

Using THC-COOH cut-off concentrations for assessing cannabis consumption frequency: a recently detected THC-COOH isomer poses an important analytical problem

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Aim: To raise forensic toxicologists' awareness that a recently detected THC-COOH isomer may compromise the use of established THC-COOH cut-off concentrations in whole blood and serum for distinguishing between occasional and regular cannabis users and for deciding on administrative and legal consequences. **Methods:** Around 300 whole blood samples from cannabis consumers were analysed with a validated LC-ESI-MS/MS method which comprises protein precipitation, column-switching chromatography and mass spectrometric detection of the analytes by electrospray ionisation in positive ion mode and selected reaction monitoring (THC-COOH: m/z 345→327 (quantifier), m/z 345→299 (qualifier)). **Results:** Under the chromatographic conditions employed, THC-COOH was adequately separated from the interfering isomer. THC-COOH concentrations ranged from 2.7 – 112.0 ng/mL (median: 24.7 ng/mL). In a second step, both peaks were integrated to determine the contribution of the isomer to the intrinsic amount of THC-COOH when no separation is achieved. The concentrations thus obtained were 3.3 – 30.3% (median: 7.8%) higher than the true THC-COOH concentrations. For the qualifier transition, which is the predominant fragmentation reaction of the isomer, concentrations were 8.4 – 57.8% (median: 20.2%) higher. **Discussion:** Our results show that measured THC-COOH blood levels may be significantly higher if this isomer is co-eluting with THC-COOH during LC-ESI-MS/MS analysis. This can lead to inter-laboratory discrepancies in reported THC-COOH concentrations and in the assessment of the cannabis consumption frequency of suspected impaired drivers. **Conclusion:** We urge forensic toxicology laboratories to search for a consensus on how to deal with this analytical issue.

1. Introduction

According to the legal regulations effective for example in Switzerland and Germany, regular cannabis users are deemed unfit to drive and are therefore not entitled to hold or obtain a driver's license. A reliable evaluation of the cannabis consumption behaviour of suspected impaired drivers is thus important for deciding on administrative and legal measures (e.g. medical assessment of fitness to drive, suspension of the driver's license). Swiss and German forensic experts currently employ whole blood and serum concentrations of the THC metabolite 11-nor-9-carboxy-THC (THC-COOH) as diagnostic marker for the cannabis consumption frequency. In Switzerland, a THC-COOH whole blood level of 40 ng/mL is used as decision limit for regular cannabis consumption ('more than twice a week'), as recommended by Fabritius *et al.* [1, 2]. In Germany, the serum cut-off concentrations proposed by Daldrup *et al.* [3] are currently applied in forensic practice. Serum THC-COOH levels lower than 5 ng/mL are associated with occasional consumption, whereas concentrations above 75 ng/mL are assumed to be indicative of regular cannabis use (provided that the blood sample is taken within an eight-day period following the last cannabis use).

Reliable assessment of the cannabis consumption frequency based on these established thresholds requires accurate determination of THC-COOH in whole blood and serum.

However, Toennes *et al.*[4] found that THC-COOH levels measured by ESI or APCI LC-MS/MS in authentic samples may be erroneously elevated due the presence of a THC-COOH isomer. This isomer exhibits a fragmentation pattern virtually identical to that of THC-COOH, with the only difference being in the relative abundances of the fragment ions [4], and has not only been detected in blood, but also in various post-mortem specimens (i.e. urine, bile, liver, lung and kidney) from cannabis users [5]. According to the latest study, the isomer is a degradation product of the labile metabolite THC-COOH-glucuronide, with serum albumin and esterases being involved in the degradation process [6]. The structure and the detailed formation mechanism of the isomer, however, have not yet been identified.

In the present work, we evaluate the overestimation of the true THC-COOH blood concentrations by LC-ESI-MS/MS analysis due to co-elution of the isomer, especially with regard of its consequences on the THC-COOH concentration-based classification of the cannabis consumption behaviour. To this aim, we analysed the data obtained from the routine LC-ESI-MS/MS measurement of around 300 forensic blood samples from cannabis consumers.

