Original Article

A REVIEW STUDY ON METHODS OF EXTRACTION & QUANTIFICATION OF CANNABINOIDS FROM BIOLOGICAL SAMPLES

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Abstract

Cannabis has become one of the most consumed illicit drugs throughout the world. Along with the time, Changes in law i. e. legalization/ decriminalization for its problematic utilization. Although, cannabis is also used for medicinal purposes to control pain, neurodegenerative disorders, etc. Cannabinoids can be identified from various biological such as blood, urine, saliva, etc for days/weeks after usage due to their retention depending on the frequency and length of exposure. In this review study, various methods for extraction and quantification such as solid phase extraction, liquid chromatography-mass spectrometry along with MEPS, LC-MS/MS, solid phase extraction, etc. of cannabinoids were studied from the body fluids. These methods not only helped in the detection of traces from biological evidence but also help in the determination of time since the occurrence.

Keywords: Cannabinoids, extraction, Quantification, biological samples, illicit drugs, etc.

INTRODUCTION

Cannabinoids are the chemical entities recovered from the *Cannabis* plant which has about 426 compounds of which 60 compounds are classified as cannabinoids. The most researched cannabinoid compounds include d-9-THC(delta-9-tetrahydrocannabinol), d-8-THC(delta-8-tetrahydrocannabinol, CBD(cannabidiol), and cannabinol [1] [2]. THC is a psychoactive compound that is mostly responsible for the hallucinogenic property and can remain in body fluids such as blood, urine, and semen for multiple days after the use of cannabinoids [3] [4]. Schwope and his colleagues have reported the detection of CBD from blood samples proving that the measurement of cannabinoids can be instrumental in forensic investigation [5]. Workplace drug testing, driving under the influence(DUI) and other criminal offenses can be solved by the detection of cannabinoids from the body fluid [3] [6]. The determination of cannabinoids was reported by many authors from body fluids which include semen[4] blood [5] urine[7] [8] oral fluids[7] [8] and sweat [9]. Apart from the body fluids, biological samples such as hairs and nails are also useful in forensic analysis for the detection of drug abuse and therefore the extraction of cannabinoids from these samples is of great importance for forensic scientists [9] [10]. Therefore, in evidence of the importance of biological entities in the detection and measurements of cannabinoids the following review gives clarity about the extraction, quantification, and analysis of biological samples such as semen, blood, urine, oral fluid, hair, and nails.

EXTRACTION

BLOOD: Solid phase extraction (SPE) and liquid-liquid extraction (LLE) comprise the bulk of techniques described for the extraction of cannabinoids from blood [11]. Due to the characteristics of the sample matrix, LLE is rapid, effective, and frequently more favourable for post-mortem blood. Recent articles describe the use of two-dimensional gas chromatography-mass spectrometry (2D GC-MS) for studying cannabinoids as the result of the conversion of standard GC-MS instrumentation to 2D GC-MS.[11]The advantages of 2D GC-MS include the ability to attain lower detection limits than regular GC-MS and the absence of the majority of undesired background interferences. Due to the complicated matrix, this is particularly significant for the examination of post-mortem blood samples[11]. Another method for extraction of cannabinoids includes disposable pipette extraction [12]. For the measurement of c9-tetrahydrocannabinol, 11-nor-9-carboxy-Δ9-tetrahydrocannabinol(THC-COOH), 11-hydroxy-Δ9-tetrahydrocannabinol, cannabinol, and cannabidiol concentrations in 100 l plasma specimens, a straightforward assay combining salting-out assisted liquid-liquid extraction sample preparation and LC-MS/MS analysis was employed.[13] The extraction Method for Testing Cannabinoids, Cocaine, and Amphetamines in Post-mortem Blood Samples uses Bond-Elute Certify cartridges for solid phase extraction, which is derivatized with N-methyl-N (trimethylsilyl)trifluoroacetamide at 808C for 30 min and is further evaluated by GC-MS.[14] Δ9-tetrahydrocannabinol (THC), 11-hydroxytetrahydrocannabinol(THC-OH),11-nor-9-carboxy-tetrahydrocannabinol(THC-COOH), and cannabidiol (CBD) were isolated using a straightforward liquid-liquid extraction (LLE) under acidic conditions from 1 mL of whole blood.[15]

