



# Inhalation and oral administration of HHC products – Quantification of (9R)-, (9S)-Hexahydrocannabinol and metabolites in plasma and detectability in on-site drug tests for urine and oral fluid

L. Lucuta<sup>a,\*</sup>, L. Schwarz<sup>a</sup>, J. Liut<sup>b</sup>, J. Hose<sup>a</sup>, L. Nauroth<sup>a</sup>, M. Juebner<sup>a,3</sup>, H. Andresen-Streichert<sup>a,3</sup>

<sup>a</sup> Department of Toxicology, Institute of Legal Medicine, University of Cologne, Faculty of Medicine and University Hospital, Cologne, Germany

<sup>b</sup> Institute of Forensic Medicine, Forensic Toxicology, University Hospital Bonn, Bonn, Germany

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## ABSTRACT

The semisynthetic cannabinoid Hexahydrocannabinol (HHC) has gained recognition among drug users. A GC-MS/MS method for the detection of (9R)- and (9S)-HHC and their respective carboxy- and hydroxy-metabolites in plasma has been developed and validated. The method was applied to authentic plasma samples obtained from a self-administration experiment. HHC was either inhaled (Vapes, 95 % HHC) or ingested (Jellies, 25 mg HHC). Maximum plasma (9R)-HHC and (9S)-HHC concentrations of 3.8 ng/mL and 2.5 ng/mL were detected 1.16 h after ingestion and approx. 65 ng/mL (9R)-HHC and 21 ng/mL (9S)-HHC were measured 0.08 h after inhalation. (9R)-OH-HHC concentrations ranged from approx. 0.3–1.4 ng/mL after ingestion, and approx. 0.2–1.8 ng/mL after inhalation. (9R)-COOH-HHC was detectable in concentrations of 0.8–17 ng/mL (ingestion) and 0.6–8.7 ng/mL (inhalation). Corresponding S-Hydroxy- and Carboxy-metabolites were detectable after ingestion ((9S)-OH-HHC: approx. 0.1–0.7 ng/mL, (9S)-COOH-HHC: approx. 0.2–0.4 ng/mL), but mainly not after inhalation. Cannabimimetic effects and respective psychomotor impairments such as (slight) vertigo as well as slight headache and dizziness, and mouth dryness could be observed after ingestion. After inhalation, one participant showed distinct impairments. On-site drug tests for cannabinoids in urine (DrugScreen®) and oral fluid (DrugWipe® 5S) were performed. DrugScreen® (cut-off: 25 ng/mL THC-COOH) gave positive results up to 10 h after ingestion and inhalation. Cross-reactivity with DrugWipe® 5S (cut-off: 5 ng/mL THC) was not observed. An immunological CEDIA™ cannabinoid assay showed good cross-reactivity with the plasma samples and gave positive results up to 6.16 h after ingestion and 4.16 h after inhalation.

## 1. Introduction

Hexahydrocannabinol (HHC) is a semisynthetic cannabinoid, which is synthesized from cannabidiol (CBD) and was firstly discovered in 1940 [1]. It has emerged as recreational drug on the US market in 2021 and has now entered the European drug market as well. The European Union Drug Agency (EUDA) posed the hexahydrocannabinol under strict control due to increased HHC-containing product seizures in the European territory. Since some HHC products are available in forms that may deliver high doses, the availability of HHC may cause implications for

public health [2]. After Austria and Switzerland lawfully restricted HHC in 2023 and at least 18 member states of the European Union listed HHC as controlled drug until March 2024, Germany included HHC in the new psychoactive substance regulations (NpSG) in June 2024.

Since HHC is structurally similar to  $\Delta^9$ -Tetrahydrocannabinol (THC) and it also binds on cannabinoid receptors, cannabimimetic drug effects are assumed for HHC. This assumption is supported by several in vivo studies [3–5] in animals as well as in vitro studies [6,7]. In most of the studies, both diastereomers (9R)-HHC and (9S)-HHC were examined, whereby a higher potency of (9R)-HHC [8,9] was assumed. Based on

\* Corresponding author.

E-mail address: [lina.lucuta@uk-koeln.de](mailto:lina.lucuta@uk-koeln.de) (L. Lucuta).

<sup>1</sup> <https://orcid.org/0000-0002-9084-9635>.

<sup>2</sup> Permanent address: Melatenguertel 60/62, 50823 Cologne, Germany.

<sup>3</sup> H. Andresen-Streichert and M. Juebner contributed equally to the manuscript.

these findings, an effect of HHC on the fitness to drive is conceivable.

Some *in vitro* studies elucidate the metabolism of HHC using human hepatic microsomal preparations or microsomal preparations obtained from different animal species [7,10,11]. *In vivo* studies regarding the metabolism of HHC in humans and corresponding effects with a greater number of participants are not published so far, only few self-administration studies were published [12–14]. Schirmer et al. [12] performed a self-administration study with two volunteers. Besides both HHC epimers, the study group detected both epimers of 11-hydroxy-hexahydrocannabinol (9R-OH-HHC and 9S-OH-HHC) in urine in relevant amounts, whereby (9R)-11-nor-9-carboxy-HHC (9R-COOH-HHC) and (9S)-11-nor-9-carboxy-HHC (9S-COOH-HHC) were identified only as minor metabolites. Mild cannabimimetic effects were described by one of the volunteers, which lasted about two hours. Serious impairments were not observed. Other studies rather focused on the pharmacokinetics in humans than on drug effects [13–15].

Ferretti et al. [16] asked HHC users to self-report effects of HHC consumption. The reported effects were mostly perceived as positive, such as euphoria and relaxation, or HHC was used by the participants because of anxiety or for pain-relief. Nevertheless, adverse effects were reported as well, including mouth dryness, sleepiness, red eyes as well as withdrawal symptoms such as sleep difficulties and depressed mood.

