Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com

11-OH-THC in hair as marker of active cannabis consumption: Estimating a reliable cut-off by evaluation of 672 THC-positive hair samples

Sara Casati [a], Ilaria Angeli [a], Alessandro Ravelli [a], Massimo Del Fabbro [b, c], Mauro Minoli [a], Marica Orioli [a, *]

[a] Laboratory of Forensic Toxicology, Department of Biomedical, Surgical and Dental Science, University of Milan, Via Luigi Mangiagalli 37, Milan, Italy
[b] Department of Biomedical, Surgical and Dental Science, University of Milan, Via Riccardo Galeazzi, 4, Milan, Italy
[c] IRCCS Orthopedic Institute Galeazzi, Milan, Italy

ARTICLE INFO

Article history:
Received 14 June 2019
Received in revised form 29 August 2019
Accepted 30 August 2019
Available online xxx

Keywords:
Hair
Cut-Off
11-Hydroxy-Δ-9-tetrahydrocannabinol
Metabolites of Δ–9–tetrahydrocannabinol
GC–MS/MS
Cannabis active use

ABSTRACT

Hair analysis for the assessment of cannabis active use from passive consumption may be failed when performed by the sole detection of compounds present in plant material as well as in cannabis smoke like Δ-9-tetrahydrocannabinol (THC), cannabidiol (CBD), and cannabinol (CBN). For this reason, the determination of 11-nor-9-carboxy-Δ-9-tetrahydrocannabinol (THC-COOH) has been proposed by the Society of Hair Testing (SoHT) in order to prove active cannabis consumption. The identification of THC-COOH in hair will continue to be complicated by its acidic nature and the critical low concentration due to the preferential incorporation of basic compounds into hair shaft. Alternatively, 11-OH-THC may be considered as a complementary marker for THC administration. Our recent study reported an accurate validated procedure for THC, CBD, CBN and 11-OH-THC in hair, based on a GC/MS-MS method in electron ionization mode. However, unlike THC-COOH, a cut-off level for 11-OH-THC in hair has not been fixed yet. For this reason, the aim of this study is to propose a concentration value for 11-OH-THC in hair analysis in order to discriminate between chronic use and external contamination. Receiver operating characteristics (ROC) analysis was applied for cut-off evaluation after 11-OH-THC quantification in a pool of 672 THC-positive hair samples. Results have shown a concentration range between 0.01–5.34 ng/mg for THC (mean 0.34 ng/mg, median 0.12), 0.00–19.2 pg/mg for THC-COOH (mean 0.72 pg/mg, median 0.19 pg/mg) and 0.01–13.33 ng/mg for 11-OH-THC (mean 1.09 ng/mg, median 0.51 ng/mg) for scalp hair and between 0.03–6.32 ng/mg for THC (mean 0.82 ng/mg, median 0.30), 0.00–42.1 pg/mg for THC-COOH (mean 2.70 pg/mg, median 1.08 pg/mg) and 0.00–7.88 ng/mg for 11-OH-THC (mean 1.70 ng/mg, median 0.89 ng/mg) for body hair. Considering these experimental data collected in our laboratory, we propose a cut-off level of 0.5 for scalp and body hair, as indicative of cannabis active consumption. The ROC curve AUCs for 11-OH-THC were 0.873 and 0.884 in 590 scalp hair and 82 body hair samples, respectively. The comparison of the results for THC-COOH (control method) and 11-OH-THC (test method) was also made by means of the Cohen's kappa statistics providing a good agreement according to both Landis & Koch and Fleiss scales. Additionally, we suggest that the detection of both THC-COOH and 11-OH-THC should be mandatory in order to prove active intake and exclude false positive results from external contamination.

