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In-source fragmentation of Δ9-THCA-A detected in hair samples of cannabis users: a simple approach to identify external contamination?

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1. Introduction

An LC-MS/MS method for the quantification of Δ9-tetrahydro-cannabinol (THC), cannabinol (CBN) and cannabidiol (CBD) in hair was established. Previously unreported additional signals (artefacts) for THC and CBN were detected in hair samples from cannabis users. The aim of this study was to shed light on the origin of these artefacts.

2. Methods

- Sample preparation was performed as shown in **Fig. 1**

LC-MS/MS (THC, CBN, CBD)

- Samples were analyzed on a Shimadzu HPLC coupled to a Sciex 5500 QTrap system using scheduled Multiple Reaction Monitoring (sMRM)
- Gradient elution using a Kinetex® C18 100 mm x 2.1 mm, 100 Å, 1.7 µm; flow rate: 0.5 mL/min
- The MS instrument was operated in atmospheric pressure chemical ionization **(APCI), positive mode**
- Identification and quantification was achieved using one quantifier and one qualifier.
- Validation experiments were performed according to the international guidelines of GTFCh[1].

LC-MS/MS (Tetrahydrocannabinolic acid (THCA-A))

- Sample preparation and LC-conditions as described above
- Ionization using **APCI, negative mode**
- Identification was achieved using three transitions

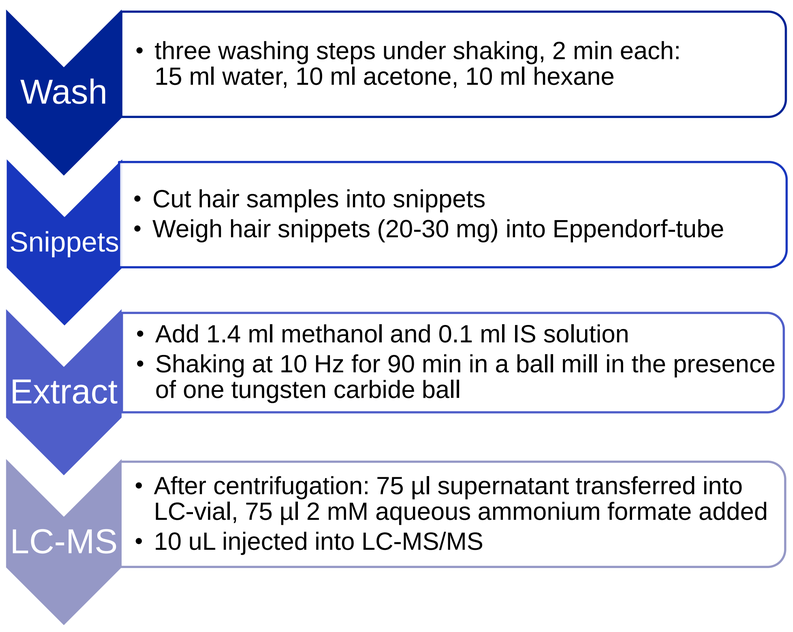

Fig.1: Scheme of the sample preparation workflow

Table 1: Validation Parameters

QC sample	Analyte	Mean value (pg/mg)	Accuracy (%)	Repeat-ability (%)	Inter-day precision (%)	Recovery (SD) %	Matrix effects (SD) %
QC_{low}	CBD	28	13	6.2	5.9	122 (5.4)	107 (13)
	CBN	26	5.7	7.1	8.1	126 (4.0)	133 (15)
	THC	23	-6.4	9.6	8.0	115 (12)	116 (23)
$QC_{med.}$	CBD	1‘419	-5.4	5.9	4.5	N/A	N/A
	CBN	1‘387	-7.6	5.6	5.2	N/A	N/A
	THC	1‘461	-2.6	5.3	4.8	N/A	N/A
QC_{high}	CBD	4‘541	0.9	5.2	4.0	114 (6.5)	130 (15)
	CBN	4‘612	2.5	4.9	7.8	125 (9.2)	126 (18)
	THC	4‘701	4.5	5.2	6.5	123 (13)	126 (22)

N/A = not determined

3. Spiking experiments

1st Experiment (**Fig.3**)
Prior to extraction, 0 pg, 8’000 pg or 16’000 pg of THC and CBN were added to the hair sample of a cannabis user.

2nd Experiment (**Fig.4**)
Prior to extraction, 0 pg, 40’000 pg or 200’000 pg of THCA-A were added to the hair sample of a cannabis user.