Another working group examined 321 cases of driving under the influence of drugs (DUID) [17]. The authors detected HHC in 17 cases, assuming a relatively low abundance among the cases. THC was concomitantly detected in 16 among those 17 cases, mostly in higher concentrations than HHC. Cross-reactivity of HHC with different on-site drug tests for THC, which are commonly used by the police during e.g. roadside traffic controls, was also investigated. The cross-reactivity for oral fluid and urine tests was rather poor.

To assess possible psychomotor effects of HHC and contribute to data on HHC and metabolite concentrations in plasma, a self-administration study of HHC products was performed. The participants were tested for their fitness to drive by psychomotor tests. Cross-reactivity of HHC with oral fluid and urine drug tests for cannabinoids (DrugWipe® 5S and DrugScreen®) and analyzer based immunological assay (CEDIA™) was investigated as well. For analyses, a quantitative gas chromatography tandem mass spectrometry (GC-MS/MS) method for the detection of (9R)-HHC and (9S)-HHC and their respective metabolites was developed and validated according to the guidelines of the Society of Forensic and Toxicological Chemistry (GTFCh) [18,19].

## 2. Materials and methods

### 2.1. Materials

d3-(±)-Δ9-Tetrahydrocannabinol (THC-d3), d3-(±)-11-Hydroxy-Δ9-Tetrahydrocannabinol (THC-OH-d3) and d3-(±)-11-nor-Carboxy-Δ9-Tetrahydrocannabinol (THC-COOH-d3) deuterated standards were purchased from LGC Promochem (Wesel, Germany); N-Methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) was obtained from Macherey-Nagel GmbH & Co. KG (Dueren, Germany); acetic acid (p.A., Rotipuran®) and methanol HPLC gradient grade (Rotisolv®) were purchased from Carl Roth GmbH (Karlsruhe, Germany); acetonitrile was obtained from VWR International (Darmstadt, Germany); ethylenediaminetetraacetic acid was obtained from Merck KGaA (Darmstadt, Germany). CEDIA™ reagent for Cannabinoids and DRI® Creatinine-Detect® test was purchased from Thermo Fisher Diagnostics GmbH (Henningsdorf, Germany). Analytical standards of (9R)-HHC, (9S)-HHC, (9R)-OH-HHC, (9S)-OH-HHC, (9R)-COOH-HHC and (9S)-COOH-HHC were purchased from Cayman Chemical (Ann Arbor, USA). HHC products were purchased in online shops (before lawfully restricted).

### 2.2. Self-administration study

Two male and two female persons (four of the authors of this

publication) participated in a self-administration study. Female participants were 28 (P1) and 31 (P3) years old, male participants 26 (P2) and 56 (P4) years. Body-mass indices (BMI) were calculated as 21,6 kg/m<sup>2</sup> (P1), 22,5 kg/m<sup>2</sup> (P2), 22,0 kg/m<sup>2</sup> (P3) and 26,5 kg/m<sup>2</sup> (P4). For blood withdrawal, indwelling cannulas were set at the beginning of the experiment. Two persons (group 1, m/f) ingested one HHC jelly each (Spacejellys, Premium HHC infused fruit jellies, annotation: 25 mg HHC/piece, Fa. HGH Medical Group GmbH), two persons (group 2, m/f) inhaled HHC from a vape pen (HHC Vapes, Lemon OG, 1 mL, annotation: 95 % HHC, Fa. Deutsche Cannabis Manufaktur). Application scheme and sample collection is shown in Fig. 1. In short, zero samples of blood and urine were collected, followed by the uptake of HHC. Group 1 ingested one HHC jelly each, group 2 took three puffs in total, whereby blood samples were withdrawn in-between. Blood samples of group 1 and 2 were collected at the same time intervals. The first blood sample was taken 5 min after the self-administration of the jelly and the first puff of the vapes, followed by two blood samples every 10 min thereafter (5 min after the 2 subsequent puffs of group 2). More blood samples were collected every 15 min for two hours and every 30 min for another 4 h, resulting in 20 blood samples including the blank sample per participant. Urine samples were continuously collected in a 1-h time interval for ten consecutive hours starting with the first sample 1 h after the self-administration. Further urine samples were collected 24, 30, 36 and 48 h after substance uptake. Hence, a total number of 13 urine samples, including blank sample, were collected per participant. Whole blood was centrifuged at 1248 × g for 10 min and plasma was separated from the red blood cells or blood clot immediately. Plasma samples and urine samples were stored at – 20 °C until analyses.

### 2.3. DrugWipe® 5 S and DrugScreen®

Drug tests for oral fluid (DrugWipe® 5S, cut-off: THC 5 ng/mL) were obtained from Securetec Detektions-Systeme AG (Neubiberg, Germany) and drug test stripes for urine (DrugScreen®, various cut-offs: THC-COOH 25 ng/mL, 150 ng/mL and 300 ng/mL) from nal von Minden GmbH (Regensburg, Germany). Oral fluid tests were applied simultaneously to blood collection samples (T1–T4, equaling sampling times from 0.08 to 0.66 h). Urine tests were applied to samples T1, T2, T4, T8, T10 (equaling sampling times 1, 2, 4, 8 and 10 h after inhalation/ingestion). Application scheme is shown in Fig. 2.

### 2.4. Evaluation of psychomotor impairments

The participants underwent testing for psychomotor impairments 0.5 and 1.5 h by medical staff after the consumption. Tests included pupil reaction test, walk and turn test, finger-finger-test and finger-nose-test according to standardized field sobriety tests (SFT tests) used by police officers during roadside control and medical staff during blood withdrawal [20–22]. Moreover time estimation has been evaluated, whereby the participants should estimate a time interval of 30 s (modified Romberg test) [23]. Other conspicuities and psychophysical conditions were documented.

### 2.5. Analyzer-based immunological testing and creatinine measurements

Semi-quantitative immunological measurements were conducted on an AU 480 Analyzer (Beckman Coulter) by CEDIA™ reagent for Cannabinoids.