1. Introduction

Cannabis is the most widely illicit drug abused worldwide and the number of cannabis users continues to rise [1]. Cannabinoids are rapidly and extensively metabolized by hepatic enzymes [2]. The main pathway involves hydroxylation of Δ-9-tetrahydrocannabinol (THC) to 11-hydroxy-Δ-9-tetrahydrocannabinol (11-OH-THC), a psychoactive metabolite, followed by further oxidation to inactive 11-nor-9-carboxy-Δ-9-tetrahydrocannabinol (THC-COOH). THC and its metabolites subsequently undergo phase II biotransformation to glucuronide conjugates [3]. However, in hair samples the sole analysis of psychoactive THC, as well as the other compounds present in plant material such as cannabidiol (CBD) and cannabinol (CBN), is not conclusive for the assessment of cannabis active use from passive consumption. For this reason, the determination of THC-COOH has been proposed by the Society of Hair Testing (SoHT) in order to prove a body passage next to the parent compound [4]. However, the identification of THC-COOH in hair will continue to be complicated by the acidic nature of this metabolite, which leads to a critical incorporation rates into the hair matrix. It is well known that the hair incorporation of neutral cannabinoids (e.g. THC and 11-OH-THC) is increased if compared to acidic metabolites [5,6]. Indeed, the pH gradient from blood to the more acidic hair matrix counteracts an effective incorporation. For this reason, very sensitive and specific techniques – i.e. gas chromatography–tandem mass spectrometry (GC/MS-MS) operating in negative chemical ionization mode (NCI) [7] – are required to identify and quantify low amounts of THC-COOH

* Corresponding author.
Email addresses: sara.casati@unimi.it (S. Casati); ilaria.angeli@unimi.it (I. Angeli); alessandro.ravelli@unimi.it (A. Ravelli); massimo.delfabbro@unimi.it (M. Del Fabbro); mauro.minoli@unimi.it (M. Minoli); marica.orioli@unimi.it (M. Orioli)

https://doi.org/10.1016/j.forsciint.2019.109951
0379-0738/

in hair. According to SoHT and European Workplace Drug Testing Society (EWDTS), a 0.2 pg/mg cut-off for THC-COOH is recommended [4,8], whereas the US Substance Abuse and Mental Health Services Administration (SAMHSA) proposes a cut-off of 0.05 pg/mg [9]. However, unlike THC-COOH, a cut-off value for 11-OH-THC in hair has not been established yet. The concentration of 11-OH-THC in hair is expected to be higher than THC-COOH , the latter being more polar than its precursor and being the drug lipophilicity directly correlated with the extent of hair deposition.

A recent study written by our group reported an accurate validated procedure for 11-OH-THC in hair, based on a GC/MS—MS method in electron ionization (EI) mode [10]. The method we set-up allows also the evaluation of THC, CBD, CBN through a single-step liquid-liquid extraction (LLE) procedure and a single GC/MS—MS acquisition in order to save time, cost and leading to a great advantage comparing to the procedure in use to detect THC-COOH. In the present study the analytical results of 672 THC-positive hair samples were evaluated for THC, 11-OH-THC and THC-COOH in accordance with Angeli et al. [10] and Minoli et al. [7] fully validated methods. Receiver operating characteristics (ROC) analysis was applied for 11-OH-THC cut-off evaluation and the Youden's index was calculated in order to measure the performance of the diagnostic test [11]. The Cohen's Kappa statistics [12], the most used agreement measure in literature, was calculated between 11-OH-THC and THC-COOH values. This test is normally used to assess the agreement between evaluators of dichotomic variables (yes/no), but in this case it has also the purpose to estimate the agreement between the identification of positive samples to cannabis by using the metabolites THC-COOH (control method) and 11-OH-THC (test method). Our aim was to identify a tentative discriminating 11-OH-THC concentration to be used as a cut-off in hair analysis, in order to distinguish active use and external contamination of cannabis.

2. Materials and methods

2.1. Sample collection

Scalp (at least 3 cm length) and body THC-positive hair samples for this study were collected from male and female (n = 672; n = 590 scalp hair and n = 82 body hair) tested for drugs chronic abuse in our Laboratory from 2015 to 2019. Diagnosis of chronic drugs abuse was requested for different purposes, such as driving license renewal, adoptions or child custody.

