Clinical Chemistry 48:2
301–306 (2002)

Drug Monitoring and Toxicology

Stability of 11-Nor-Δ^9-carboxy-tetrahydrocannabinol Glucuronide in Plasma and Urine Assessed by Liquid Chromatography–Tandem Mass Spectrometry

Gisela Skopp[1*] and Lucia Pötsch[2]

Background: **Unconjugated 11-nor-Δ^9-carboxy-tetrahydrocannabinol (THCCOOH) in blood and urine has been proposed as a valuable marker, but the glucuronide (THCCOOglu) is present in considerably higher concentrations than the parent drug. Acyl glucuronides have been shown to be potentially reactive conjugates, which may affect the in vitro metabolite pattern.**

Methods: **Extraction procedures and a liquid chromatography–tandem mass spectrometry assay were developed and validated to investigate the stability of THCCOOglu in urine and plasma. Plasma and urine samples with added THCCOOglu were stored at −20, 4, 20, and 40 °C up to 10 days.**

Results: **The glucuronide was stable at −20 °C in both matrices, whereas THCCOOglu concentrations decreased at all other storage conditions. For a given storage time and temperature, the decrease in plasma was higher than that in urine. At 20 °C, a marked change in concentration could be observed within 2 days of storage. Degradation of THCCOOglu followed an apparent first-order process and led to the formation of THCCOOH. The sum of the molar concentrations of both analytes corresponded only to the initial THCCOOglu in plasma and urine samples stored at 4 °C.**

Conclusions: **The in vitro degradation of THCCOOglu prevents clinical conclusions based on the metabolite pattern or the concentration of unconjugated THCCOOH in samples stored at ≥4 °C for prolonged periods.**

© 2002 American Association for Clinical Chemistry

In clinical and forensic chemistry, drug stability during storage is an important consideration for the interpretation of drug concentration or its metabolite pattern. A few studies on the stability of cannabinoid components have been performed in authentic plasma or urine samples as well as in drug-free specimens to which cannabinoids had been added *(1–9)*.

In stored blood samples, mainly Δ^9-tetrahydrocannabinol (THC)[3] has been investigated because it represents the major psychoactive constituent of hashish and marijuana *(1–3)*. The amount of 11-nor-Δ^9-carboxy-tetrahydrocannabinol glucuronide (THCCOOglu) has been estimated from the difference of total and free 11-nor-Δ^9-carboxy-tetrahydrocannabinol (THCCOOH), with the ratio ranging from 2:1 to 1:10 *(10, 11)*. Recently the amount of unconjugated THCCOOH has been proposed as a criterion to classify heavy, frequent drug consumption *(12, 13)*. Mathematical models have been presented to predict the time of marijuana exposure or the degree of impairment based on the analyte profile *(13, 14)*.

In urine samples, THCCOOH was predominantly chosen as the target analyte representing the major metabolite of THC excreted in urine *(15)*, although THCCOOH exists primarily as the glucuronide *(16)*. Free THCCOOH is present in minute amounts in urine for only a few days after marijuana smoking *(17)*. Therefore, its detection was suggested as an indication of recent consumption *(17, 18)*.

THCCOOglu is an ester-linked β-glucuronide, and acyl glucuronides have been shown to be generally unstable *(19, 20)*. These findings led us to investigate whether free THCCOOH, which is proposed as a valuable marker for interpretation, may be liberated from THC-

[1] Institut für Rechtsmedizin und Verkehrsmedizin der Universität Heidelberg, Vossstrasse 2, D-69115 Heidelberg, Germany.
[2] Institut für Rechtsmedizin der Universität Mainz, Am Pulverturm 3, 55131 Mainz, Germany.
*Author for correspondence: Fax 49-6221-565252; e-mail gisela_skopp@med.uni-heidelberg.de.
Received July 23, 2001; accepted November 14, 2001.