Therefore, 400 µL plasma were transferred into a reagent glass and mixed with 400 µL 0.1 mol/L EDTA solution (29,25 g ethylenediaminetetraacetic acid, 900 mL water, adjusted to pH 7 by sodium hydroxide). Urine samples were not diluted. The analyses were performed according to the manufacturer's instructions. Cut-offs were 11 ng/mL for serum/plasma and 20 ng/mL for urine.

Quantitative creatinine measurements were also conducted on an AU 480 Analyzer by a DRI® Creatinine-Detect® test. The method is based

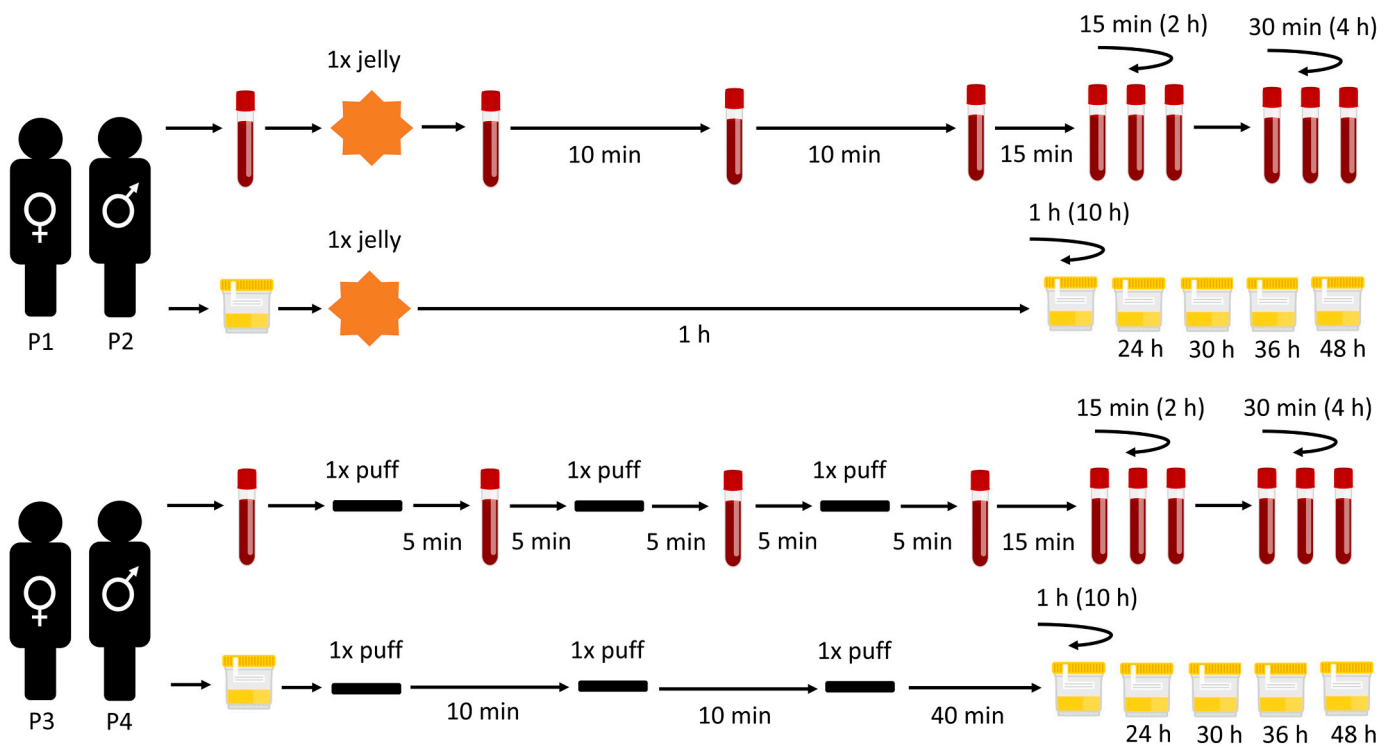


Fig. 1. Application scheme of HHC products (HHC jelly or puffs from vape pen) to participants and sample collection procedure.