2.2. Sample preparation

The methods normally used in our laboratory to detect THC, 11-OH-THC [13] and THC-COOH [7] were applied to 672 THC-positive hair samples. In brief, hair samples were reduced in short cuts (2–4 mm) and 50 mg were washed with dichloromethane (3 mL) and dried. The internal standard (THC-d3) was added to the samples and treated with 1 mL NaOH solution (1 M) for 20 min at 90 °C. Then, samples were added with 4 mL n-hexane/ethyl acetate (9:1, v/v). After centrifugation, the organic layer was separated and dried under a stream of nitrogen. The dried residue was then derivatized with MSTFA and 2 µL aliquot was injected into GC/MS-MS system for THC and 11-OH-THC analysis. The remaining basic solution was used for THC-COOH extraction. After pH adjusting to 3.5, the solution was added with THC-COOH-d3 and 4 mL n-hexane/ethyl acetate (9:1, v/v). After extraction, the residue was derivatized by adding PFPA and HFIP at 70 °C and then reconstituted in ethyl acetate. 1 µL aliquot was injected into GC/MS-MS system for THC-COOH determination.

2.3. Equipment

Our previously published methods were applied for the identification and quantification of THC, 11-OH-THC (LOD 0.03 pg/mg, LOQ 0.1 pg/mg) [10] and THC-COOH ((LOD 0.01 pg/mg, LOQ 0.04 pg/mg) [7] in scalp hair. Briefly, GC/MS-MS analysis were performed on an Agilent (Palo Alto, CA, USA) 7000B MSD gas chromatograph interfaced with a triple quadrupole detector operating in EI (THC and 11-OH-THC) and NCI (THC-COOH) mode by multiple ion monitoring (MRM). The GC separation was carried out on a capillary column DB5-MS (15 m x0.25 mm i.d., 0.25 µm film thickness).

2.4. Statistical analysis

Statistical analysis was conducted using MedCalc Statistical ver. 18.1 (MedCalc Software, Ostend, Belgium; https://www.medcalc.org, 2019). Sensitivity, specificity, predictive value, accuracy and receiver operating characteristic (ROC) analysis were applied to optimize the cut-off value. Area under ROC curves (AUROC) were obtained and the difference between the areas was analyzed using the method of DeLong et al. [14] and the Youden's index was calculated in order to measure the performance of the diagnostic test [11]. The comparison of the results for THC-COOH (control method) and 11-OH-THC (test method) was also made by means of the Cohen's kappa statistics [12] in order to evaluate their agreement according to the Landis and Koch scales [15] and the Fleiss scale [16].

3. Results and discussion

3.1. Comparison of the results for THC, THC-COOH, and 11-THC-OH from THC-positive hair samples

A total of 672 THC-positive hair samples (THC > 0.01 ng/mg hair [10]) were included in the calculations, THC-COOH [7] and 11-OH-THC [10] have been quantified (≥ LLOQ) in 513 and 578 scalp hair samples and in 73 and 81 body hair samples, respectively. Both metabolites could be detected from more than half of these samples providing a definitive proof of active cannabis use. In 38.5% of the whole THC-positive cases, neither 11−OH-THC nor THC-COOH were detectable. These data mean that an environmental exposure to cannabis smoke or an external contamination cannot be excluded by the sole detection of THC in hair. However, 11-OH-THC was more frequently detected than THC-COOH both in scalp hair and body hair samples group. Arithmetic means and medians, 95% confidence intervals (CI) based on all samples including the 11-OH-THC and THC-COOH negative results as well as the concentrations range relative to THC, 11-OH-THC and THC-COOH for each group were calculated. The results are displayed in Table 1 and 2. The calculated mean values of the two metabolites concentrations were considerably different: in scalp hair, THC-COOH mean value was found to be 34% lower than 11-OH-THC, whilst it was 58% higher in body hair samples. Moreover, the levels of both THC-COOH and 11-OH-THC found in body hair samples were much higher than those found in scalp hair samples (3.7 and 1.5 times, respectively). According to Cirimele et al. [17], higher cannabinoids concentrations have been found in body hair versus scalp hair. These differences are explained by an increased incorporation from sweat or sebum during the longer telogen stage, representation of another time period due to the

Table 1
Median, arithmetic mean, 95% confidence intervals (CI) and range concentration of THC, THC-COOH, 11-OH-THC in scalp hair samples. (* : > cut-off).