[3] Nonstandard abbreviations: THC, Δ^9-tetrahydrocannabinol; THCCOOH, 11-nor-Δ^9-carboxy-tetrahydrocannabinol; THCCOOglu, 11-nor-Δ^9-carboxy-tetrahydrocannabinol glucuronide; LC-MS/MS, liquid chromatography–tandem mass spectrometry; and MRM, multiple reaction monitoring.

COOglu during storage or transport to the laboratory. The stability of THCCOOglu in blood and urine has not been thoroughly investigated. Suitable analytical techniques are now available to analyze such compounds directly. Therefore, the aim of the present study was to develop and validate a liquid chromatography–tandem mass spectrometry (LC-MS/MS) method for the simultaneous determination of free and glucuronidated THCCOOH in plasma and urine and to apply this method to a study of the short-term stability of THCCOOglu as a function of storage time and temperature.

Materials and Methods

EXPERIMENTAL DESIGN

Drug-free samples of urine (pH 6.2; 1040 mg/L creatinine) and heparinized fresh whole blood (150 mL; S-Monovette; Sarstedt) were obtained from a single healthy volunteer, and plasma was separated from blood after centrifugation. We added 300 and 400 μg/L, respectively, to the plasma and urine samples. To suppress bacterial growth in urine samples, we added 1 g/L sodium azide as a preservative. Aliquots (2.5 mL) of samples with added THCCOOglu were placed in crimp-top vials (type I glass A, aluminum seals, and Teflon liners) and stored protected from light up to 10 days as outlined in Table 1. The samples were heated to 40 °C to calculate additional kinetic data. The storage temperature was monitored by a microprocessor-controlled meter. Determination of bound and free THCCOOH in each experimental sample involved liquid–liquid or solid-phase extraction procedures and analysis by LC-MS/MS in the multiple-reaction monitoring (MRM) mode.

CHEMICALS

Deuterated and nondeuterated THCCOOglu (10 mg/L) was purchased from Alltech Associates, and deuterated (100 mg/L) and nondeuterated (1 g/L) THCCOOH was supplied by Cerilliant. For solid-phase extraction, HF Bond Elut Certify extraction columns (300 mg) were used (Varian). Acetonitrile, methanol, acetone, acetic acid, ammonium hydroxide, and ammonium acetate were from Roth. Diisopropyl ether was supplied by Merck, and doubly distilled water was obtained from Braun. All chemicals were of HPLC or analytical grade.

Table 1. Storage conditions for supplemented plasma and urine.

Matrix	Storage temperature, °C	Analysis time post storage, days
Urine	−20	0, 10
	4	0, 2, 4, 6, 8, 10
	20	0, 2, 4, 6, 8, 10
	40	0, 1, 2, 3, 4, 6
Plasma	−20	0, 10
	4	0, 2, 4, 6, 8, 10
	20	0, 1, 2, 3, 4, 5, 6, 8, 10
	40	0, 1, 2, 3, 4, 5, 6

SAMPLE PREPARATION

Plasma. Deuterated calibrators (50 ng of THCCOOglu-d_3 and 50 ng of THCCOOH-d_3) and 500 μL of water were added to 500 μL of plasma containing THCCOOglu. After vortex-mixing (60 s), the samples were centrifuged for 10 min at 3000*g*, and the supernatant was transferred to the preconditioned (3 mL of methanol, 3 mL of water) extraction column. After the samples had passed through the bed by gravity, the column was washed with 500 μL of water. After the addition of 40 μL of acetone, the sorbent was dried for 30 min under reduced pressure (80 mmHg). The analytes were eluted with 1500 μL of methanol, transferred to a silanized vial, and evaporated to dryness under nitrogen at 40 °C. The residue was reconstituted in 50 μL of the mobile phase, and 10 μL was injected into the LC-MS/MS system.

Urine. Deuterated calibrators (100 ng of THCCOOglu-d_3 and 100 ng of THCCOOH-d_3) and 25 μL of acetic acid were added to 500 μL of urine containing THCCOOglu. After vortex-mixing (60 s), 1000 μL of diisopropyl ether was added, and the mixture was vigorously shaken for 20 min. The mixture was centrifuged for 10 min at 3000*g*, and 900 μL of the clear supernatant was transferred to a silanized vial and processed as described for plasma.

