Analysis of THC and THC-COOH in plasma and urine using online extraction LC-MS/MS

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Introduction

Cannabis sativa is a drug of abuse largely used. Tetrahydrocannabinol (THC) is the most abundant component in the plant. After smoke assumption, THC is absorbed and distributed in blood; subsequently it is rapidly metabolized to THC-COOH, conjugated with glucuronic acid and excreted in urine.
LC-MS/MS is a useful tool to establish the assumption of Cannabis by the assessment of THC and/or THC-COOH in plasma and urine.
Furthermore, LC-MS/MS can be coupled with new techniques for the on-line sample purification which allow to inject plasma or urine without any sample preparation.

Goal

To develop a method for the analysis of THC and THC-COOH in human plasma and urine performing an on-line sample purification using a TurboFlow™ system coupled with a triple quadrupole mass spectrometer.

FIGURE 1. TurboFlow chromatography - a system for on-line sample preparation and sample purification

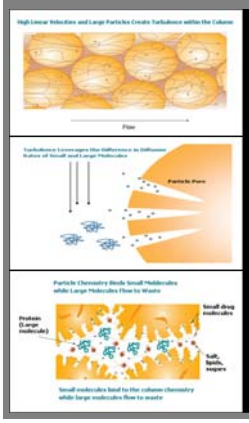


Figure 1a. Large particle columns (30 µm or larger) allow high flow rate with low back pressure.

Figure 1b. Small molecules diffuse into particle pores, large molecules (from matrix) do not diffuse and are washed to waste.

Figure 1c. The separation is bimodal, since additional retention time is achieved with different column chemistries.

Methods

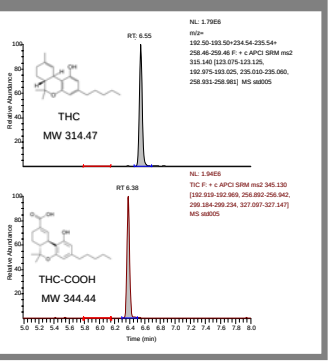
An online extraction TurboFlow™ system (Thermo Scientific) was employed, principles of a TurboFlow system are shown in Fig 1.

Cyclone-P™ column was used for sample purification while chromatographic separation was performed on an Hypersil Gold C18 50x3 5µm column (Thermo Scientific). Both for purification and separation, water and methanol were used as mobile phases. MS/MS analysis were carried out on a TSQ Quantum Access triple quadrupole mass spectrometer (Thermo Scientific) equipped with an Ion Max™ source.

Ion source: APCI +
Discharge Current: 4.5 kV
Sheath gas pressure: 5 (arbitrary units)
Auxiliary gas pressure: 1 (arbitrary units)
Ion transfer tube temperature: 300°C
Scan type: SRM
THC: m/z 315 → 193, 235, 259;
THC-COOH: m/z 345 → 193; 257, 299, 327;

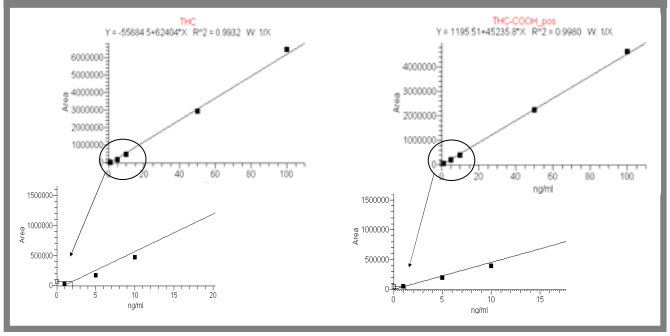
10µl of the samples were analyzed, plasma was injected without any sample preparation while urine has been hydrolyzed with basic treatment (NaOH), neutral pH were restored before the analysis.

FIGURE 2. Chromatographic conditions and typical ion chromatogram obtained



TurboFlow Column: Cyclone-P™
Analytical Column: Hypersil Gold C18 50x3mm 5um
Mobile Phases: A) H_2O
B) MeOH
C) Magic Mix
D) CH_3CN

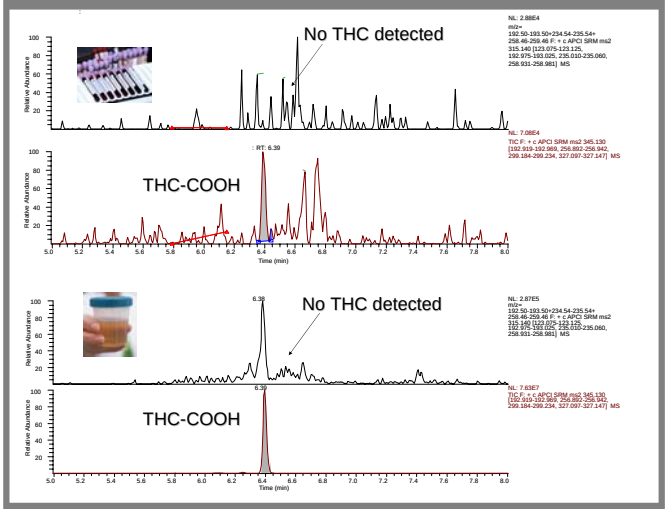
FIGURE 3. Calibration curve obtained in the range 1-100 ng/ml



Results and Discussion

Composition of mobile phases, chromatographic columns, gradient and a typical ion chromatogram obtained are shown in Fig 2; the total run time, including on-line extraction and chromatographic separation is 9.5 minutes per sample. Calibration curves for THC and THC-COOH were linear over the concentration range 1-100ng/ml with a R^2>0.99 (Fig 3) and LOQ of 5 ng/ml. Ion chromatograms obtained after the injection of biological samples are shown in Fig 4.
Plasma analysis showed a slight amount of THC-COOH (5.2ng/ml) while THC was not detected. The absence of THC can be explained by the fact that, in the case of smoked marijuana, plasmatic levels of THC increase rapidly in the first few minutes after inhaling, often to levels above 100 ng/ml. It then declines quickly within an hour [1].
As expected, in urine only THC-COOH was found (>100 ng/ml) in fact the main urinary metabolite is THC-COOH-glucuronide which gives unconjugates form after basic hydrolysis..

FIGURE 4. Ion chromatograms obtained after the injection of biological samples i.e. urine and plasma



Conclusions

The method enables the forensic toxicologist to assess the presence of THC and THC-COOH in plasma and urine with sensitivity and specificity. Since no sample preparation is required, as consequence, significant time is saved in the absence of SPE or liquid/liquid sample preparation.

References

[1] M. Huestis , J. Henningfield and E. Cone,"Blood Cannabinoids. I. Absorption of THC and Formation of 11-OH-THC and THCCOOH During and After Smoking Marijuana", *Journal of Analytic Toxicology*, Vol. 16: 276-282 (1992).