Compound	Mean	Median	95%CI	Range
THC (ng/mg) (N=672, 100% positive*)	0.34	0.12	0.28, 0.40	0.01 – 5.34
THC-COOH (pg/mg) (N=291, 43% positive*)	0.72	0.19	0.60, 0.83	0.00 – 19.2
11-OH-THC (pg/mg) (N=292, 43.5% positive*)	1.09	0.51	0.96, 1.22	0.01 – 13.33

Table 2
Mean, median, 95% confidence intervals (CI) and range concentration of THC, THC-COOH, 11-OH-THC in body hair samples. (* : > cut-off).

Compound	Mean	Median	95% CI	Range
THC (ng/mg) (N=82, 100% positive*)	0.82	0.30	0.50, 1.15	0.03 – 6.32
THC-COOH (pg/mg) (N=63, 76.8% positive*)	2.70	1.08	1.43, 3.97	0.00 – 42.1
11-OH-THC (pg/mg) (N=60, 73.2% positive*)	1.70	0.89	1.29, 2.10	0.00 – 7.88

different growth cycle, differences in pigmentation and less exposition to light, weather and cosmetic treatments.

In addition, the mean values relative to 11-OH-THC in scalp and body hair are more similar to each other than those relative to THC-COOH.

Furthermore, the mean concentration ratios 11-OH-THC/THC and THC-COOH/THC were evaluated in positive hair samples (THC-COOH/11-OH-THC > cut-off) as follow: 0.98 (±1.89, standard deviation (SD)) and 1.10 (±2.23, SD) for scalp hair, 0.17 (±0.11, SD) and 0.65 (±0.67, SD) for body hair, respectively. We can observe a very high variability between the different subjects, however THC-COOH/THC ratio is slightly higher in both scalp and body hair groups than 11-OH-THC/THC. More data are necessary in order to evaluate the possible correlation between THC and its different metabolites hair incorporation.

Moreover, to see if the results might be biased correlated, the Pearson product-moment correlation coefficient (PPMCC) between THC-COOH and 11-OH-THC was calculated showing a correlation coefficient of 0.25. Since PPMCC is a value between +1 and −1, where 1 is total positive linear correlation, 0 is no linear correlation and −1 is total negative linear correlation, a PPMCC of 0.25 indicates a slightly low correlation. In parallel, median concentration ratios of THC to 11-OH-THC and THC to THC-COOH of 0.56% and 0.31% for positive hair samples were found and may serve as an indicator of chronic abuse of cannabinoids.

3.2. ROC analysis and method performance

For the estimation of an applicable cut-off value for the differentiation between active use and external contamination of cannabis a ROC analysis was performed. The test was performed considering THC-COOH as the true parameter with a cut-off equal to 0.2 pg/mg according to SoHT [4]. The results of the ROC analysis are shown in Fig. 1.

The ROC curve AUCs for 11-OH-THC were 0.873 with a standard error (SE) of 0.01 and 95% confidence interval (CI) ranging from 0.844 to 0.899 (p value <0.001) for 590 scalp hair samples and 0.884 with a SE of 0.05 and 95% CI ranging from 0.795 to 0.944 (p value <0.001) for 82 body hair samples. That means that 87% and 88% of positives ranked before a uniformly drawn random negative. To determine the optimal cut-off, a minimum sensitivity and specificity of 80% was defined. The Youden's index suggests cut-off values of 0.51 and 0.52 pg/mg for scalp and body hair specimens, respectively. In the Fig. 2, data of the negative and positive groups are displayed as dots on two vertical axes. A horizontal line indicates the cut-off point with the best separation (minimal false negative and false positive results) between the two groups. For these cut-off values, we found a sensitivity of 81.4% and a specificity of 82.2% for scalp hair samples, 90.5% and 89.5% for body hair samples. The positive predictive value (PPV) and the negative predictive value (NPV) were 81.4% and 82.2% for scalp hair, and 90.5% and 84.2% for body hair samples. The accuracy of the tests was 81.8% and 89.0% for scalp hair and body hair, respectively.