For both extraction procedures, we investigated the stability of THCCOOglu during sample preparation and injection by analyzing three plasma and three urine samples fortified with 500 μg/L THCCOOglu.

LC-MS/MS ANALYSIS

Analysis was performed using a API 365 mass spectrometer (Applied Biosystems) with a TurboIon ionization source operated in the positive-ion mode. The device was interfaced to a quaternary HPLC pump equipped with an autosampler (series 200; Perkin-Elmer). The samples were chromatographed on a Zorbax Eclipse XDB C8 column [150 × 2.1 mm (i.d.); particle size, 5 μm; Ziemer] with acetonitrile–methanol–20 mmol/L ammonium acetate buffer, pH 4.0 (41:41:18 by volume) as the mobile phase at a flow rate of 270 μL/min. All LC-MS/MS data were recorded in the MRM mode. Quantification was based on the most prominent parent-product transitions: for THCCOOglu, *m/z* 521→345; for THCCOOglu-d_3, *m/z* 524→348; for THCCOOH, *m/z* 345→327; for THCCOOH-d_3, *m/z* 348→330 (Apple Macintosh G3 Power PC with Masschrom 1.1.1 software; Applied Biosystems). Internal standard calibration was based on the peak-area ratio of the particular analyte and its respective internal standard, which was referenced to the calibration curve. The six-point calibration curve covered a concentration range of 10–500 μg/L for each analyte in plasma or urine (calibrators: 10, 20, 50, 100, 200, and 500 μg/L of plasma or urine and a blank).

DATA ANALYSIS

The calibration lines were assessed for linearity of response by regression analysis. The limit of detection and the limit of

quantification were determined from the calibration curve according to DIN 32645 *(21)*. Recovery was tested by repeated extractions (n = 10) of drug-free plasma and urine to which 10 or 500 μg/L of THCCOOglu and THCCOOH had been added. Precision was based on plasma or urine samples to which 100 μg/L of each analyte had been added (n = 10) analyzed on 5 different days. The SD derived from these data was used to establish the stability of the glucuronide. The compound was considered stable for a specified time period if the difference between the initial concentration, c_0, and the concentration, c_t, did not exceed the critical difference d: $d = c_0 - c_t \leq 2 \cdot \sqrt{2} \cdot SD$ *(22)*. The extraction efficiency for THCCOOH and its glucuronide was calculated by comparing the peak areas of extracted calibrators with the peak areas of calibrators injected directly into the LC-MS/MS system. Each series was run in duplicate, and all samples were extracted three times. Values given are the means (n = 6).

The reaction kinetics were investigated by graphic representation. In apparent first-order reaction kinetics, the rate constant for the hydrolysis of THCCOOglu was obtained from the slope of the linear regression line obtained by plotting the natural logarithm of the drug concentration vs the time of storage.

Results

Representative chromatograms of extracted plasma samples are shown in Fig. 1. The retention time was 2.48 min for THCCOOglu and 4.01 min for THCCOOH. A 7-min run was sufficient to elute both analytes and all endogenous compounds. The stability of THCCOOglu for both extraction procedures and during injection into the LC-MS/MS system was ensured. THCCOOH was not detectable in any of these samples. The mean recoveries for THCCOOglu and THCCOOH were 43% and 75%, respectively, for plasma and 52% and 87%, respectively, for urine. With concentrations of 10–500 μg/L a strong linear relationship was observed for both analytes ($r > 0.99$). Data for recovery, precision, limit of detection, and limit of quantification are summarized in Table 2.

THCCOOglu appeared to be stable in both urine and plasma samples at a storage temperature of −20 °C during the entire observation period. At any other storage temperature chosen, the concentration of THCCOOglu decreased steadily during the observation period (Fig. 2).

The rate of degradation increased in plasma and urine samples with increasing temperature (Fig. 2). At a given temperature the decrease in THCCOOglu concentration was higher in plasma than in urine. For example, ~25% of the initial concentration of THCCOOglu was detectable in plasma on day 10 at a storage temperature of 20 °C, whereas in urine ~50% was still present. In samples stored at 4, 20, and 40 °C, THCCOOglu degraded by an apparent first-order process. The rate constants for THCCOOglu degradation in plasma and urine are given in Table 3.

