemp oil contents of up to 75% in lip balm and bar soap sold in Northern America [6]. Only one direct measurement of THC content in final products has been conducted: Health Canada [6] found 1–5 μg/g THC in a product.

This report describes a case of driving under the influence of drugs, in which the delinquent claimed that the positive cannabinoid findings in his blood were due to topical absorption of THC following application of a hemp containing cream. An excretion experiment was conducted to evaluate if this scenario was possible.

2. Case report

A male driver (age 43, 83 kg) was behaving conspicuously at a traffic stop. The police officers documented; walk unsafe; deficiency in concentration and orientation. The officers had conducted a speed control. The accused had driven with 117 km/h instead of 80 km/h being allowed. The accused claimed he had not
consumed any cannabis products by inhalation or orally. However, he had frequently used a hemp oil containing cream. He had used this product because of bruises at different body parts (back, arms, legs, neck) some time including the 3 days before the incident.

The two products were 1. The salve ‘Extra konpná mast 100’ (Sativa Medical, Czech Republic) and 2. A self-made production from a ‘farmer’s market’ in the Czech Republic. Fig. 1 shows pictures of both products.

A blood sample taken 35 min after the control and was brought to the laboratory. The plasma sample was determined to contain 7.3 ng/mL THC, 3.5 ng/mL 11-hydroxy-THC and 44.6 ng/mL 11-nor-9-carboxy-THC (THC-COOH). The appearance of 11-hydroxy-THC and the high concentration of THC (compared to the concentration of the metabolite THC-COOH) favored an active and contemporary cannabis consumption in the hours before blood sampling.

3. Material and methods

3.1. Material

In this experiment, three volunteers (25, 26 and 34 years) put on both salves on their skin over a period of 3 days every 2–4 h. The application was extensive (50–100 cm²), less than 1 g per application. Each volunteer put the products on different parts of the body (neck, arm/leg and trunk, respectively). Prior to the experiment, a blood sample and a urine sample of each participant confirmed negative for all cannabinoids. After the first application blood and urine samples of the participants were taken every 2–4 h until 15 h after the last application (both n = 10 urine and blood samples, respectively). One volunteer additionally applied the products on irritated skin.

3.2. Methods

3.2.1. Sample preparation

20 mg of each product was extracted twice with 2 mL of methanol/water (80:20, v/v) after addition of 100 µL of the internal standard solution containing 100 ng/mL THC-d₃. Extracts were derivatized and analyzed as follows. Two calibration curves were established. For Vaseline calibration curve 1 and the quantification of the lower concentrated product (product 1), 20 mg of blank Vaseline was spiked with 5 absolute amounts of 0.02–0.5 µg to reach final concentrations ranging between 1 and 25 ng THC/mg.

For Vaseline calibration curve 2 and the quantification of the higher concentrated product (product 1), 20 mg of blank Vaseline was spiked with 5 absolute amounts of 0.5–5 µg to reach final concentrations ranging between 25 and 250 ng THC/mg. Both calibration curves showed satisfactory linearity (R² > 0.99). Each calibration point showed accuracy >90%.

Blood was centrifuged as soon as possible after the collection from the subject. 1 mL of serum was spiked with 100 µL of the internal standard solution containing 100 ng/mL THC-d₃, 100 ng/mL 11-hydroxy-THC-d₃, and 200 ng/mL THC-COOH-d₉ (from Sigma, St. Louis, Missouri) and with 4 mL of a mixture of n-hexane and ethyl acetate (90:10, v/v). Afterwards samples were extracted by vortexing for 1 min. Following centrifugation, the organic phase was transferred into another test tube and evaporated to dryness under a nitrogen stream at 60–80 °C. The residue was redissolved in a mixture of 50 µL MSTFA, 20 µL pyridine and 130 µL iso-octane. Derivatization took place for 30 min at 90 °C.

1 mL of urine was spiked with 100 µL of the internal standard solution containing 200 ng/mL THC-COOH-d₉ (from Sigma, St. Louis, Missouri) and with 200 µL of 1N NaOH to hydrolyze the glucuronide conjugate for 15 min at 55 °C. Afterwards pH was adjusted to 4.0 by addition of HCl (0.1 M). The samples were extracted with 4 mL of a mixture of n-hexane and ethyl acetate (90:10, v/v) by vortexing for 1 min. Following centrifugation, the organic phase was transferred into another test tube and evaporated to dryness in a nitrogen stream at 60–80°C. The residue was redissolved in a mixture of 50 µL MSTFA, 20 µL pyridine and 130 µL iso-octane. Derivatization took place for 30 min at 90 °C.