SALIVA: When an individual administers cannabis either by smoking, inhaling, or spraying it into the mouth, the oral mucosa is observed to be highly contaminated for a short time right after intake. Contamination increases. Deposits in the oral cavity from external exposure are the primary source of THC OF concentrations. But there is a problem with OF collection for cannabis testing because the drug is known to cause dry mouth. For this reason, the specimen volume may be limited or significantly less in quantity for identification and extraction.[16] All free unconjugated cannabinoids were detected by both LLE(liquid -liquid extraction) and SPE(solid-phase extraction) methods.[17] A saliva sample collected 30 min after marijuana smoking was subject to SPME and traditional liquid−liquid extraction analysis.[18]

URINE: The most commonly used extraction methods are solid phase extraction, liquid chromatography-mass spectrometry along with MEPS. [19] The other methods which are recently been used are molecularly imprinted solid phase extraction, disposable pipette extraction, disperse liquid-liquid molecular extraction, packed-in tube solid phase extraction, and hollow fiber membrane solid phase extraction.[20] In concern with dried urine samples extraction using methanol has been observed to have relatively high extraction efficiency and it is the simple and cost-effective method of extraction. This technique involves taking a small number of biological fluids on a filter card and drying the sample. [21]The recent most specific and sensitive method for extraction of cannabinoids from urine is using a β-glucuronidase enzyme for digestion supported with liquid extraction. [22]

SWEAT: Liquid-liquid extraction [methanol (0.2 mol/L): sodium acetate buffer (pH 5.0) = 3:1] was used for extracting cannabinoids from sweat patches. Dried eluates were derivatized with trifluoroacetic acid.[16]

SEMEN: The collection of semen is usually done by masturbation, according to WHO guidelines[23], [24]. Every participant in a study provides written informed permission. After collection of the semen, the semen is left to liquefy for at least 30 to 60 minutes.[23] [25]. The sperm is then processed to form a percoll gradient before analysis. The proteins in the semen will be digested to generate peptides.[24] After the liquefaction and processing follow, the process of semen analysis is done by using high-performance liquid chromatography/tandem mass spectrometry (LC-MS/MS). This occurs by lipid extraction of the semen with chloroform: methanol (2:1, v/v). Using a single quadrupole API-150 EX mass spectrometer (Applied Biosystems, Foster City, CA) in conjunction with a PerkinElmer liquid chromatography system, the organic phase was dried before being examined by liquid chromatography-electrospray ionization mass spectrometry. [23]

HAIR: Al-Zahrani used a novel derivatizing method to aid in the detection of THC-COOH in the hair matrices, the FMP-TS derivatizing reagent to form the N-methyl pyridinium ether derivatives of the hydroxyl group of

cannabinoids (CBN, THC, and THC-COOH), in combination with esterification of the carboxyl group in THC-COOH. Strata X-A 33-µm polymeric strong anion exchange cartridge is employed for the extraction of the desired analyte.[26] Heinl mentioned that alkali digestion, mostly by NaOH gives free cannabinoids. The analytes obtained after digestion can be extracted using liquid-liquid extraction (n-hexane/ethyl acetate (9/1, v/v)) or solid-phase extraction. Solid-phase microextraction and solid-phase dynamic extraction are also developed.[27] Cobo-Golpe and his colleagues used solid phase extraction using mixed-mode cation exchange-reversed phase cartridges Oasis MAX cartridge to extract THC, CBN, CBD, OHTHC, THCCOOH, and diOHTHC. Samples were reconstituted using MeOH and NaOH. [28] Tobias Kieliba et al. also employed alkali digestion using NaOH, followed by solid phase extraction (SPE), using a mixed-mode anion exchange sorbent. For THC extraction, employment of HR-XA extraction cartridge of SPE was done.[29]

NAILS: Various techniques used to detect and quantify substances in nails include gas chromatography, mass-spectrometry, nuclear activation, x-ray fluorescence and emission, and atomic absorption and emission.[30] The most commonly used technique is gas chromatography.[31] Nikolaos P. Lemos determined the number of cannabinoids in nails using radioimmunoassay (RIA) and gas chromatography-mass spectrometry (GC-MS).[32] Cobo-Golpe]introduced a new method with 99.5% to 109.8% accuracy, They did alkaline hydrolysis followed by solid phase extraction and liquid chromatography with tandem mass spectrometry (LC-MS-MS) to analyse the Cannabinoids and their primary metabolites.[28]