As expected, degradation of THCCOOglu during stor-

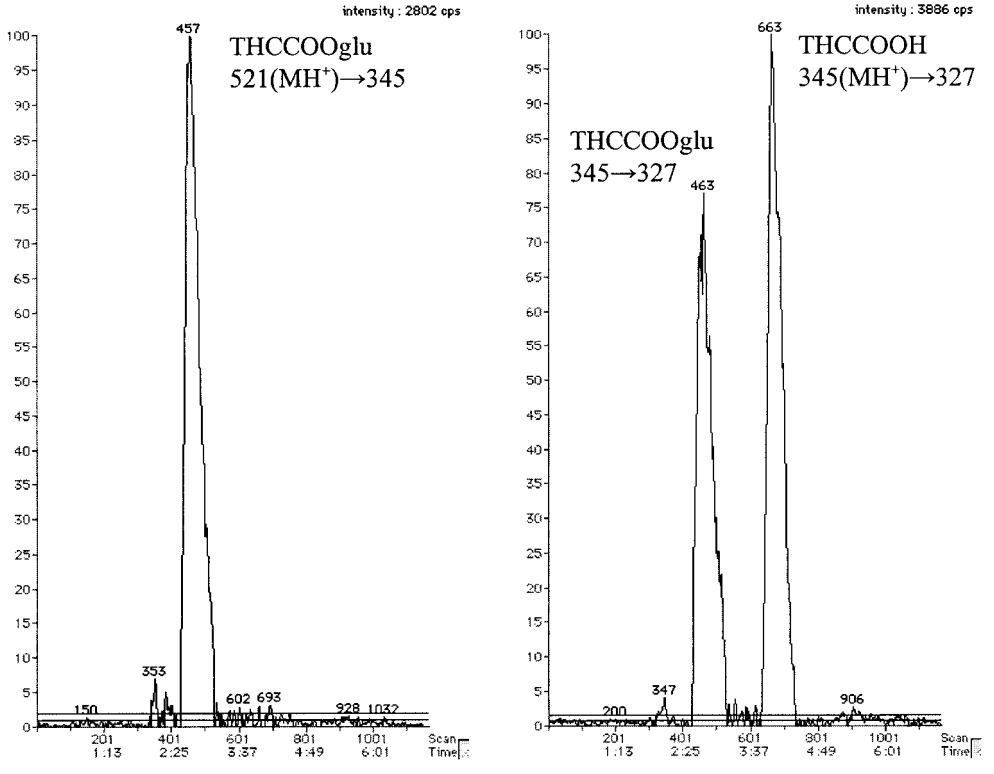

Fig. 1. LC-MS/MS chromatograms of a plasma extract (100 μg/L THCCOOglu and THCCOOH).
MRM transitions are indicated.

Table 2. Analytical recovery (mean ± SD), precision (100 μg/L; CV, %), limit of detection, and limit of quantification of the assay for THCCOOglu and THCCOOH in plasma and urine.

Matrix	Analyte	Recovery (mean ± SD), μg/L	CV, %		LOD,[a] μg/L	LOQ, μg/L
			Within-run	Between-run		
Urine	THCCOOH		2.0	4.1	1.4	6.0
	10 μg/L	10.2 ± 0.5				
	500 μg/L	499.5 ± 3.4				
	THCCOOglu		2.6	3.7	1.4	6.6
	10 μg/L	10.4 ± 0.9				
	500 μg/L	500.1 ± 6.1				
Plasma	THCCOOH		1.8	4.4	0.6	2.4
	10 μg/L	10.6 ± 0.5				
	500 μg/L	501.5 ± 8.6				
	THCCOOglu		1.9	5.3	1.1	4.5
	10 μg/L	10.3 ± 0.6				
	500 μg/L	504.0 ± 9.0				

[a] LOD, limit of detection; LOQ, limit of quantification.

age led to the formation of THCCOOH. Formation of free THCCOOH increased with increasing storage temperature in both plasma and urine (Fig. 3). At 20 and 40 °C, higher amounts of THCCOOH were liberated from its glucuronide in urine compared with plasma. However, the sum of the molar concentrations of bound and free THCCOOH at any time interval of storage corresponded to the initial concentration of THCCOOglu only in plasma and urine samples stored at 4 °C.