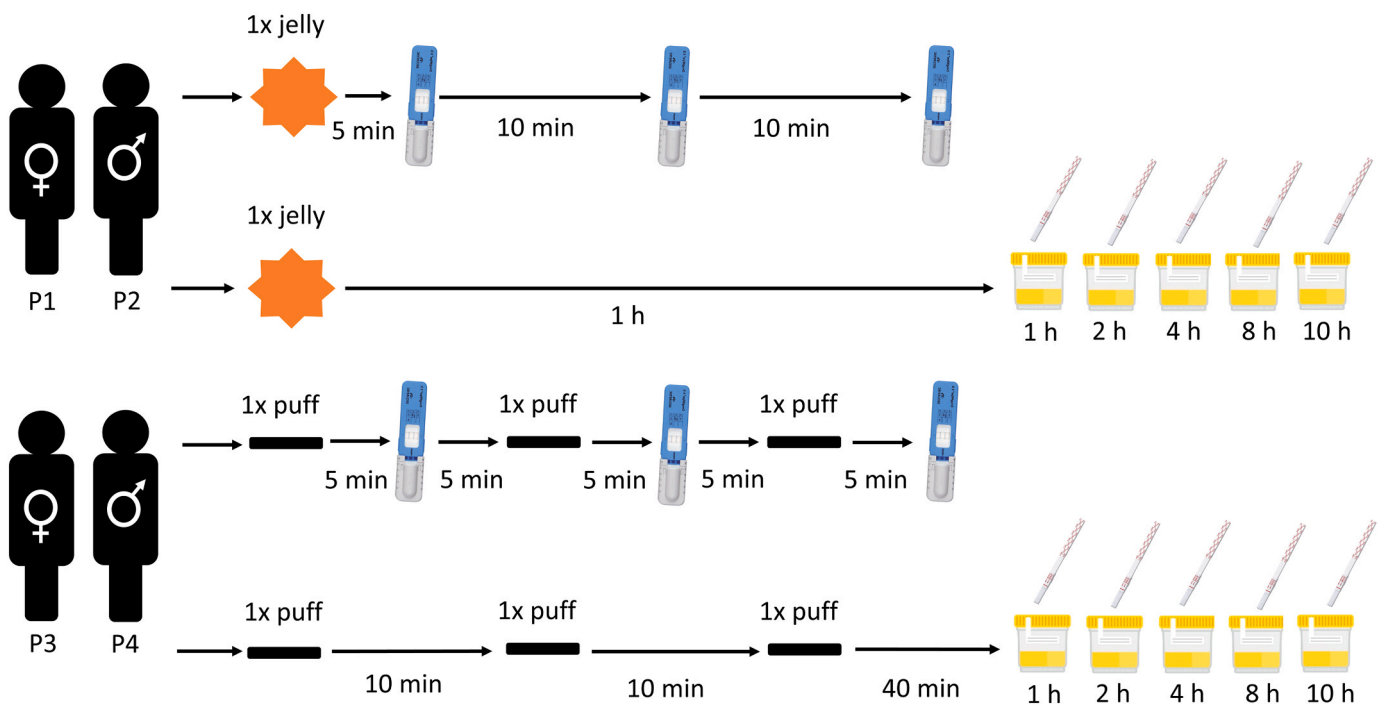


Fig. 2. Application scheme of HHC products (HHC jelly or puffs from vape pen) to participants and application time points of DrugScreen® and DrugWipe® 5S tests.

on the Jaffe-reaction, whereby creatinine is determined color metrically via alkali-picrate which forms the red Janovsky-complex. The immunological methods are validated for serum/plasma and/or urine for standard routine analysis in the laboratory according to the guidelines of the GTFCh [19].

## 2.6. Calibrators and quality control samples

All samples were prepared with 250  $\mu$ L plasma. Blank plasma was provided by six different volunteers. THC-d3, THC-OH-d3 and THC-COOH-d3 were used as internal standard. 3 ng/mL of THC-d3/THC-OH-d3 and 10 ng/mL of THC-COOH-d3 were spiked in each sample. For calibrator and quality control (QC) samples 20  $\mu$ L of the respective standard solution were added. Calibration ranged from 0.3 to 20 ng/mL

for (9R)-HHC and (9R)-HHC-COOH and from 0.5 to 20 ng/mL for (9S)-HHC, (9S)-HHC-OH, (9R)-HHC-OH and (9S)-HHC-COOH, using seven calibrators for each analyte. Quality control samples were prepared at levels of 0.8 and 16 ng/mL.

## 2.7. Automated solid phase extraction (SPE) and GC-MS/MS

Automated solid phase extraction (SPE) was performed via the GERSTEL-MPS 2 system (GERSTEL GmbH & Co. KG, Muehlheim an der Ruhr, Germany) connected to a GC-MS/MS. Cartridges (Chromabond C18ec 1 mL/100 mg, Macherey-Nagel GmbH & Co. KG, Dueren, Germany) were conditioned with 2 mL methanol, 2 mL ultra-pure water and 2 mL acetic acid (0.1 mol/L). 250  $\mu$ L acetic acid (10 %) were added to the sample and mixed by the syringe, followed by loading on the cartridge. Washing was performed by adding 2 mL acetic acid (0.1 mol/L) and 2 mL acetonitrile/water (40:60, v/v) mixture. Afterwards, samples were dried under nitrogen (4 min), eluted by adding 200  $\mu$ L acetonitrile twice. The extract was narrowed under shaking (70 °C; 6.5 min at 8 kPa), subsequently derivatized in 30  $\mu$ L MSTFA to the corresponding TMS derivatives and shaken for 1 min (40 °C, 69 g) before injection. The injection volume was 1  $\mu$ L using splitless mode.

Measurements were performed on a 7890B GC system equipped with a 7000B Triple Quadrupole MS (Agilent Technologies, Santa Clara, USA). Chromatographic separation was performed on a Zebron ZB-5MSi column (2  $\times$  15 m, I.D. 0.25 mm  $\times$  0.25  $\mu$ m, Phenomenex, Torrance, USA). Pulsed-splitless injection was performed (6.8 kPa until 0.5 min, total splitless time 1.0 min, 250 °C injection temperature). Helium was used as carrier gas (1.2 mL/min). Column temperature program was set as 100 °C (1 min), 25 °C/min to 310 °C; postrun 310 °C (midpoint backflush, 2 min). For mass spectrometry, conditions were as follows: electron ionization was used; nitrogen as collision gas (1.5 mL/min collision flow) with helium quench flow (2.25 mL/min), 290 °C MS-transferline, source temperature 230 °C, both quadrupoles 150 °C. Dwell time was 30 ms for each analyte (Table 1).

## 2.8. Method validation

The method was validated according to the guidelines of the GTFCh [19]. Statistical data were evaluated using Valistat® software (Arvecon GmbH, Walldorf, Germany).

For linearity, six separately prepared calibration series underwent

**Table 1**

MRM method with the relevant collision energy, resolution, mass transitions and retention times.

	Collision energy [eV]	MS1/MS2 resolution	Target [m/z] Qualifier [m/z]	Retention time [min]
(9R)-HHC-TMS	15	Unit	388.2 $\rightarrow$ 303.2	8.97
	15		345.2 $\rightarrow$ 265.0	
(9S)-HHC-TMS	15	Unit	388.2 $\rightarrow$ 303.2	9.19
	15		345.2 $\rightarrow$ 265.0	
(9R)-HHC-OH-TMS	15	Unit	476.5 $\rightarrow$ 420.2	9.99
	15		476.5 $\rightarrow$ 343.2	
(9S)-HHC-OH-TMS	15	Unit	476.5 $\rightarrow$ 420.2	10.09
	15		476.5 $\rightarrow$ 343.2	
(9R)-HHC-COOH-TMS	15	Unit	490.2 $\rightarrow$ 329.1	10.28
	15		490.2 $\rightarrow$ 434.2	
(9S)-HHC-COOH-TMS	15	Unit	490.2 $\rightarrow$ 329.1	10.33
	15		490.2 $\rightarrow$ 434.2	
THC-TMS-d3	15	Unit	374.0 $\rightarrow$ 292.0	9.12
	15		389.0 $\rightarrow$ 306.0	
THC-OH-TMS-d3	15	Unit	374.0 $\rightarrow$ 292.0	9.95
	15		374.0 $\rightarrow$ 268.0	
THC-COOH-TMS-d3	20	Unit	476.0 $\rightarrow$ 358.0	10.47
	15		374.0 $\rightarrow$ 292.0	