QUANTIFICATION

BLOOD: Liquid chromatography-tandem mass spectrometry can be employed for the quantification of THC, and five other cannabinoids.[12] For the quantification of Δ9-tetrahydrocannabinol (THC), 11-hydroxytetrahydrocannabinol(THC-OH),11-nor-9-carboxy-tetrahydrocannabinol (THC-COOH), and cannabidiol (CBD), an extremely sensitive and selective liquid chromatography (LC) method was devised.[15] To accomplish parallel reaction monitoring (PRM) quantification in positive polarity with a negative polarity switching for THC-OH and THC-COOH, HRMS on an Orbitrap-based equipment was used.[6] Thirteen cannabinoids components were quantified using a liquid chromatography-tandem mass spectrometry approach, including CBD, CBDA, CBDV, CBN, CBG, CBGA, CBC, THC, THCA, THCV, 11-OH-THC, THC-COOH, and THC-COOH-glu.[33] To quantify THC, CBG, and THCV in whole blood, a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was created. It also measures THC, CBN, CBD, 11-OH-THC, THCCOOH, and THCCOOH-gluc.[34]

SALIVA: A sensitive method was determined for quantifying a wide range of cannabinoids in oral fluid (OF) using liquid chromatography-tandem mass spectrometry (LC-MS/MS). [18] Laboratory-based cannabinoid immunoassay screening or chromatographic confirmation was determined that OF can be collected by passive drool, expectoration, or commercial collection devices. The combined extracts were then purified by liquid-liquid or solid-phase extraction. [16] Another laboratory-based screening, THC, was identified in OF by thin-layer chromatography and colorimetric development in early reports.

URINE: The simple, sensitive, cost-effective, rapid, and most commonly used method for the isolation and quantification of analytes from complex fluids like urine is Gas chromatography-mass spectrometry. [35] The previously reported analytical methods include Gas Chromatography-Mass Spectrometry [35], Liquid Chromatography- Mass spectrometry, Solid phase extraction- liquid chromatography-Tandem Mass Spectrometry [36], Liquid chromatography coupled with quadrupole time of flight Mass Spectrometry [22], Ultra-high pressure liquid chromatography (more sensitive and rapid) [37], High-performance liquid chromatography-tandem mass spectrometry. [36]The simple liquid-liquid extraction procedure along with GCMS showed acceptable accuracy and specificity in comparison with LCMS/MS using solid-phase extraction. [35] The detection and quantification of several synthetic cannabinoids were done using liquid chromatography coupled with high-resolution mass spectrometry. [37] Recently for the quantification of identification of the same molecules, the combination of the LC-MS - MEPS along with molecularly imprinted polymers is used, but this technique of MEPS doesn't have any significant impact on the quantification of CBD, CBN, and metabolites. [38,39]

SWEAT: A Hewlett-Packard 6890 gas chromatograph (fused-silica capillary column) with a mass selective detector (HP 5973) using NICI was used for sample analysis. Methane (99.99% purity) was used as reagent gasgas [9]

SEMEN: While there are many metabolites of THC, delta-9 THC, THC- COOH, and 11- OH -THC are a few of them found in the semen. The quantification range of these metabolites is 0.50–50 ng/mL, 5.0– 500 ng/mL, and 1.0–100 ng/mL, respectively. [25]

HAIR: Al-Zahrani, et al. validated LC-MS/MS techniques for the detection of the main cannabinoids such as CBN, THC, and THC-COOH. [26] Heinl employed alkali digested analyte to derivatization with silylation reagents employed for GC-MS separation.[27] Cobo-Golpe et al performed quantification y LC-MS/MS after reconstituting the sample with MeOH and NaOH. [28] Tobias Kieliba et al., the quantification and simultaneous determination of THC-COOH along with OH-THC, THC, CBD, and CBN in human hair by GC-MS/MS with electron ionization along with automated sample preparation. Quantification of THC-COOH is done using fluorinated reagents derivatizing followed by GC-MS with negative chemical ionization (NCI) [29]

NAILS: Mean concentrations of cannabinoid, A9-tetrahydrocannabinol were determined using RIA, 11-nor-Ag-tetrahydrocannabinol-9- carboxylic acid from fingernails was quantified using GC-MS. [32] Cobo-Golpe quantified CBN, TCH, and THCCOOH from fingers and toenails.[28]