Discussion

The analytical procedures described for the isolation and quantification of THCCOOglu and THCCOOH in plasma and urine were very rapid and offered a low limit of quantification and excellent precision when applied to a large number of specimens. The use of deuterated internal standards corrected for the overall extraction efficiency, which averaged 43% and 52% for THCCOOglu in plasma and urine, respectively. A major advantage of the present method was the ability to analyze samples for the unchanged compound directly. We found only one published report *(23)* describing the measurement of THCCOOglu in urine that used a method developed with authentic urine samples, apparently because of the lack of appropriate standards for THCCOOglu. Until now, the classic procedure was to assay samples for THCCOOH by gas chromatography–mass spectrometry and then, after a hydrolytic procedure, to determine the "total" drug. The amount of conjugated drug was calculated from the difference of the two procedures. However, the results obtained by this method may be influenced by chemical hydrolysis/degradation of the drug itself or incomplete enzymatic hydrolysis. In authentic serum samples, different concentrations of THCCOOH have been observed after different extraction procedures, which has been attributed to chemical hydrolysis of the glucuronide during sample preparation *(24)*. To our knowledge, a validation of hydrolytic procedures for THCCOOglu in body fluids has not yet been performed.

The present data stress the influence of time and

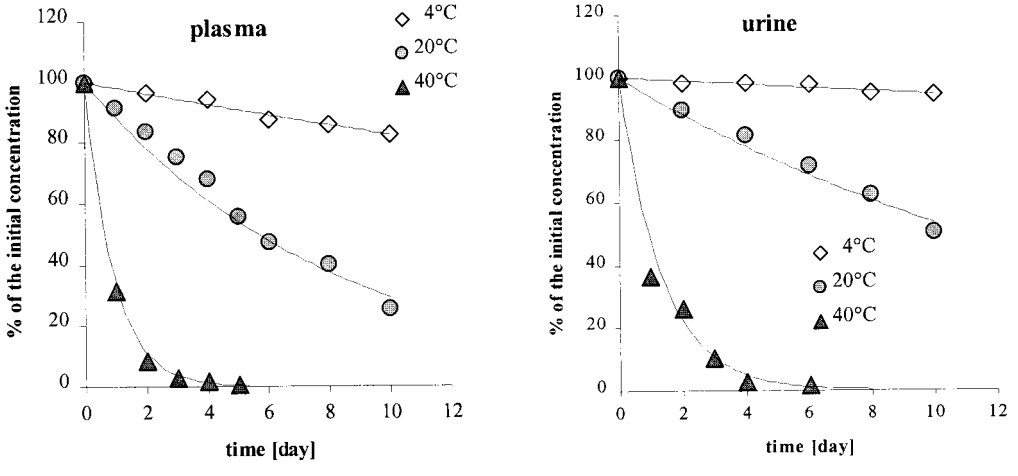

Fig. 2. Recovery of THCCOOglu as a percentage of the initial concentration (μg/L) in plasma and urine stored at 4, 20, and 40 °C.

Table 3. Rate constant and correlation coefficient for THCCOOglu in plasma and urine stored at 4, 20, and 40 °C.