statistical tests for outliers (Grubbs test), homogeneity of variances (F-test) and linearity (Mandel F-test). Samples of each QC concentration were analyzed in duplicates on eight consecutive days for bias (trueness), time-different intermediate precision, repeatability and extraction efficiency. The extraction efficiency was evaluated by the ratio between regressions of calibration samples according to the GTFCh guideline (alternative method 2). Limit of detection (LOD) and limit of quantification (LOQ) were determined based on signal-to-noise ratio. Freeze-thaw stability was evaluated based on the average of the QC samples within a 90 % confidence interval [19]. For selectivity, cannabinoids (THC, THC-OH, THC-COOH, CBD) as well as synthetic cannabinoids (5F-AB-PINACA, 5F-ADB, 5F-MDMB-PICA, 4CN-Cumyl-BINACA) and other drugs of abuse (amphetamines, cocaine, opiates (morphine, codeine), methadone, buprenorphine) were examined.

## 3. Results

Validation of the GC-MS/MS method was performed successfully. Corresponding data are displayed in Table 2.

The results of analyzer based immunological testing procedures and on-site drug tests (DrugWipe® 5S and DrugScreen®) are shown in supplement (Tables 1a–4a). In short, DrugScreen® (cut-off: 25 ng/mL THC-COOH) gave positive results up to 10 h after ingestion and inhalation. Cross-reactivity with DrugWipe® 5S (cut-off: 5 ng/mL THC) was not observed. DrugScreen® tests with higher cut-off value of 150 ng/mL were partly positive, whereby tests with a cut-off value of 300 ng/mL were all negative.

Both HHC epimers and respective metabolites were detected in the plasma samples of the participants (Fig. 3). Peak plasma (9R)-HHC (3.8 and 2.2 ng/mL) and (9S)-HHC (2.5 and 1.6 ng/mL) concentrations were detected 1.16 h after the ingestion of HHC jellies for both participants of group 1 (P1 and P2). For the other participants (group 2), peak plasma concentrations of 17 ng/mL and 65 ng/mL ((9R)-HHC) and 5.6 and 21 ng/mL ((9S)-HHC) were achieved 0.08 h after inhalation.

(9R)-HHC-COOH concentrations of maximum 15 ng/mL and 17 ng/mL were measured 4.25 and 5.25 h, respectively, after ingestion. Subsequent to inhalative uptake, maximum concentrations of (9R)-HHC-COOH were 1.9 ng/mL and 8.7 ng/mL. (9S)-HHC-COOH was not detectable in plasma samples of both participants after inhalative uptake and only detectable in low concentrations (maximum approx. 0.4 ng/mL) after ingestion.

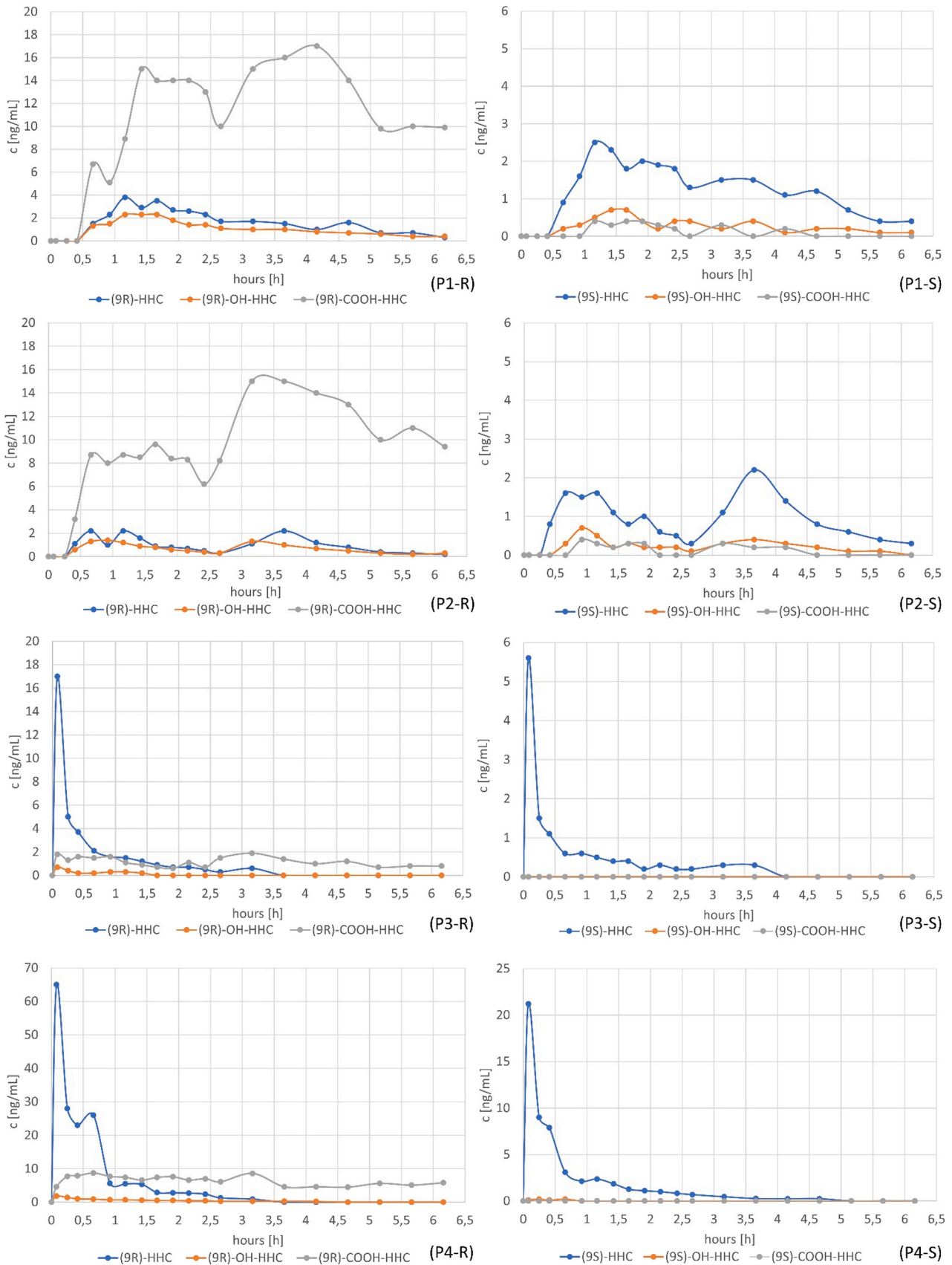
Maximum (9R)-HHC-OH concentrations were measured 0.92 h