2. Material and Methods

In our laboratory, whole blood concentrations of THC and its metabolites 11-OH-THC and THC-COOH are routinely determined by a previously published column-switching LC-MS/MS method [7] with minor modifications. Briefly, 200 μ L of whole blood samples are spiked with 20 μ L of internal standard solution (2 ng THC- d_3 and 11-OH-THC- d_3 , 10 ng THC-COOH- d_3) and proteins are precipitated by adding 600 μ L of acetonitrile. After mixing and centrifugation, the supernatant is evaporated to dryness at 50°C under nitrogen and reconstituted in 200 μ L of acetonitrile/water/formic acid, 60/40/0.1; v/v/v. Five microliters of the prepared samples are injected onto a trapping column (Phenomenex Mercury Synergi 4 μ m Polar RP 80 Å, 20 x 2.0 mm) and eluted in backflush mode to the analytical column (Phenomenex Kinetex 2.6 μ m C8 100 Å, 50 x 2.1 mm) by running a gradient of 30 – 97.5% B over 9 min, at a flow rate of 300 μ L/min (total run time: 12 min). Mobile phase A is 0.1% formic acid in water and mobile phase B is 0.1% formic acid in acetonitrile. Mass spectrometric detection of the analytes is accomplished with a Sciex 4500 QTRAP[®] operated in positive ESI and SRM mode. Linear ranges are 0.5 – 20 ng/mL for THC and 11-OH-THC, and 2.5 – 100 ng/mL for THC-COOH.

3. Results and Discussion

Our LC-ESI-MS/MS achieves partial separation of THC-COOH and its isomer (Fig. 1). As observed by Toennes *et al.* [4], the isomer elutes later than THC-COOH and exhibits both transitions monitored for THC-COOH (m/z 345 \rightarrow 327 (quantifier), 345 \rightarrow 299 (qualifier)), but with a different ion ratio. It was detected in all of the 297 whole blood samples considered for this study, with the isomer/THC-COOH concentration ratios being variable among samples.

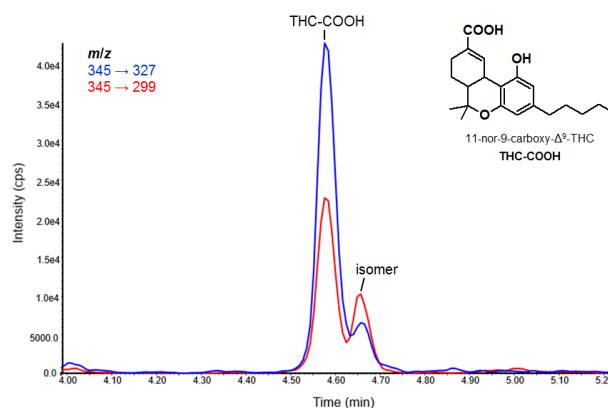


Fig. 1. Chemical structure of THC-COOH and SRM ion chromatogram of an authentic whole blood sample from a cannabis consumer.

For routine determination of THC-COOH, only the main THC-COOH peak is integrated (~4.47-4.63 min). THC-COOH levels in the 297 whole blood samples considered for this study ranged from 2.7 to 112.0 ng/mL (median: 24.7 ng/mL). The 40 ng/mL cut-off for regular cannabis was exceeded by 27.3% ($n = 81$) of the samples.

To make a quantitative estimate of the contribution of the isomer to the intrinsic amount of THC-COOH when chromatographic co-elution occurs, we integrated both the THC-COOH and isomer peak. For the quantifier transition, the concentrations thus obtained were 3.3 – 30.3% (median: 7.8%) higher than the true THC-COOH concentrations and consequently an additional 3.4% ($n = 10$) of the cannabis consumers had THC-COOH blood levels > 40 ng/mL. For the qualifier transition, the predominant fragmentation reaction of the isomer, concentrations were 8.4 – 57.8% (median: 20.2%) higher and an additional 8.4% ($n = 25$) of the samples exceeded the 40 ng/mL cut-off for regular cannabis use.

Our results show that co-elution of THC-COOH and the isomer during LC-ESI-MS/MS analysis can lead to significant overestimation of the THC-COOH blood levels and can thus give rise to an erroneous suspicion of regular cannabis use. To date, however, it is still unclear whether the detection of this isomer in biological samples from cannabis consumers is only achievable with ESI or APCI LC-MS/MS methods, and whether and to what extent the detection depends on the extraction technique [4].

4. Conclusion

The possible overestimation of THC-COOH blood levels due to the presence of a THC-COOH isomer can lead to inter-laboratory discrepancies in reported THC-COOH concentrations and in the assessment of the cannabis consumption frequency of suspected impaired drivers. In our opinion, forensic toxicology laboratories should verify whether their analytical methods currently used for the determination of THC-COOH are affected and should come to a consensus on how to deal with the analytical issues posed by the isomer.

5. References

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