Storage temperature, °C	Matrix	k, day^{-1}	r
4	Urine	0.006	0.952
20		0.063	0.987
40		0.747	0.968
4	Plasma	0.019	0.988
20		0.123	0.985
40		1.096	0.992

temperature on the THCCOOglu concentration in stored urine and plasma samples. The rigorous experimental design chosen allowed us to study degradation of the glucuronide under fixed conditions except for temperature and the particular biological matrix. THCCOOglu was stable only at a storage temperature of −20 °C for both plasma and urine. In contrast to stable ether glucuronides, such as morphine glucuronides *(25)*, acyl glucuronides have been reported to be highly reactive because of their intrinsic susceptibility to nucleophilic attack via the ester group linking the parent drug and the glucuronic acid moiety *(19)*. Acyl glucuronides can thus undergo hydrolysis, altering the disposition of the drug in vivo or the metabolite profile in vitro.

In plasma and urine samples, deconjugation of THCCOOglu occurred, even at 4 °C storage. The present results indicate that in vitro THCCOOglu degradation is attributable mainly to chemical hydrolysis but is also influenced by additional processes. In a recent publication, the addition of fluoride/oxalate to plasma samples slowed the hydrolysis of THCCOOglu *(26)*, which indicates that THCCOOglu may be hydrolyzed by enzymatic and chemical mechanisms. In urine, the degradation of THCCOOglu was lower than in plasma, which indicates that plasma proteins may also be involved. A similar observation was reported for acyl glucuronides based on a comparison of their disappearance in buffer and in solutions of buffered serum albumin *(27, 28)*. Human serum albumin was found to catalyze the hydrolysis of acyl glucuronides, depending on the chemical structure of the aglycone moiety *(29, 30)*. Furthermore, in vitro degradation of the glucuronide or THCCOOH seems evident, as indicated by the sums of their molar amounts in samples stored at 20 and 40 °C.

Instability may be deemed to exist when the mean value measured in the particular sample differs from the limits of the analytical precision *(22)*. The application of this stringent approach to the experimental data showed that THCCOOglu is stable at a storage temperature of 4 °C for ~10 days in urine and 7 days in plasma. Nevertheless, it should be considered that, during these time periods, small amounts of THCCOOH had formed in vitro. At 20 °C, THCCOOglu showed a change in concentration within 2 days of storage.

Stability data derived from a particular biological matrix, linked to the assay precision, are important for reliable interpretation of the analytical results in clinical chemistry and toxicology. Distinguishing genuine metabolite concentrations from results that are influenced by the release of the aglycone during sample storage is important, especially in forensic interpretation of cannabinoid concentrations. This should be reflected not only in stability studies, but should also be considered whenever the ratio of free to conjugated THCCOOH or the amount of free THCCOOH is used to estimate the extent of cannabis use or time of drug consumption *(13, 14, 17, 18)*. To our knowledge, this problem has not been addressed in any quality management protocol. Preliminary investigations on authentic plasma samples derived from a clinical study (n = 88) for THCCOOglu and THCCOOH by LC-MS/MS analysis revealed concentration ranges of 3–875 μg/L (mean, 190 μg/L) and 1–286 μg/L (mean, 52 μg/L), respectively (unpublished results). From these data it can be concluded that the concentration of THCCOOglu in

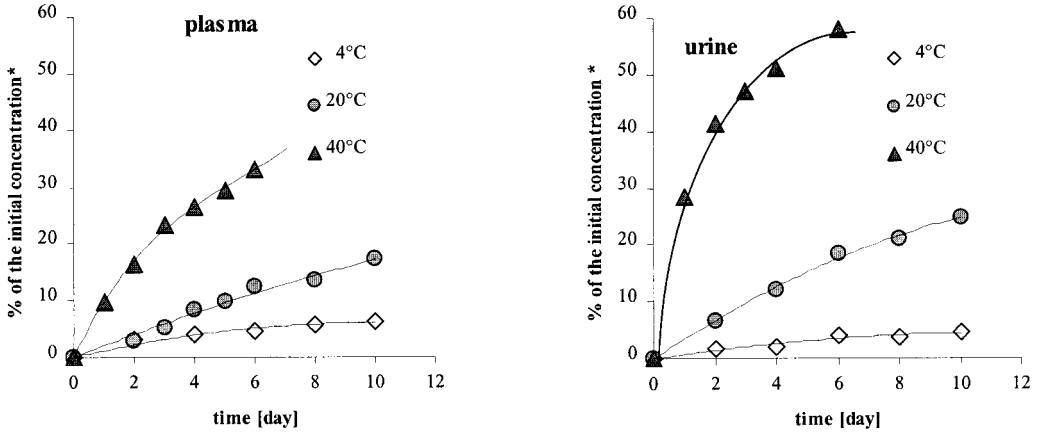

Fig. 3. Formation of THCCOOH in plasma and urine stored at 4, 20, and 40 °C as a percentage of the initial concentration of THCCOOglu.
*, initial concentration is the molar concentration.