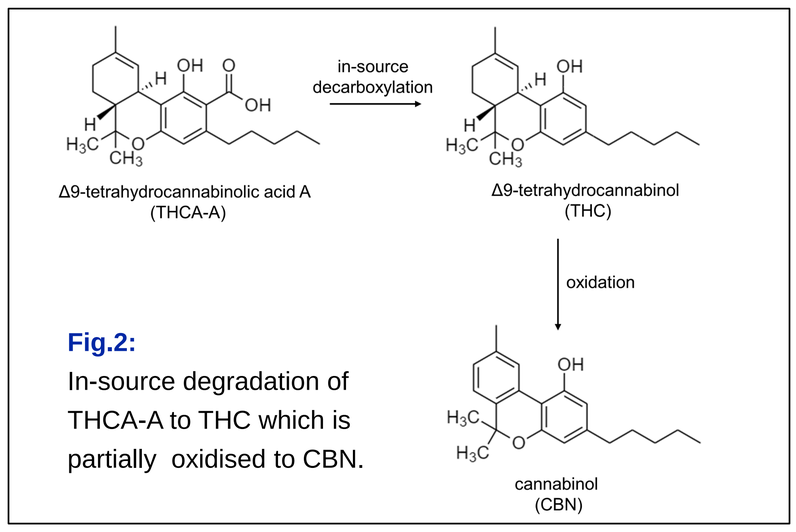

Fig.2:
In-source degradation of THCA-A to THC which is partially oxidised to CBN.

Conclusion

The data indicates in-source fragmentation of the thermo-labile THCA-A to THC which is to some extent further oxidized to CBN using APCI-LC-MS (**Fig. 2**). Therefore, in-source degradation of THCA-A should be taken into account for the analysis of hair samples from cannabis users which are typically contaminated with THCA-A. For APCI-LC-MS methods with poor LC-separation between THCA-A and THC (and/or CBN), data should be interpreted with caution as in-source degradation of THCA-A may lead to falsely increased THC/CBN-concentrations. The data suggests that the presence of these artefacts and the artefact-to-analyte ratios may be useful indirect markers for the detection of external contamination.

4. Results and Discussion

- Validation parameters for the identification and quantification of THC, CBN and CBD are listed in **Table 1**.
- Signals of artefacts were observed at 9.8 min for THC and CBN in hair samples from cannabis users in APCI LC-MS.
- THCA-A was detected at the same retention time as the signals of the artefact (9.8 min).
- After spiking CBN and THC, the intensities of the artefacts remained unchanged in the hair sample of a cannabis user (**Fig. 3**).
- After spiking THCA-A, the signals intensities of the artefacts increased in the hair sample of a cannabis user (**Fig. 4**).
- Enhanced product ion (EPI) spectra revealed similar fragmentation patterns for CBN and THC and the respective artefacts, respectively (**Fig. 5**).
- The herein described in-source fragmentation of the thermo-labile THCA-A may be a useful indirect marker for external contamination as THCA-A derives from external contamination.

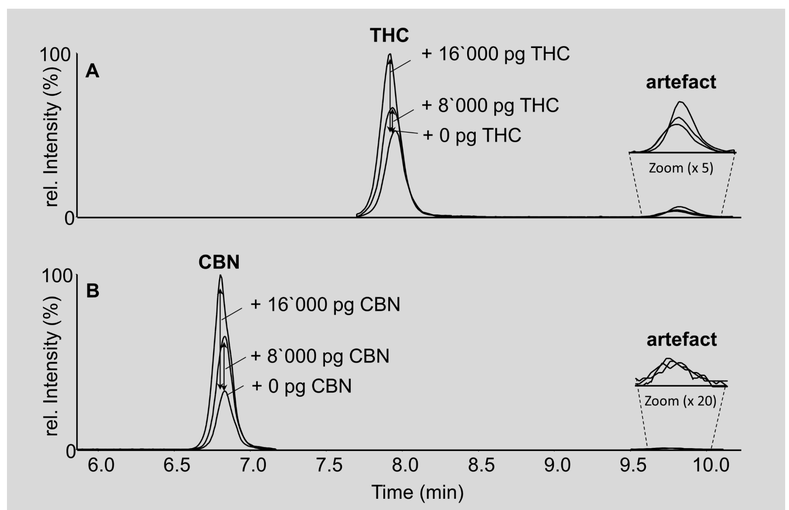

Fig.3: MS-Signals for the first MRM transition of (A) THC and (B) CBN after spiking 0 pg, 8’000 pg and 16’000 pg of CBN and THC, respectively, into the hair sample from a cannabis user.