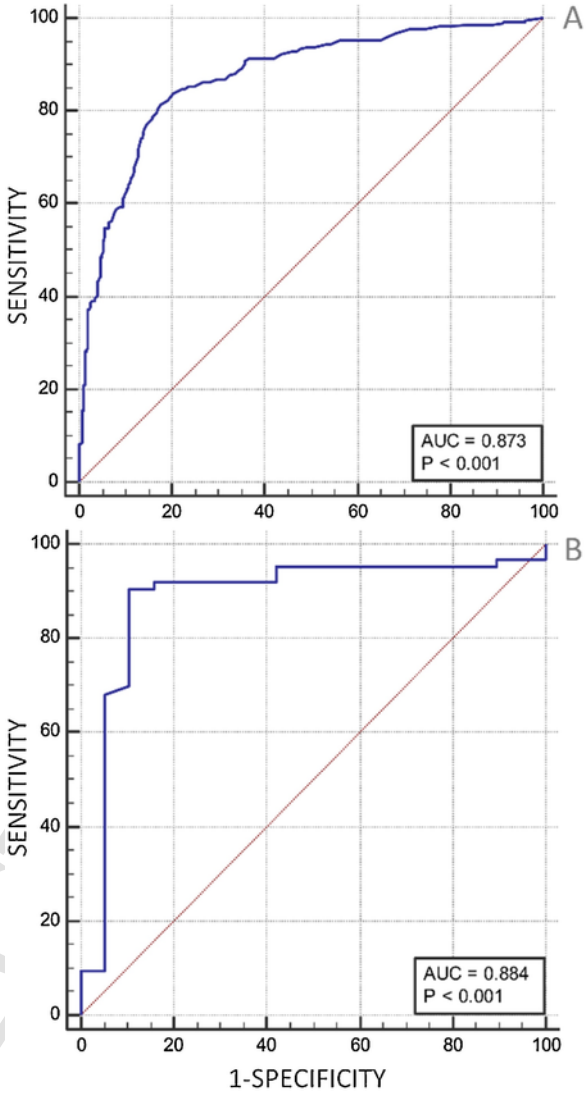

Fig. 1. Receiver operating characteristic (ROC) graphs for 11−OH-THC measured in scalp hair (A) and body hair (B) samples.

3.3. Cohen's kappa analysis

By considering a threshold equal to 0.2 pg/mg for THC-COOH and of 0.51 or 0.52 pg/mg for 11-OH-THC for a sample to be considered positive, in a total of 590 scalp hair and 82 body hair

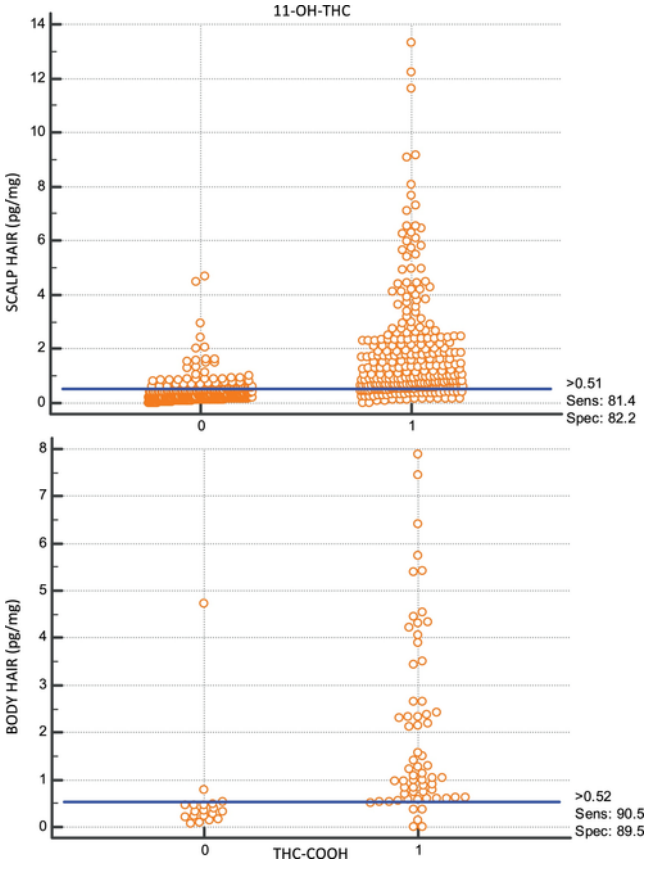

Fig. 2. Data dot diagrams of negative (0) and positive (1) groups at the 11−OH-THC cut-off point of 0.51 pg/mg for scalp hair (A) and 0.52 pg/mg for body hair (B) specimens. The corresponding test characteristics sensitivity and specificity are shown at the right side.