plasma is always several times higher than the concentration of THCCOOH. Two days are frequently required for the transport of a blood sample to a forensic laboratory, and at least 5% of THCCOOH may have been liberated from the initial THCCOOglu before analysis. Therefore, a mathematical model based on carefully collected and stored samples may fail if applied to blood specimens with an unknown history *(14)*.

In light of the LC-MS/MS results obtained in urine, the presence of free THCCOOH as a reliable marker to identify recent *Cannabis* consumption seems questionable. To overcome preanalytical problems in the practice of forensic toxicology, it would be necessary to cool the samples during transportation. Furthermore, any meaningful measurement of free THCCOOH must be done on samples that have been properly preserved in the frozen state until analysis.

This work was supported by the Deutsche Forschungsgemeinschaft (Grant Sk48/2-2).

References

1. Levine B, Smith ML. Stability of drugs of abuse in biological specimens. Forensic Sci Rev 1990;2:147–57.
2. Johnson JR, Jennison TA, Peat MA, Foltz RL. Stability of Δ-9-tetrahydrocannabinol (THC), 11-hydroxy-THC and 11-nor-9-carboxy-THC in blood and plasma. J Anal Toxicol 1984;8:202–4.
3. Christophersen AS. Tetrahydrocannabinol stability in whole blood: plastic versus glass containers. J Anal Toxicol 1986;10:129–31.
4. Bond GD, Chand P, Walia AS, Liu RH. Observation of reduced concentration of Δ9-THC-carboxylic acid in urine specimen containers using internal barcode levels. J Anal Toxicol 1990;14:389–90.
5. Joern WA. Surface adsorption of the urinary marijuana carboxy metabolite: the problem and a partial solution. J Anal Toxicol 1992;16:401.
6. Dugan S, Bogema S, Schwartz RW, Lappas NT. Stability of drugs of abuse in urine samples stored at −20 °C. J Anal Toxicol 1994;18:391–6.
7. Romberg RW, Past MR. Reanalysis of forensic urine specimens containing benzoylecgonine and THC-COOH. J Forensic Sci 1994; 39:479–85.
8. Roth KDW, Siegel NA, Johnson RW, Litauszki L, Salvati L, Harrington CA, et al. Investigations of the effects of solution composition and container material type on the loss of 11-nor-Δ^9-THC-9-carboxylic acid. J Anal Toxicol 1996;20:291–300.
9. Golding-Fraga S, Diaz-Flores Estevez JF, Diaz Romero C. Stability of cannabinoids in urine in three storage temperatures. Ann Clin Lab Sci 1998;28:160–2.
10. Kelly P, Jones RT. Metabolism of tetrahydrocannabinol in frequent and infrequent marijuana users. J Anal Toxicol 1992;16:228–35.
11. Law B, Mason PA, Moffat AC, Gleadle RI, King LJ. Forensic aspects of the metabolism and excretion of cannabinoids following oral ingestion of *Cannabis* resin. J Pharm Pharmacol 1984; 36:289–94.
12. Wall ME, Taylor HL. Conjugation of acidic metabolites of Δ^8- and Δ^9-THC in man. In: Harvey DJ, Paton W, Nahas GG, eds. Marihuana 84′. Proceedings of the Oxford Symposium on Cannabis. Oxford: IRL Press, 1985:69–76.
13. Daldrup T, Meininger I. Der Cannabis Influencing Factor (CIF). In: Berghaus G, Krüger HP, eds. Cannabis im Strassenverkehr. Stuttgart: Fischer, 1998:193–5.