CONCENTRATION

BLOOD: For five cannabinoids (THC, CBD, CBN, 11-OH-THC, and THC-COOH), the Limit Of Detection (LOD) was estimated to be 0.25 ng/mL.[11] The Lower Limit Of Quantification(LLOQ) for THC, CBN, and 11-OH-THC was confirmed to be 0.25 ng/mL. Since they did not exhibit adequate accuracy or precision at 0.25 ng/mL, the LLOQ for THC-COOH and CBD was set at 0.5 ng/mL.[11] For cannabinoids extracted by disposable pipette extraction method, LODs for THC, CBG, THCV, and THCVCOOH were 0.5 g/L, 0.25 g/L for 11-OH-THC, THCCOOH, CBD, CBN, and THC-glucuronide, and 1.25 g/L for THCCOOH-glucuronide.[12] For THC and THCCOOH, the linear ranges were 0.5-100 g/L; for 11-OH-THC, CBD, CBN, and THC-glucuronide; for CBG, THCV, and THCVCOOH; and for THCCOOH-glucuronide, the linear ranges were 0.5-50 g/L. [12] For THC, THCV, CBG, etc quantified by liquid chromatography-tandem mass spectrometry, the LOQ range was within 0.5 to 2 μg/L in whole blood.[34]

SALIVA: Delta-9-tetrahydrocannabinol OF concentrations obtained from liquid chromatography-tandem mass spectroscopy was found to be > 1000 μg/L shortly after smoking.[28] Immuno analysis of Sweat/OF THC Direct ELISA method (THC cutoff = four μg/L), confirming OF cannabinoids by 2-dimensional GC-MS with a THC cutoff of 1 μg/L. [18] The level of Δ9-THC by SPME was found to be 9.54 ng/mL for the saliva sample.[18]

URINE: As per the guidelines of the Substance Abuse and Mental Health Services Administration and European guidelines for workplace drug testing in urine the range of cannabis metabolites for laboratory screening tests is 50ng/ml and the recommended concentration cut-off for THC-COOH is 15ng/ml and its lower limit of quantification is from 1ng/ml to 10ng/ml. [38] The recent enzyme extraction method has a significant value of 2ng/ml LLOQ which is very specific compared to all other methods used. [22]

SWEAT: LOD and LOQ for THC in human sweat were 0.2 and 0.4ng/patch, respectively. The concentration of THC in sweat patches worn for consecutive 24-h periods by a cannabis user during excretion of THC from previously self-administered cannabis was found in the range of 0.90 to 3.11 ng/patch. Application of the proposed SAMHSA cutoff of 1 ng/ patch revealed that patches worn for consecutive 24-h intervals were positive up to the 5^{th} day of cannabinoid abstinence. [39]

SEMEN: In an experimental setup consisting of 12 men who were heavy marijuana users, semen assays were performed for ten men. Two participants ' semen samples could not be assayed because of low sample volume. These two participants suffered from hypospermia. Of these ten males whose assay was performed, THC was found in only two samples in concentrations of 0.87 and 0.97 ng/mL. Thus, it is puzzling why all semen samples

did not contain THC metabolites. So far, only one study was recorded that studied the direct presence of THC and its metabolites in semen directly. However, it should be noted that experimental setups have allowed for the identification of endocannabinoid receptors in human testes and sperm.[25]

HAIR: Al-Zahrani, et al. obtained the limit of detection (LOD) for CBN (2pg/mg) and THC20 pg/mg) and the limit of quantification (LOQ) for CBN0.1pg/mg THC0.2pg/mg. The obtained concentrations of other cannabinoids range from CBN (0.022–2.562 ng/mg), THC (0.049–0.431 ng/mg), and THC-COOH (0.222–4.867 pg/mg). [26] Hein et al. obtained the limit of quantification (LOQ) for THC (0.01 ng/mg), CBN (0.06 ng/mg), and CBD (0.03 ng/mg). These LOQ values obtained are lower than required for THC (0.02 ng/mg), in medical psychological assessments in Germany, and also for the Society of Hair Testing of THC (0.1 ng/mg). [27] Cobo-Golpe et al found in the range from LOQ to 20000 pg/mg for all the analytes except diOHTHC. The LOQ of CBN and THC was found to be 40 pg/mg and 100 pg/mg for the rest of the hair. Except for diOHTHC (100 pg/mg), LOD was found to be 40 pg/mg for all analytes). [28] Tobias Kieliba et al. found that the limit of detection (LOD) of THC-COOH and OH-THC was 0.2 pg/mg and for THC, CBD, and CBN 2 pg/mg. [29]