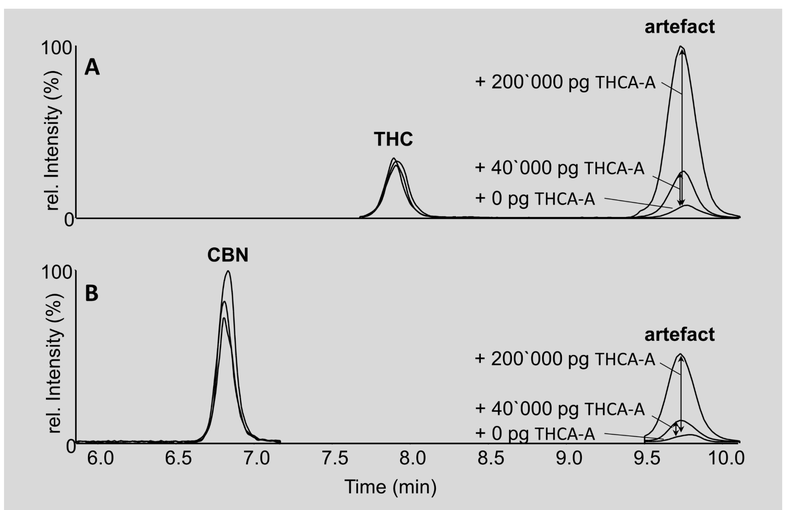

Fig.4: MS-Signals for the first transition of (A) THC and (B) CBN after spiking 0 pg, 40’000 pg and 200’000 pg of THCA-A into the hair sample from a cannabis user.

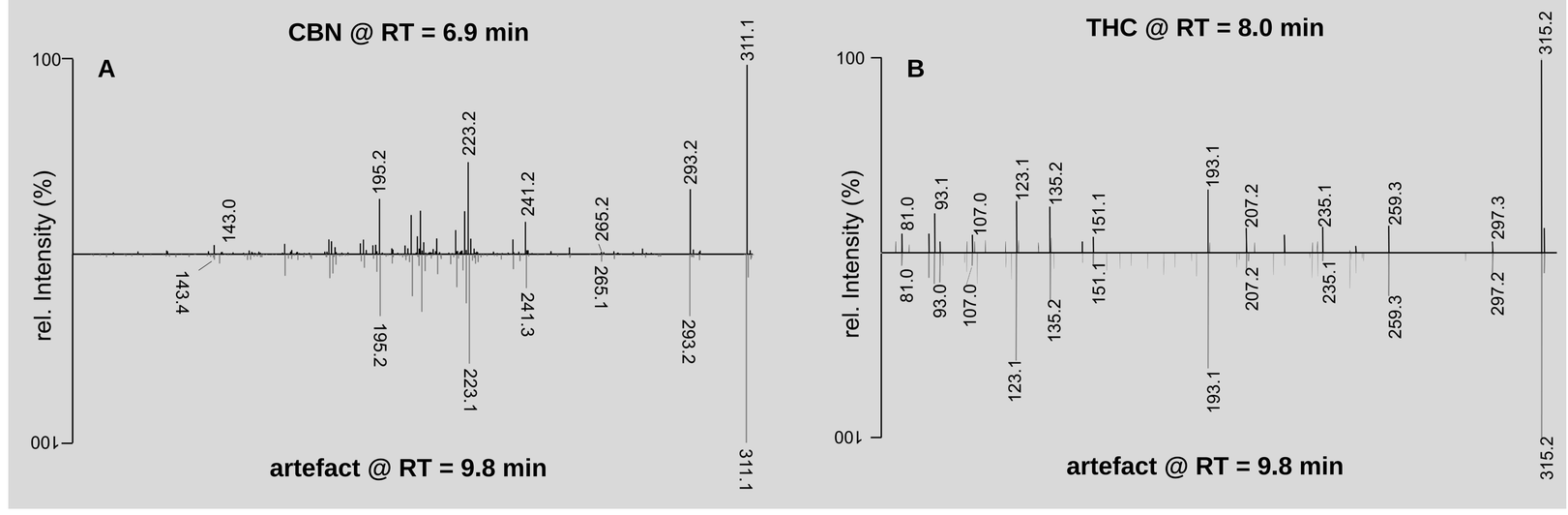

Fig. 5: EPI spectra for (**A**) CBN and (**B**) THC measured at the retention time of the analyte and the respective artefact.

Reference

1. Peters, F. T. et al. Anhang B zur Richtlinie der GTFCh zur Qualitätssicherung bei forensisch-toxikologischen Untersuchungen. Anforderungen an die Validierung von Analysenmethoden. Toxichem Krimtech 76, 185 (2009)

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