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 absorption, 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.

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 mm 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;
10 μl 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.

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 1-100 ng/ml with a R²>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 (5.2 ng/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.

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