NAILS: 1.03 ng/mg was the mean cannabinoid concentration in fingernails determined using RIA in 6 known cannabis users, and 1.44 ng/mg was the mean concentration of A9-tetrahydrocannabinol in fingernails of 14 cannabis users. The average 11-nor-Ag-tetrahydrocannabinol-9- carboxylic acid concentration in fingernails from 3 known cannabis users extracted in acidic pH was 19.85 ng/mg using GC-MS. [32] The LOQ was 20 pg/mg for CBN and THC and 100 pg/mg for the rest of the analytes in the nails. LOD in nails was found to be 10 pg/mg for THC, 20 pg/mg for CBN, and 50 pg/mg for THCCOOH. When they compared the concentration obtained between finger and toenails, fingernails were 8–28.9 times higher than toenails.[28] Toenails treated with antifungal agents had lower concentrations than those found untreated. CBN was found to be 52 times lower and THC 228 times lower.[28]

CONCLUSION

This study emphasis various advanced techniques to extract and quantify the illicit drugs from various biological fluids. These techniques increase the probability to attain recovery at lower detection with quantification limitations. Above mentioned methods propose an appealing for extraction of drugs due to their quick response even in a minimal amount of sample and solvents used. As per the best knowledge of authors, these are the methods for quantification and extraction of cannabinoids present in various biological fluids.

CONFLICT OF INTEREST: NA

SOURCE OF FUNDING: NA

ETHICAL CONSIDERATION: NA

REFERENCES

[1] G. Lafaye, L. Karila, L. Blecha, A. Benyamina, Cannabis, cannabinoids, and health, Dialogues in Clinical Neuroscience. (2022).

[2] Z. Atakan, Cannabis, a complex plant: different compounds and different effects on individuals, Therapeutic Advances in Psychopharmacology. 2 (2012) 241–254.

[3] K. Sivashanmugan, K. Squire, A. Tan, Y. Zhao, J.A. Kraai, G.L. Rorrer, A.X. Wang, Trace detection of tetrahydrocannabinol in body fluid via surface-enhanced Raman scattering and principal component analysis, ACS Sensors. 4 (2019) 1109–1117.

[4] M.S. Lee, A. Lanes, E.S. Ginsburg, J.H. Fox, Delta-9 THC can be detected and quantified in the semen of men who are chronic users of inhaled cannabis, Journal of Assisted Reproduction and Genetics. 37 (2020) 1497–1504.

[5] D.M. Schwope, E.L. Karschner, D.A. Gorelick, M.A. Huestis, Identification of recent cannabis use: whole-blood and plasma free and glucuronidated cannabinoid pharmacokinetics following controlled smoked cannabis administration, Clinical Chemistry. 57 (2011) 1406–1414.

[6] M. Sharma, N. Sharma, M. Muddassir, Q.I. Rahman, U.N. Dwivedi, S. Akhtar, Structure-based pharmacophore modeling, virtual screening and simulation studies for the identification of potent anticancerous phytochemical lead targeting cyclin-dependent kinase 2, Journal of Biomolecular Structure and Dynamics. (2021) 1–18.

[7] E. Gerace, S.P. Bakanova, D. Di Corcia, A. Salomone, M. Vincenti, Determination of cannabinoids in urine, oral fluid and hair samples after repeated intake of CBD-rich cannabis by smoking, Forensic Science International. 318 (2021) 110561.

[8] R.S. Niedbala, K.W. Kardos, D.F. Fritch, S. Kardos, T. Fries, J. Waga, J. Robb, E.J. Cone, Detection of marijuana use by oral fluid and urine analysis following single-dose administration of smoked and oral marijuana, Journal of Analytical Toxicology. 25 (2001) 289–303.

[9] M.A. Huestis, K.B. Scheidweiler, T. Saito, N. Fortner, T. Abraham, R.A. Gustafson, M.L. Smith, Excretion of Δ9-tetrahydrocannabinol in sweat, Forensic Science International. 174 (2008) 173–177.