**Table 2**

Validation data for HHC epimers and corresponding metabolites.

Analyte	LOD [ng/mL]	LOQ [ng/mL]	Trueness Bias [%] (QC <sub>low</sub> /QC <sub>high</sub> )	RSD <sub>T</sub> [%] (QC <sub>low</sub> /QC <sub>high</sub> )	RSD <sub>r</sub> [%] (QC <sub>low</sub> /QC <sub>high</sub> )	EE [%]
(9R)-HHC	0.3	0.3	- 2.0/3.0	8.3/6.6	6.3/3.9	50.3
(9S)-HHC	0.3	0.5	- 0.3/5.8	11.5/4.9	10.4/1.9	55.1
(9R)-HHC-OH	0.2	0.4	2.4/3.7	6.8/5.0	4.9/2.2	61.5
(9S)-HHC-OH	0.1	0.4	0.8/- 0.1	7.9/7.9	6.0/4.9	63.0
(9R)-HHC-COOH	0.2	0.3	1.9/7.0	9.0/3.7	6.9/2.8	67.0
(9S)-HHC-COOH	0.2	0.4	9.0/9.4	3.5/3.9	3.5/3.3	63.7

\* RSD<sub>T</sub> = time different intermediate precision, RSD<sub>r</sub> = repeatability, EE = Extraction Efficiency, QC<sub>low</sub> = quality control sample (low concentration), QC<sub>high</sub> = quality control sample (high concentration).



**Fig. 3.** Concentration curves of (9R)-HHC, (9R)-OH-HHC, (9R)-COOH-HHC and (9S)-HHC, (9S)-OH-HHC, (9S)-COOH-HHC in plasma samples of four participants after ingestion of HHC jellys (P1 and P2) or inhalation of HHC vape (P3 and P4).

(1.4 ng/mL) and 1.16 h (2.3 ng/mL) after ingestion, and 0.08 h (0.7 ng/mL and 1.8 ng/mL) after inhalation. (9S)-HHC-OH was detectable in low concentrations after ingestion of HHC (maximum 0.7 ng/mL) in both participants, but only in one of two participants (shortly) after inhalation.

Psychomotor impairments which were tested 0.5 and 1.5 h after uptake of HHC jellies and after (first) inhalation are shown in Table 3.

#### 4. Discussion

The developed GC-MS/MS method was successfully applied for chromatographic separation of HHC epimers as well as their metabolites and validated for identification and quantification. Interferences with THC and its metabolites did not occur.

The required validation parameter all met the requirements of the applicable guidelines of GTFCh [18]. Extraction efficiency was rather low, but above 50 % for all analytes. The results for the extraction efficiency can be explained by the use of a relatively high amount of organic solvent (acetonitrile) in the washing step for the SPE. For the analysis of THC and its metabolites, this step is necessary to remove present glucuronides from the sample. It is known, that silylation of co-extracted THC-COO glucuronide can result in the formation of THC-COOH and thus, to artificially higher concentrations of the carboxylic metabolite [24]. As HHC-metabolites are structurally similar to THC-metabolites, the washing step was performed likewise it is performed for THC measurements. An adaption of this step to improve extraction efficiency was not performed due to adequate accuracy and sensitivity of the method.

Our results for accuracy, repeatability and time different intermediate precision of (9R)- and (9S)-HHC were comparable to Hoefert et al. [17]. They found an accuracy of > 86.2 % and > 87.8 %, repeatability of < 6.2 % and < 6.5 %, and a time different intermediate precision of < 9.0 % and < 10.0 % respectively. HHC metabolites were not included in their study.

HHC and its respective metabolites were detected in plasma samples collected during the self-administration study. Independent from the route of administration (inhalation or ingestion), (9R)-HHC and metabolite concentrations were abundant in higher concentrations compared to (9S)-HHC and its metabolites, which was also described in foregoing studies [13,17,25].

Moreover, the route of administration seems to influence the metabolic pattern. After inhalation, (9R)-HHC and respective R-metabolites were detected in plasma samples of both participants, as well as (9S)-HHC. (9S)-COOH-HHC was not detected in both participants, (9S)-OH-HHC was detected only in small concentrations in one of the participants. This is overall in line with a comparable study, where (9R)-COOH-HHC and (9R)-OH-HHC were detected in blood samples of two volunteers, who inhaled HHC. (9S)-COOH-HHC was only detected below LOQ in this study, whereby (9S)-OH-HHC was not detected (LOQ = 2.5 ng/mL, LOD = 1 ng/mL) [13]. Remarkably, all R- and S-forms of HHC and respective metabolites were detectable after ingestion of HHC jelly in the presented self-administration study (LOD (S-OH-HHC): 0.1 ng/mL). This indicates that the route of administration (e.g. a first-pass effect after ingestion) seems to have an influence on analytical findings.