With each batch, blank plasma and urine samples were analyzed. Furthermore, quality control samples in plasma and

![Fig. 1. (a–d) Pictures of product 1 (a–c) and 2.](image-url)
urine in the concentrations 1.6 ng/mL and 16 ng/mL for THC and 11-hydroxy-THC and 16 ng/mL and 160 ng/mL for THC-COOH were measured.

3.2.2. Gas chromatographic and mass spectrometric parameters

Cannabinoids (THC, 11-hydroxy-THC and THC-COOH) were determined by a gas chromatographic mass spectrometric method with the following specifications: The analytical column was a HP-5MS Ultra Inert column (length: 30 m; 0.25 mm ID; particle size: 0.25 µm; Agilent Technologies, Santa Clara, CA, USA). Temperature program was as follows: initially 1 min at 160 °C; with 15 °C/min to 250 °C; hold at 250 °C for 3 min; with 13/° C/min to 300 °C; hold at 300 °C for 5 min. Total runtime was 18 min and 50 s. Detector temperature was 250 °C, injector temperature was 260 °C. Helium was used as carrier gas with a flow rate of 1 mL/min. 2 µL were injected splitless. Substances were detected in SIM (single ion monitoring) mode with the following target and qualifier masses of the trimethylsilyl (TMS)-derivatives: THC-TMS: m/z 371 (target), 386 and 303. THC-d5-TMS: 374 (target), 389, 306. 11-hydroxy-THC-TMS: 371 (target), 459, 474, 11-hydroxy-THC-d5-TMS: 374 (target), 462, 477. THC-COOH-d1-TMS: 371 (target), 473, 488. THC-COOH-d5-d1-TMS: 380 (target), 479, 497.

The method was validated according to forensic guidelines [7]. Validation parameters were as follows: Selectivity was given in Vaseline, plasma and urine (n = 6 blank matrices). Analytical limits for plasma were LoD (THC) = 0.40 ng/mL; LoD (11-hydroxy-THC) = 0.28 ng/mL; LoD (THC-COOH) = 1.6 ng/mL. LoD for THC-COOH in urine was 1.2 ng/mL. Analytical limits were determined after DIN 32646 measuring increasing concentrations of THC (0.4–1.0 ng/mL), 11-hydroxy-THC (0.4–1.0 ng/mL) in plasma and THC-COOH (1.0–5.0 ng/mL) in plasma and urine. Calibration range was 0.5–25 ng/mL for THC and 11-hydroxy-THC and 2.5–200 ng/mL for THC-COOH. No weighing of the linear calibration model was necessary.

Accuracy and precision data was taken by analyzing quality control samples in low (1.6 ng/mL for THC and 11-hydroxy-THC and 16 ng/mL for THC-COOH) and high (16 ng/mL for THC and 11-hydroxy-THC and 160 ng/mL for THC-COOH) concentrations in duplicates at 8 consecutive days. Bias was 9.2% and -11.7%, respectively for THC; -4.4% and -8.5%, respectively, for 11-hydroxy-THC and -9.4% and 5.0%, respectively for THC-COOH. Intraday precision was 4.4% and 1.6%, respectively, for THC; 1.8% and 1.9%, respectively, for 11-hydroxy-THC and 3.6% and 1.8%, respectively for THC-COOH. Inter day precision was 5.8% and 6.1%, respectively, for THC; 4.6% and 4.6%, respectively, for 1-hydroxy-THC and 5.0% and 6.0%, respectively, for THC-COOH.

Recovery was >90% for THC and 11-hydroxy-THC at the two concentrations of the quality controls. Recovery for THC-COOH in plasma was 48% at 16 ng/mL and 52% at 160 ng/mL, respectively. Recovery of THC-COOH in urine was 47% at 16 ng/mL and 48% at 160 ng/mL, respectively. Stability (decrease of peak areas ≤10%) in the autosampler was given over a period of 24 h. Carryover was eliminated by analyzing the highest calibrator and showing no signal in the following blank sample. Ion ratios of the substances were monitored. Acceptable ranges for qualifier/target ion ratios for THC were 45.8–68.8% for qualifier m/z 303 and 62.1–93.1% for qualifier m/z 386. Acceptable ranges for qualifier/target ion ratios for 11-hydroxy-THC were 2.8–4.2% for qualifier m/z 459 and 2.3–3.5% for qualifier m/z 474. Acceptable ranges for qualifier/target ion ratios for THC-COOH were 32.0–48.0% for qualifier m/z 473 and 18.6–28.0% for qualifier m/z 488.