14. Huestis MA, Henningfield JE, Cone EJ. Blood cannabinoids. II. Models for the prediction of time of marijuana exposure from plasma concentrations of Δ^9-tetrahydrocannabinol and 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol. J Anal Toxicol 1992;16:283–90.
15. Agurell S, Halldin M, Lindgren JE, Ohlsson A, Widman M, Gillespie H, et al. Pharmacokinetics and metabolism of Δ^1-tetrahydrocannabinol and other cannabinoids with emphasis in man. Pharmacol Rev 1986;38:21–43.
16. Williams PL, Moffat AC. Identification in human urine of Δ^9-tetrahydrocannabinol-11oic acid glucuronide: a tetrahydrocannabinol metabolite. J Pharm Pharmacol 1980;32:445–8.
17. Skopp G, Pötsch L, Ganssmann B, Mauden M, Richter B, Aderjan R, et al. Freie und glucuronidierte Cannabinoide im Urin—Untersuchungen zur Einschätzung des Konsumverhaltens. Rechtsmedizin 1999;10:21–8.
18. Alburges ME, Peat MA. Profiles of Δ-9-tetrahydrocannabinol metabolites in urine of marijuana users: preliminary observations by high performance liquid chromatography-radio immunoassay. J Forensic Sci 1986;31:695–706.
19. Faed EM. Properties of acyl glucuronides: implications for studies of the pharmacokinetics and metabolism of acidic drugs. Drug Metab Rev 1984;15:1213–49.
20. Shipkova M, Schütz E, Armstrong W, Niedmann PD, Oellerich M, Wieland E. Determination of the acyl glucuronide metabolite of mycophenolic acid in human plasma by HPLC and Emit. Clin Chem 2000;46:365–72.
21. Deutsches Institut für Normung. DIN 32645 Chemische Analytik—Nachweis-, Erfassungs- und Bestimmungsgrenze Berlin: Beuth, 1994:3–10.
22. Stamm D. A new concept for quality control of clinical laboratory investigations in the light of clinical requirements and based on reference method values. J Clin Chem Clin Biochem 1982;20: 817–24.
23. Weinmann W, Vogt S, Goerke R, Müller C, Bromberger A. Simultaneous determination of THC-COOH and THC-COOH-glucuronide in urine samples by LC/MS/MS. Forensic Sci Int 2000;113:381–7.
24. Mauden M, Skopp G, Mattern R, Aderjan R. GC/MS-Bestimmung von THCCOOH im Serum. Vergleich verschiedener Aufarbeitungsmethoden und Einfluss von THCCOOH-Glucuronid. Blutalkohol 2000;37:48–56.
25. Skopp G, Pötsch L, Klingmann A, Mattern R. Stability of morphine, morphine-3-glucuronide, and morphine-6-glucuronide in fresh blood and plasma and postmortem blood samples. J Anal Toxicol 2001;25:2–7.
26. Toennes SW, Kauert GF. Importance of Vacutainer selection in forensic toxicological analysis of drugs of abuse. J Anal Toxicol 2001;25:339–43.
27. Williams AM, Dickinson RG. Studies on the reactivity of acyl glucuronides. VI. Modulation of reversible and covalent interaction of diflunisal acyl glucuronide and its isomers with human plasma in vitro. Biochem Pharmacol 1994;47:457–67.
28. Sallustio BC, Fairchild BA, Pannall PR. Interaction of human serum albumin with the electrophilic metabolite 1-*O*-gemfibrozil-β-D-glucuronide. Drug Metab Dispos 1997;25:55–60.
29. Dubois-Presle N, Lapicque F, Maurice MH, Fournel-Gigleux S, Magdalou J, Abiteboul M, et al. Stereoselective esterase activity of human serum albumin toward ketoprofen glucuronide. Mol Pharmacol 1995;47:647–53.
30. Salvi A, Carrupt PA, Mayer JM, Testa B. Esterase-like activity of human serum albumin toward prodrug esters of nicotinic acid. Drug Metab Dispos 1997;25:395–8.