Another factor that might influence the higher concentrations of (9R)-HHC in blood after consumption of HHC might be the different abundance of the epimers in the products. One publication described the R-isomer to be more abundant than the S-isomer in HHC products [26], which could be a possible explanation for the concentration differences of the R- and S- parent compounds and also metabolites in plasma.

A higher abundance of (R)-HHC in edibles or vape pens appears feasible due to the assumed higher potency of (R)-HHC and thus, stronger effects. In our study, a quantitative analysis of the abundance of (R)-HHC and (S)-HHC in the products was not performed due to the

**Table 3**  
Psychomotor impairments of the participants 0.5 and 1.5 h after ingestion (P1 and P2, group 1) or inhalation (P3 and P4, group 2) of HHC. Conspicuous are displayed bold.

	Walk-and-turn			Finger-Test		Finger-Nose-Test		Pupil reaction		Pupils (width)		Time-estimation		Other conspicuities		Statements by participants	
P1 (0.5 h)	secure			insecure	secure	secure	prompt	normal	29 s as 30 s	none	none	none	none	none	feeling as if everything is packed "in cotton"		
P1 (1.5 h)	slightly insecure			insecure	secure	secure	prompt	normal	21 s as 30 s	continuously claims that she has troubles estimating the actual clock time							
P2 (0.5 h)	secure			secure	secure	secure	prompt	normal	30 s as 30 s	none	slight headache, mouth dryness						
P2 (1.5 h)	secure			secure	secure	secure	prompt	normal	22 s as 30 s	none	slight headache, slight dizziness						
P3 (0.5 h)	Insecure (7 steps instead of 6, asks twice for procedure)			insecure	secure	secure	prompt	normal	27 s as 30 s	slight vertigo during standing							
P3 (1.5 h)	secure			secure	secure (slightly slowed)	secure (slightly slowed)	prompt	normal	30 s as 30 s	none	none						
P4 (0.5 h)	insecure (vertigo)			insecure	secure	secure	slightly delayed	normal	s s	slowed behavior, glazed eyes, vertigo during standing	dizziness, feeling high, 1 am feeling strongly impaired, I would not drive, mouth dryness						
P4 (1.5 h)	insecure			insecure	insecure	insecure	prompt	normal	30 s as 30 s	none	Feeling better, headache						

lack of a suitable sample preparation method for the matrices. This results in a limitation with regard to the interpretation, that the concentration differences of the epimers in blood may arise from different abundance of the epimers in the consumed HHC-products.

Nonetheless, not only the abundance of HHC epimers in the products, but the chemical properties of the stereoisomers themselves and corresponding distinct metabolism were described to influence the abundance of (9R)-HHC or (9S)-HHC in blood as well. Hence, Di Trana et al. [14], who performed a study on the metabolism of HHC after the inhalation of HHC preparations containing the S- and R-isomer in equal shares. Accordingly, (9R)-HHC appeared in higher concentrations in blood, followed by slower elimination compared to (9S)-HHC.

Comparing the presented HHC-data to studies on  $\Delta$ 9-THC ingestion and inhalation, both, similarities and differences can be observed. A study on the metabolism of  $\Delta$ 9-THC after ingestion of gummies, described mean peak  $\Delta$ 9-THC plasma concentrations between 3.2 and 5.5 ng/mL 0.58–1.5 h after ingestion of 10 mg THC [27]. Assuming similar characteristics of  $\Delta$ 9-THC and (9R)-HHC plasma concentrations of (9R)-HHC 1.16 h after ingestion were in a comparable range (3.8 ng/mL and 2.2 ng/mL) in the present study, but after uptake of 25 mg HHC. On the contrary, concentrations of the carboxy metabolite were distinct with maximum concentrations of 15 and 17 ng/mL (9R)-COOH-HHC after 3.25 and 4.25 h in the present study and between 35 and 44 ng/mL  $\Delta$ 9-THC-COOH on average after 1.4–2.4 h in literature [27]. Concentration differences may be explained by interindividual differences in general, the small sample cohort in the present study or by differences in the metabolism of HHC. Another possible factor might be a lower dose than labeled being present in the respective product.

After inhalation,  $\Delta$ 9-THC is rapidly absorbed and already detectable during smoking and vaping [28,29]. (9R)- and (9S)-HHC were detectable in peak concentrations in the first plasma sample, withdrawn 0.08 h after the first inhalation, which is comparable to  $\Delta$ 9-THC. Peak concentrations, especially of (9R)-HHC, were highly variable between both participants ((9R)-HHC: approx. 65 ng/mL and 17 ng/mL) directly after the first vape, thus comparable to peak plasma concentrations of  $\Delta$ 9-THC after vaping [28]. Different amounts of vaporized HHC can explain differences in peak concentrations. P4 was able to deeply inhale the steam and hold it in the closed mouth for several seconds followed by slow exhalation, whereby P3 strongly coughed directly after the inhalation of the first puff. Interestingly, HHC concentrations continuously decreased, although blood samples were withdrawn in-between the three puffs. Again, the amount of HHC vaporized might explain the decrease, since both participants only shortly inhaled during the two consecutive puffs due to irritation of the throat and moderate psychoactive effects of P3 (e.g. slight dizziness, slight vertigo) or strong psychoactive effects for P4 (e.g. dizziness, feeling high, vertigo), respectively. It is assumed, that the inhaled amount during the second and third puff was rather low, so that the fast concentration decreases in the blood due to distribution processes could not be compensated.

Psychomotor impairments were objectively recorded 0.5 and 1.5 h after the uptake of both, HHC jellies and inhalative uptake. After inhalation, first psychoactive effects were already observed shortly after the first puff. After oral uptake, slightly stronger effects were objectively reported 1.5 h after the ingestion in comparison to the first recording after 0.5 h. With regard to peak plasma concentrations, which were reached 1.16 h after ingestion, and slowed absorption of substances after oral uptake, a delayed appearance of slightly stronger perceptible effects is reasonable after the jellies. Delayed appearance of psychoactive effects is also described after oral  $\Delta$ 9-THC ingestion in literature [30]. After inhalation, cannabimimetic effects were also described by both participants. Psychomotor impairments were more severe 0.5 h after the first inhalation, which is comparable to similar studies of inhalative  $\Delta$ 9-THC consumption, describing stronger effects shortly after inhalation [31,32]. Effects like mouth dryness and feeling high after HHC consumption were also reported in literature [16], whereby only mild cannabimimetic effects were described by the participant of a

self-administration study after the vaping of 15 mg of HHC [12]. Although the subjective feeling of effects can differ between individuals, the findings of the present study, especially regarding one of the test persons, allow the assumption that HHC consumption can impair the ability to drive – comparably to  $\Delta$ 9-THC.

