TECHNICAL NOTE

imcs
integrated micro-chromatography systems

A summary of "Accurate Quantitation of 11-nor-Δ9-tetrahydrocannabinol-9-carboxylic acid (THCA) in Urine using IMCSzyme®"

Overview:

Marijuana is a commonly used illicit drug and the active constituent, tetrahydrocannabinol (THC), is rapidly oxidized and metabolized into 11-nor-Δ9-tetrahydrocannabinol-9-carboxylic acid (THCA), which is conjugated to form glucuronides in biological systems. In order to accurately quantify these metabolites by LC-MS/MS from urine samples, the glucuronide conjugates are hydrolyzed. The use of IMCSzyme® for hydrolysis of THCA-glucuronide against crude enzyme was investigated.

Material and Methods

Drug standards (Cerilliant) and crude enzyme solution (Perkin Elmer) that contains both β-glucuronidase and sulfatase enzymes were provided with courtesy from LabSource, LLC. The novel recombinant β-glucuronidase enzyme (IMCSzyme) was from IMCS LLC. 50 - 200 µL of urine was hydrolyzed with 150 µL of hydrolysis solution (containing buffer, enzyme, and internal standards) with shaking. Incubation temperatures ranging from room temperature to 55 ˚C were evaluated for IMCSzyme and at 60 ˚C for the crude enzyme hydrolysis solution. The hydrolysis times ranged from 0 - 15 minutes for IMCSzyme and 60 minutes for crude enzyme. The hydrolyzed samples were processed with acetonitrile crash, and then diluted for LC-MS/MS analysis.

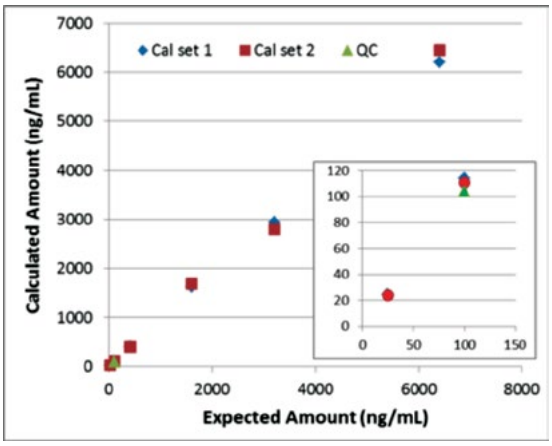

Figure 1. *Calibration curve of THCA (25-6400 ng/mL) in negative urine using IMCSzyme™. The correction of the calculated amount with the expected amount was greater than 0.99. (Insert) The magnified region of the first two calibration points*

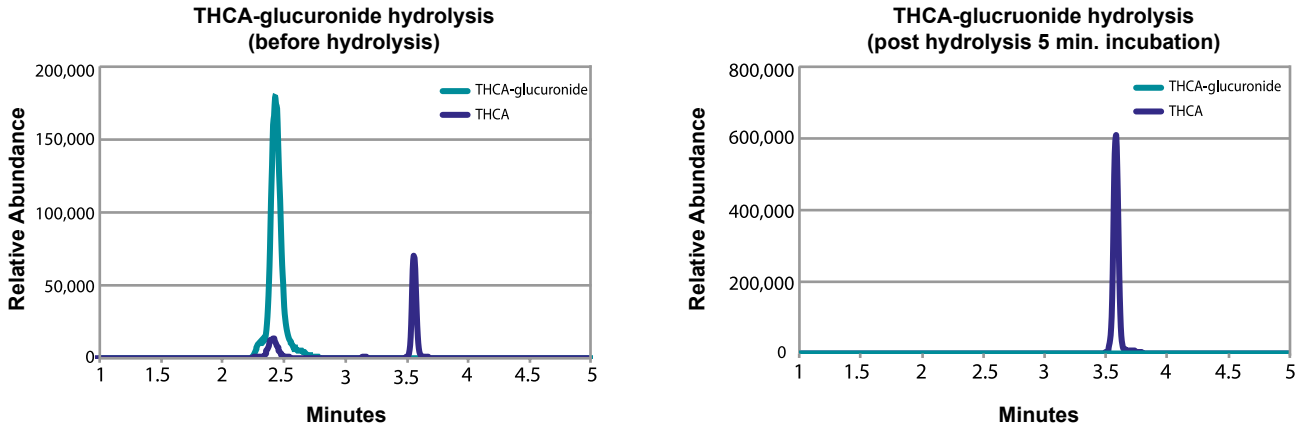

Figure 2. *Chromatograms of THCA-glucuronide (1000 ng/mL) and THCA in negative urine using IMCSzyme®. The detection of THCA in the unhydrolyzed sample (left panel), indicates some inherent instability of the analyte and the rapid hydrolysis of the IMCSzyme despite the immediate addition of acetonitrile (ACN) to precipitate the protein. After 5 minute incubation at 65 ˚C, the glucuronide peak is nearly undetected with a full conversion to the parent compound, THCA (right panel). For larger sample processing, there is an inherent 10-15 minute delay from the addition of enzyme, buffer and internal standard to the addition of ACN. Similar hydrolysis results were observed for samples that were not incubated at 65 ˚C, but at room temperature for 15 minutes prior to addition of ACN.*

Results

To validate the use of IMCSzyme, the calibration and quality control curves generated from neat urine spiked with THCA was assessed. The actual experimental values correlate well with the expected concentrations with R2 value > 0.99 (Figure 1). Quality control sample was hydrolyzed using neat urine spiked with 100 ppb of THCA-glucuronide (Figure 1, insert). The hydrolysis recovery was tested by monitoring both the THCA-glucuronide and THCA prior (Figure 2, left)and post (Figure 2B, right) hydrolysis. Several authentic patient urine samples (n = 88) that tested positive for THCA were hydrolyzed and analyzed using IMCSzyme or crude enzyme. The results demonstrated that calculated amounts of THCA were in agreement using both enzymes (R2 > 0.99) (Figure 3).

Conclusions

Use of IMCSzyme shortened the incubation time from 60 minutes down to minimal incubation without compromising the accuracy.

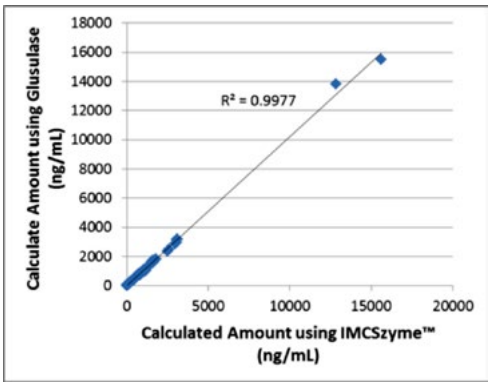

Figure 3. *Calculated amount of THCA in 88 authentic patient urine samples using IMCSzyme, compared to crude enzyme. The correlation of results using IMCSzymewith those using crude enzyme was greater than 0.99. The success of this blind study signifies the validity of this fast IMCSzyme compared to the crude enzyme.*

Technical summary from study performed by Andrew Lee at IMCS, LLC.