4. Results

In both salve products THC could be detected at a concentration of 1.7 ng/mL (product 1, see chromatogram in Fig. 2) and 102 ng/mg (product 2).

5. Discussion

Substances penetrate into the outer layer of the skin and permeate from one layer to another. Substances are transferred through the main barrier of the skin, the Stratum corneum, by passive diffusion, into the underlying viable layers of the epidermis. Finally, substances are absorbed through the vascular system into the blood stream [8]. Uptake of a substance is proportional to the compound’s concentration gradient between the formulation and the basal layer of the stratum corneum. The extent of the uptake varies widely with the physicochemical properties of the xenobiotic, the formulation and application of the product, and with skin conditions. The most important characteristic of a substance is the inclination to distribute between aqueous and lipid compartments in tissues which can be scaled by the octanol/water coefficient (Kow). In general, the higher the Kow and lipophilicity of a substance and the smaller the molecular weight of a substance, the faster the penetration of the stratum corneum is. The lipid matrix is not only the major pathway for lipophilic compounds, it also functions as a reservoir. To reach the systemic circulation, the compound needs to permeate the aqueous epidermis underneath. Uptake through hair follicles or sebaceous and perspiratory glands may also be a route of absorption for THC [9]. Bast [10] has shown that substances with Kow >2000 show lower permeation coefficients into the aqueous layer. For THC, Kow values ranging between 6000 and 63,000,000 have been described [11,12]. Therefore, in general THC with its high Kow and high molecular weight should present a substance with a low transdermal uptake factor as many topically applied cosmetic ingredients do [11]. In fact, there should be built up a THC reservoir in stratum corneum.

Depending on formulation and skin condition, Touitou et al. [9] demonstrated that in vitro that between application of a product and the detection of THC in blood there should be a time delay of approximately 8 h. In this study volunteers were evaluated up to 15 h after the last application which took place 3 days after the first application. Penetration enhancing compounds like dimethylsulfoxide or Tween can increase absorption rates by a maximum of the 10-fold to 20-fold [8]. For a given compound and formulation transfer rates also vary with anatomical location and skin conditions. Skin of the arms is 3–4 fold thicker than facial skin [8]. In this study the subjects applied the products to the same body locations the driver claimed having used. Furthermore, one volunteer additionally applied the cream on irritated skin because studies have shown that the strongest skin-related enhancement of transdermal uptake may be caused by compromised skin, e.g., chapped, irritated, sunburned or injured skin. Such conditions may cause an up to ten-fold increased permeability [8].
Two studies [9,13] could be found in the literature on transdermal delivery of THC. One study demonstrated the positive effect of enhancers like DMSO or oleic acid on transdermal uptake of THC. The other study was criticized by several researchers including Hadgraft who estimated the transdermal uptake factor for THC with 3–4% [14]. Based on these studies Pless and Leson [6] used a transdermal bioavailability factor of 5% within 24 h. Based on this assumption they calculated a mean THC intake of less than 1 µg per day when using commercially available hemp oil products. The worst case scenario, the full body being in contact with 100% hemp oil, would lead to a higher uptake factor (16.25%) and a maximum intake of 11 µg THC per day.

However, THC contents of the two products used in this study were low compared to the assumed contents of products in the study of Pless and Leson. Product 1 had 1.7 ng THC per mg and product 2 had 102 ng/mg. Assuming an applied amount of 1 g for each application, 102 µg of THC would have been applied on the skin by each application of the higher concentrated product. Assuming a maximum of 5 applications per day and a bioavailability factor of 5% used by Pless and Leson, a maximum of 51 µg per application and 25.5 µg per day would have reached the blood circulation. The accused in this case had allegedly used the creams from time to time including the 3 days before the incident so our scenario of an application five times daily would have exceeded the application frequency of the driver. The body weight of the accused did not differ from the body weights of the test persons. It should be kept in mind that THC is a photo labile and oxidizable substance [9]. Thus, some degradation of topically applied THC will occur immediately after application. A large part of the salve can be absorbed by clothes or washed away by showering or bathing especially by using surfactants containing cosmetics. Therefore, even after 3 days of regular application of the two products there was neither a positive blood nor a positive urine result on THC or on its metabolites. An exclusive application of these two products was therefore not able to produce the measured concentrations of cannabinoids in blood. Application on irritated skin did not lead to positive results neither.

6. Conclusion

It is very unlikely that the exclusive (frequent) application of THC containing cosmetic products resulted in the subject’s positive drug test for cannabinoids in blood or in urine.

References