The concomitant application of oral fluid tests during the study revealed no cross-reactivity with HHC or metabolites since all test results were negative. A cut-off value of 5 ng/mL for THC in oral fluid is given for the DrugWipe® 5S by the manufacturer. Our findings are in line with the findings of Hoefert et al. [17], who detected a cross-reactivity of HHC with the DrugWipe® 5S test only at higher concentrations of (9R)-HHC (250 ng/mL) and (9R)-HHC-OH (50 ng/mL), respectively. Cross-reactivity for the carboxy-metabolite was not observed [17]. Following the results of Kobidze et al. [13], concentrations of > 250 ng/mL (R)-HHC in oral fluid after inhalation of HHC seem possible, but appeared in only one of the samples of their study after the intake of 25 mg HHC. Thus, oral fluid concentrations after the consumption of HHC need to be subject to further investigation. Based on current knowledge, oral fluid tests appear poorly suited for detection of HHC consumption due to poor cross-reactivity.

On the contrary to oral fluid tests, cross-reactivity with the DrugScreen® urine test stripes was given in the present study, dependent on the cut-off value of the test. For DrugScreen® test with a cut-off value of 25 ng/mL THC-COOH, urine samples showed positive test results 1, 2, 4 and 8 h after consumption, except for P3, who inhaled HHC. Since the latter showed much lower parent compound and metabolite concentrations in blood, lower amounts are also expected in the urine samples, possibly explaining the discrepancies to P4, who also inhaled HHC. Moreover, creatinine value in the first sample of P3 (1 h after inhalation) was rather low (12 mg/dL), showing a dilution of the urine. Thus, the negative test result could be additionally caused by dilution of the urine (low creatinine measured). Cross-reactivity at higher cut-off values (150 ng/mL or 300 ng/mL) appeared occasionally or was not given. [13] measured urine concentrations of maximum 12.8 ng/mL for (9R)-HHC and 17.0 ng/mL for (9S)-HHC and rather low abundance of carboxy- and hydroxy-metabolites after HHC inhalation (dose: 25 mg HHC). Assuming suchlike concentrations in urine, poor detectability at higher cut-off DrugScreen® test stripes appear reasonable. Based on the results in this study, the authors assume that a preliminary drug test with a cutoff of 25 ng/mL is suitable to detect a recent single consumption of HHC in terms of using on-site urine drug tests.

Despite the mentioned cross-reactivity with urine on-site drug tests, the urine samples showed good cross-reactivity with CEDIA™ Assay on an AU 480 Analyzer (cut-off: 20 ng/mL). Participants were tested positive up to 48 h after consumption. For plasma samples, CEDIA™ assay (cut-off: 11 ng/mL) was positive firstly 0.66 h after ingestion and 0.08 h after inhalation, except for P3, where all samples showed negative results. Cross-reactivity with an ELISA assay (Immunoanalysis, Fa. Abbot) was described in literature, especially of the (R)-carboxy metabolite [17,25]. Kronstrand et al. [25] described a cross-reactivity of 120 % (R)-HHC-COOH and 48 % (S)-HHC-COOH in plasma with ELISA assay (threshold: 8.3 ng/mL and 20.7 ng/mL). Hoefert et al. [17] also described good cross-reactivity with (R)-HHC-OH at 10 ng/mL and (R)-HHC-COOH at 5 ng/mL, but only at 250 ng/mL for (9R)-HHC. Following the assumptions of the previous publications, the negative immunological analyses of the plasma samples of P3 can be explained by the low abundance of (R)-HHC-COOH and (R)-HHC-OH, respectively.

## 5. Conclusion

A method for the detection and quantification of HHC epimers and its respective metabolites was developed and validated. After uptake of HHC products (R)- and (S)-epimers appeared in varying proportions, depending on the route of administration. After single uptake, the carboxy-metabolite appeared rather as a minor metabolite, differing from THC metabolism. Nonetheless, the carboxy-metabolites are

important to detect since chronic consumption might lead to accumulation, as known for THC-COOH. The detectability of a HHC consumption with devices for on-site drug testing was given for urine, depending on the cut-off concentration and time interval after consumption. Respective oral fluid tests did not indicate HHC consumption. Analyzer based immunological analyses for THC in urine and plasma showed mostly good cross-reactivity with HHC.

Following the presented data, strong cannabimimetic effects after HHC consumption can occur, leading to possible serious impairments of the psychomotor function of the consumer. The impairments are dependent on dose, route of administration and habituation, but must not be underestimated concerning traffic offences.

### Ethics approval

An approval by an ethics committee is not required for self-experiments.

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### CRediT authorship contribution statement

**Hilke Andresen-Streichert:** Writing – review & editing, Supervision. **Lina Lucuta:** Writing – original draft, Project administration, Data curation, Conceptualization. **Lukas Schwarz:** Validation, Data curation. **Jeremai Hose:** Writing – review & editing. **Jennifer Liut:** Writing – review & editing. **Martin Juebner:** Writing – review & editing, Supervision. **Luzie Nauroth:** Writing – review & editing.

### Declaration of Generative AI and AI-assisted technologies in the writing process

The authors declare that generative AI and AI-assisted technologies were not used in the writing process.

### Declaration of Competing Interest

The authors have no relevant non-financial or financial conflicts of interest to disclose.

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### Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.forsciint.2025.112437](https://doi.org/10.1016/j.forsciint.2025.112437).

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