samples evaluated the Cohen's Kappa statistics, was 0.64 and 0.71, respectively. The result of the Cohen's kappa test is a number between a negative value and 1, where 1 indicates 100% agreement and <0 indicates no agreement at all. There are two scales for interpreting the results of the Kappa statistics. The Landis and Koch scale rates the results as follows: k<0: no agreement; 0 ≤ k<0.20: slight agreement; 0.20 ≤ k<0.40: fair agreement; 0.40 ≤ k<0.60: moderate agreement; 0.60 ≤ k<0.80: substantial agreement; 0.80 ≤ k<1: almost perfect agreement; 1: perfect agreement [15]. The Fleiss scale rates the results as follows: k<0.40: poor agreement; 0.40 ≤ k<0.75: fair to good agreement; k≥0.75: excellent agreement [16]. Our results can be interpreted as a substantial agreement according to the Landis and Koch scale, and a good agreement according to the Fleiss scale, between the two diagnostic markers. In detail, 238 samples resulted positive and 245 negative for both metabolites, 54 samples were negative for THC-COOH and positive for 11-OH-THC, and 53 were positive for THC-COOH and negative for 11-OH-THC in scalp hair samples group (Table 3). Results for body hair samples are shown in Table 4.

4. Conclusions

Cannabis users recently continues to rise and hair analysis for THC is regularly performed in laboratories worldwide for clinical and forensic purposes. Unlike its more common acidic metabolite THC-COOH, a cut-off value for 11-OH-THC in hair has not been fixed up to now. We propose tentative cut-off value for 11-OH-THC in order to prove active intake and exclude false positive results from external contamination. 672 THC-positive hair samples have been evaluated as previously reported. Our results suggest cut-off values of 0.51 pg/mg for scalp hair and 0.52 body hair specimens, which could be unified into 0.5 pg/mg. We showed that including 11-OH-THC in addition to THC-COOH into analysis may improve detection of cannabis consumption and avoid false positive and false negative risk. In our opinion, the hereby proposed criterium can be productively utilized and results strongly suggest 11-THC-OH to be a suitable marker to reliably prove cannabis use. In conclusion, we suggest that the detection of both THC-COOH and 11-OH-THC should be mandatory in order to prove active intake and exclude false positive result from external contamination.

Table 3
Schematic outcomes of the scalp hair samples analysis.

11-OH-THC	THC-COOH		Total
	Positive	Negative	
Positive	238	54	292
Negative	53	245	298
Total	291	299	590

Table 4
Schematic outcomes of the body hair samples analysis.

11-OH-THC	THC-COOH		Total
	Positive	Negative	
Positive	57	3	60
Negative	6	16	22
Total	63	19	82

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CRediT authorship contribution statement

Sara Casati: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Visualization. **Ilaria Angeli:** Methodology, Investigation, Conceptualization. **Alessandro Ravelli:** Methodology, Visualization. **Massimo Del Fabbro:** Software, Formal analysis, Writing - review & editing. **Mauro Minoli:** Conceptualization, Methodology, Validation. **Marica Orioli:** Conceptualization, Validation, Resources, Data curation, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

None.

Acknowledgements

The authors would like to thank Prof. Giorgio Binelli for his contribute to the manuscript.