[10] M. Cobo-Golpe, A. de-Castro-Ríos, A. Cruz, M. López-Rivadulla, E. Lendoiro, Determination and distribution of cannabinoids in nail and hair samples, Journal of Analytical Toxicology. 45 (2021) 969–975.

[11] R. Andrews, S. Paterson, A validated method for the analysis of cannabinoids in post-mortem blood using liquid–liquid extraction and two-dimensional gas chromatography–mass spectrometry, Forensic Science International. 222 (2012) 111–117.

[12] K. Scheidweiler, M. Newmeyer, A. Barnes, M. Huestis, Quantification of Cannabinoids and their Free and Glucuronide Metabolites in Whole Blood by Disposable Pipette Extraction and Liquid Chromatography Tandem Mass Spectrometry, Journal of Chromatography A. 1453 (2016).

[13] P. Frei, S. Frauchiger, E. Scheurer, K. Mercer-Chalmers-Bender, Quantitative determination of five cannabinoids in blood and urine by gas chromatography tandem mass spectrometry applying automated on-line solid phase extraction, Drug Test Anal. 14 (2022).

[14] F.S. Pelição, M.D. Peres, J.F. Pissinate, B.S. De Martinis, A One-Step Extraction Procedure for the Screening of Cocaine, Amphetamines and Cannabinoids in Postmortem Blood Samples, Journal of Analytical Toxicology. 38 (2014) 341–348.

[15] T. Joye, C. Widmer, B. Favrat, M. Augsburger, A. Thomas, Parallel Reaction Monitoring-Based Quantification of Cannabinoids in Whole Blood, J Anal Toxicol. 44 (2020) 541–548.

[16] D. Lee, M.A. Huestis, Current knowledge on cannabinoids in oral fluid, Drug Test Anal. 6 (2014) 88–111.

[17] M. Martin-Fabritius, C. Staub, P. Mangin, C. Giroud, Analysis of cannabinoids in oral fluid by liquid chromatography–tandem mass spectrometry, Forensic Toxicology. 31 (2013) 151–163.

[18] B.J. Hall, M. Satterfield-Doerr, A.R. Parikh, J.S. Brodbelt, Determination of cannabinoids in water and human saliva by solid-phase microextraction and quadrupole ion trap gas chromatography/mass spectrometry, Anal Chem. 70 (1998) 1788–1796.

[19] M. Sergi, C. Montesano, S. Odoardi, L. Mainero Rocca, G. Fabrizi, D. Compagnone, R. Curini, Micro extraction by packed sorbent coupled to liquid chromatography tandem mass spectrometry for the rapid and sensitive determination of cannabinoids in oral fluids, J Chromatogr A. 1301 (2013) 139–146.

[20] F. Gaunitz, T. Kieliba, M. Thevis, K. Mercer-Chalmers-Bender, Solid-phase extraction-liquid chromatography-tandem mass spectrometry method for the qualitative analysis of 61 synthetic cannabinoid metabolites in urine, Drug Test Anal. 12 (2020) 27–40.

[21] M. Moretti, F. Freni, C. Carelli, C. Previderé, P. Grignani, C. Vignali, M. Cobo-Golpe, L. Morini, Analysis of Cannabinoids and Metabolites in Dried Urine Spots (DUS), Molecules. 26 (2021) 5334.

[22] D. Borg, A. Tverdovsky, R. Stripp, A Fast and Comprehensive Analysis of 32 Synthetic Cannabinoids Using Agilent Triple Quadrupole LC-MS-MS, J Anal Toxicol. 41 (2017) 6–16.

[23] F. Francavilla, N. Battista, A. Barbonetti, M.R.C. Vassallo, C. Rapino, C. Antonangelo, N. Pasquariello, G. Catanzaro, B. Barboni, M. Maccarrone, Characterization of the endocannabinoid system in human spermatozoa and involvement of transient receptor potential vanilloid 1 receptor in their fertilizing ability, Endocrinology. 150 (2009) 4692–4700.

[24] S. de Mateo, J.M. Estanyol, R. Oliva, Methods for the Analysis of the Sperm Proteome, in: D.T. Carrell, K.I. Aston (Eds.), Spermatogenesis: Methods and Protocols, Humana Press, Totowa, NJ, 2013: pp. 411–422.

[25] M.S. Lee, A. Lanes, E.S. Ginsburg, J.H. Fox, Delta-9 THC can be detected and quantified in the semen of men who are chronic users of inhaled cannabis, J Assist Reprod Genet. 37 (2020) 1497–1504.

