Rapid quantification of free and glucuronidated THC-COOH in human urine using coated well plates and column-switching LC-MS/MS

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Introduction:

Cannabis is one of the most widely abused substances throughout the world. Not surprisingly, it is the most commonly detected illicit drug in workplace urine drug tests. The primary psychoactive component of cannabis is Δ^9-tetrahydrocannabinol (THC). 11-nor-9-carboxy-THC (THC-COOH) is the major metabolite of THC excreted in urine, primarily as glucuronide conjugate. Generally, cannabis consumption is detected by measuring the total concentration of THC-COOH in urine after enzymatic and/or alkaline hydrolysis of THC-COOH-glucuronide. As variable and incomplete hydrolysis can lead to erroneous results, direct measurement of free and glucuronidated THC-COOH is preferable. In addition, the THC-COOH-glucuronide/THC-COOH ratio in urine is supposed to be useful for assessing the extent of cannabis consumption. For these reasons a novel high-throughput LC-MS/MS method which allows for rapid and simultaneous quantification of both metabolites in human urine was developed.

Methods:

Sample preparation is achieved with Tecan AC Extraction Plates™. The inner surface of each well of these 96 well plates is partly coated with a sorptive material which acts as an extraction phase for relatively non-polar small molecules from aqueous solution. Sample processing is performed according to the workflow illustrated in Figure 1 using the following solutions:

- modifier buffer: H_2O/MeCN 90:10 (v/v) + 2% HCOOH
- wash solution: H_2O/MeCN 90:10 (v/v) + 1% HCOOH
- elution solvent: H_2O/MeCN 10:90 (v/v) + 0.2% HCOOH

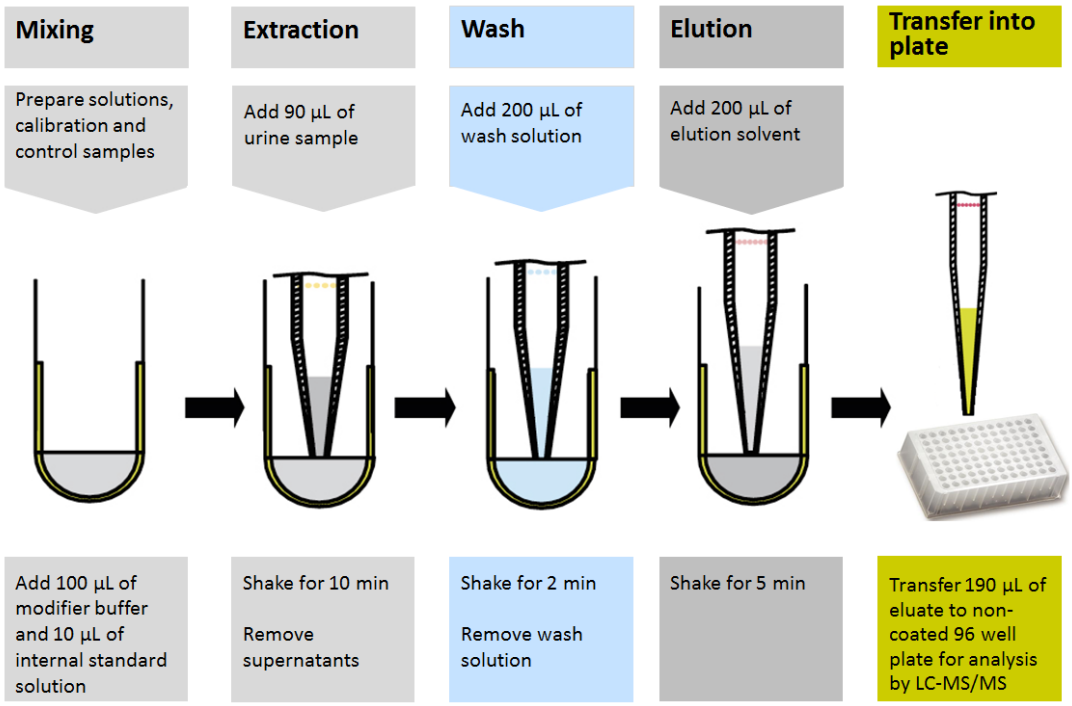

Figure 1. Sample preparation workflow with the Tecan AC Extraction Plate™.

40 µL of the processed sample is injected onto a trapping column (Phenomenex Synergi Polar RP, 10 x 2.0 mm) and eluted in backflush mode to the analytical column (Phenomenex Kinetex PFP, 30 x 2.1 mm) by a ballistic gradient. Detection of the analytes is accomplished by a hybrid triple quadrupole/linear ion trap tandem mass spectrometer (AB Sciex, 5500 QTrap) operated in positive electrospray ionization and selected reaction monitoring mode (two transitions per compound).

Results:

The method was validated according to the FDA guidelines on bioanalytical method validation. Details on the method validation will be presented. Both compounds are analyzed within 2.5 min with appropriate linear range (5.0 to 500 ng/mL), sensitivity and selectivity. So far, the method has been successfully applied to the analysis of more than 400 authentic urine samples originating from traffic controls or accidents, demonstrating the reliability of this technique in the clinical and forensic field.

Conclusions:

Tecan AC Extraction Plate™ allows for a simple sample preparation protocol which requires pipetting and shaking steps only and therefore can be automated using a robotic system. By combination of extraction with these coated well plates and column-switching LC-MS/MS

analysis a fast, robust and highly specific approach for the direct quantification of THC-COOH and THC-COOH-glucuronide in urine was developed. Its capacity for automation and high-throughput renders the presented method very attractive for workplace drug testing without previous enzymatic hydrolysis.