References

[1] UNODC, World Drug Report 2018: Opioid Crisis, Prescription Drug Abuse Expands; Cocaine and Opium Hit Record Highs, in:

https://www.unodc.org/unodc/en/frontpage/2018/June/world-drug-report-2018_-opioid-crisis--prescription-drug-abuse-expands-cocaine-and-opium-hit-record-highs.html, 2018.
[2] S. Agurell, M. Halldin, J.E. Lindgren, A. Ohlsson, M. Widman, H. Gillespie, L. Hollister, Pharmacokinetics and metabolism of delta 1-tetrahydrocannabinol and other cannabinoids with emphasis on man, Pharmacol. Rev. 38 (1986) 21–43.
[3] H. M.A., Human Cannabinoid Pharmacokinetics, Chem. Biodivers. 4 (2007) 1770–1804 http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=emed8&NEWS=N&AN=2007441321.
[4] G.A.A. Cooper, R. Kronstrand, P. Kintz, Society of Hair Testing guidelines for drug testing in hair, Forensic Sci. Int. 218 (2012) 20–24, https://doi.org/10.1016/j.forsciint.2011.10.024.
[5] M.A. Huestis, R.A. Gustafson, E.T. Moolchan, A. Barnes, J.A. Bourland, S.A. Sweeney, E.F. Hayes, P.M. Carpenter, M.L. Smith, Cannabinoid concentrations in hair from documented cannabis users, Forensic Sci. Int. 169 (2007) 129–136, https://doi.org/10.1016/j.forsciint.2006.08.005.
[6] M. Uhl, H. Sachs, Cannabinoids in hair: strategy to prove marijuana/hashish consumption, Forensic Sci. Int. 145 (2004) 143–147, https://doi.org/10.1016/J.FORSCIINT.2004.04.029.
[7] M. Minoli, I. Angeli, A. Ravelli, F. Gigli, F. Lodi, Detection and quantification of 11-nor-Δ9-tetrahydrocannabinol-9-carboxylic acid in hair by GC/MS/MS in Negative Chemical Ionization mode (NCI) with a simple and rapid liquid/liquid extraction, Forensic Sci. Int. 218 (2012) 49–52, https://doi.org/10.1016/j.forsciint.2011.10.014.
[8] A. Salomone, L. Tsanaclis, R. Agius, P. Kintz, M.R. Baumgartner, European guidelines for workplace drug and alcohol testing in hair, Drug Test. Anal. 8 (2016) 996–1004, https://doi.org/10.1002/dta.1999.
[9] D.M. Bush, The U. S. Mandatory Guidelines for Federal Workplace Drug Testing Programs: Current Status and Future Considerations, 174, 2008111–119, https://doi.org/10.1016/j.forsciint.2007.03.008.
[10] I. Angeli, S. Casati, A. Ravelli, M. Minoli, M. Orioli, A novel single-step GC–MS/MS method for cannabinoids and 11-OH-THC metabolite analysis in hair, J. Pharm. Biomed. Anal. 155 (2018) 1–6, https://doi.org/10.1016/j.jpba.2018.03.031.
[11] W. Youden, Index for rating diagnostic tests, Cancer3 (1950) 32–35.
[12] J. Cohen, A coefficient of agreement for nominal scales, Educ. Psychol. Meas. XX (1960) 37–46.
[13] I. Angeli, S. Casati, A. Ravelli, M. Minoli, M. Orioli, A novel single-step GC–MS/MS method for cannabinoids and 11-OH-THC metabolite analysis in hair, J. Pharm. Biomed. Anal. 155 (2018) 1–6, https://doi.org/10.1016/j.jpba.2018.03.031.
[14] E.R. DeLong, D.M. DeLong, D.L. Clarke-Pearson, Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach, Int. Biometric Soc. 44 (1988) 837–845.
[15] R. Landis, G. Koch, An Application of Hierarchical Kappa-type Statistics in the Assessment of Majority Agreement among Multiple Observers Author (s): J. Richard Landis and Gary G. Koch Published by : International Biometric Society Stable URL : https://www.jstor.org/stab, Biometrics 33 (1977) 363–374.
[16] J.L. Fleiss, Measuring nominal scale agreement among many raters, Psychol. Bull. 76 (1971) 378–382, https://doi.org/10.1037/h0031619.
[17] V. Cirimele, P. Kintz, P. Mangin, Forensic science interniitiond testing human hair for cannabis, Forensic Sci. Int. 70 (1995) 175–182.