[26] M.A. Al-Zahrani, A.I. Al-Asmari, F.F. Al-Zahrani, H.J. Torrance, D.G. Watson, Quantification of cannabinoids in human hair using a modified derivatization procedure and liquid chromatography-tandem mass spectrometry, Drug Test Anal. 13 (2021) 1095–1107.

[27] S. Heinl, O. Lerch, F. Erdmann, Automated GC–MS Determination of Δ9-Tetrahydrocannabinol, Cannabinol and Cannabidiol in Hair†, Journal of Analytical Toxicology. 40 (2016) 498–503.

[28] M. Cobo-Golpe, A. de-Castro-Ríos, A. Cruz, M. López-Rivadulla, E. Lendoiro, Determination and Distribution of Cannabinoids in Nail and Hair Samples, J Anal Toxicol. 45 (2021) 969–975.

[29] T. Kieliba, O. Lerch, H. Andresen-Streichert, M.A. Rothschild, J. Beike, Simultaneous quantification of THC-COOH, OH-THC, and further cannabinoids in human hair by gas chromatography-tandem mass spectrometry with electron ionization applying automated sample preparation, Drug Test Anal. 11 (2019) 267–278.

[30] C.R. Daniel, B.M. Piraccini, A. Tosti, The nail and hair in forensic science, J Am Acad Dermatol. 50 (2004) 258–261.

[31] A.J. Jenkins, Y.H. Caplan, eds., Drug Testing in Alternate Biological Specimens, Humana Press, Totowa, NJ, 2008.

[32] N.P. Lemos, R.A. Anderson, J.R. Robertson, Nail Analysis for Drugs of Abuse: Extraction and Determination of Cannabis in Fingernails by RIA and GC-MS, Journal of Analytical Toxicology. 23 (1999) 147–152.

[33] M.J. Roslawski, R.P. Remmel, A. Karanam, I.E. Leppik, S.E. Marino, A.K. Birnbaum, Simultaneous Quantification of 13 Cannabinoids and Metabolites in Human Plasma by Liquid Chromatography Tandem Mass Spectrometry in Adult Epilepsy Patients, Therapeutic Drug Monitoring. 41 (2019) 357–370.

[34] J. Hubbard, B. Smith, P. Sobolesky, S. Kim, M. Hoffman, J. Stone, M. Huestis, D. Grelotti, I. Grant, T. Marcotte, R. Fitzgerald, Validation of a liquid chromatography tandem mass spectrometry (LC-MS/MS) method to detect cannabinoids in whole blood and breath, Clinical Chemistry and Laboratory Medicine (CCLM). 58 (2019).

[35] L.M. Rosendo, T. Rosado, P. Oliveira, A.Y. Simão, C. Margalho, S. Costa, L.A. Passarinha, M. Barroso, E. Gallardo, The Determination of Cannabinoids in Urine Samples Using Microextraction by Packed Sorbent and Gas Chromatography-Mass Spectrometry, Molecules. 27 (2022) 5503.

[36] J. Sánchez-González, S. Odoardi, A.M. Bermejo, P. Bermejo-Barrera, F.S. Romolo, A. Moreda-Piñeiro, S. strano rossi, Development of a micro-solid-phase extraction molecularly imprinted polymer technique for synthetic cannabinoids assessment in urine followed by liquid chromatography–tandem mass spectrometry, Journal of Chromatography A. 1550 (2018).

[37] T. Berg, L. Kaur, A. Risnes, S.M. Havig, R. Karinen, Determination of a selection of synthetic cannabinoids and metabolites in urine by UHPSFC-MS/MS and by UHPLC-MS/MS, Drug Testing and Analysis. 8 (2016) 708–722.

[38] D. Pon, I.J. Fenyvesi, A Validated Method for the Detection and Quantitation of Synthetic Cannabinoids in Whole Blood and Urine, and its Application to Postmortem Cases in Johannesburg, South Africa, South African Journal of Chemistry. 71 (2018) 24–29.

[39] T. Saito, A. Wtsadik, K.B. Scheidweiler, N. Fortner, S. Takeichi, M.A. Huestis, Validated gas chromatographic-negative ion chemical ionization mass spectrometric method for delta (9)-tetrahydrocannabinol in sweat patches, Clin Chem. 50 (2004) 2083–2090.