In Vitro Glucuronidation of Δ^9-Tetrahydrocannabinol-D3 by UDP-Glucuronosyl-Transferases detected using LC/MS-MS

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Introduction

From several studies it has been shown, that Δ^9-tetrahydrocannabinol-glucuronide is a phase II metabolite of Δ^9-tetrahydrocannabinol (THC) formed and excreted in urine in humans [1]. There is not much known either about the extent of glucuronidation in the metabolic degradation and elimination of Δ^9-tetrahydrocannabinol (THC) or about which one of the enzymes of the UDP-Glucuronosyl-Transferase (UGT) family is forming the THC-conjugate. Therefore, we studied seven commercially available variants of the UGTs regarding their enzymatic activity to THC-D3 (Figure 1).

In order to detect and monitor the formation of the glucuronide either GC/MS [2] or LC/MS could be considered as an analytical method. We have developed an LC/MS-MS method which has the advantage of obviating the need for enzymatic hydrolysis prior to measurement, as required with the known GC/MS method [3].

Ideally, isotopically labelled THC glucuronide would be necessary if the LC/MS-MS method is to be used for quantitative analysis. For this reason THC-D3 was used as substrate to test a new pathway to synthesize a standard of the THC-glucuronide-D3.

Materials and Methods

The enzymes UGT and the UGT Reaction Mix Solution A (UDPGA Cofactor) and Solution B (5x-UGT Buffer Mix with Alamethicin) were purchased from BD Bioscience (Woburn, MA, USA).

THC-D3 standard was obtained from LGC Promochem GmbH (Wesel, Germany).

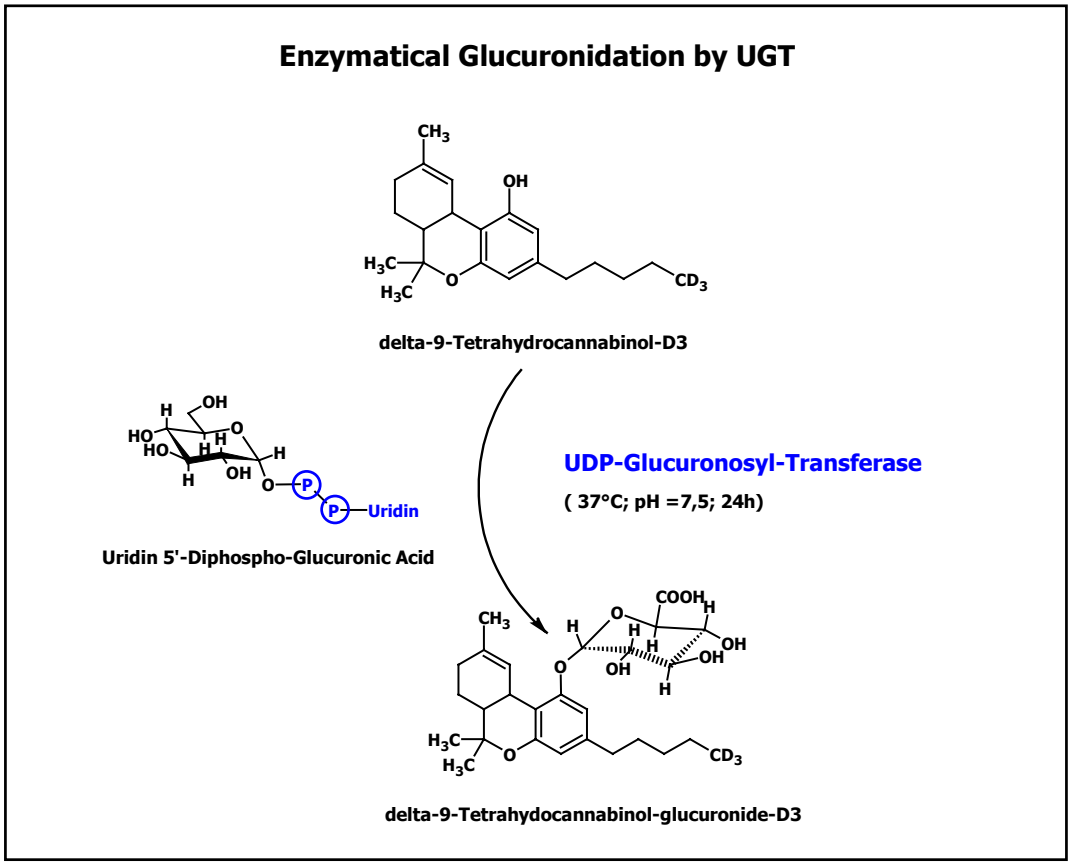

Figure 1. Enzymatical Glucuronidation by UGT

THC-glucuronide was purchased from El Sohly Laboratories (Oxford, Mississippi, USA).

The UGT-glucuronidation assay was performed at 37°C in Tris-Buffer-Solution (pH = 7.5) containing Reaction Mix Solutions A and B and stopped after 24 h hours. After separation from protein, the THC-Glucuronide-D3 product was obtained by liquid-liquid extraction with tert-Butylmethylether. Then the solvent was evaporated under N_2 and the residue was dissolved in HPLC solvent and injected into the LC/MS/MS system.

Multiple reaction monitoring (MRM) of the transition m/z 492.2 > 316.2 was used in the negative mode of an ESI-LC/MS-MS (triple quad mass spectrometer 4000 Q Trap, Applied Biosystems) for the detection of THC-glucuronide-D3 (Figure 2).

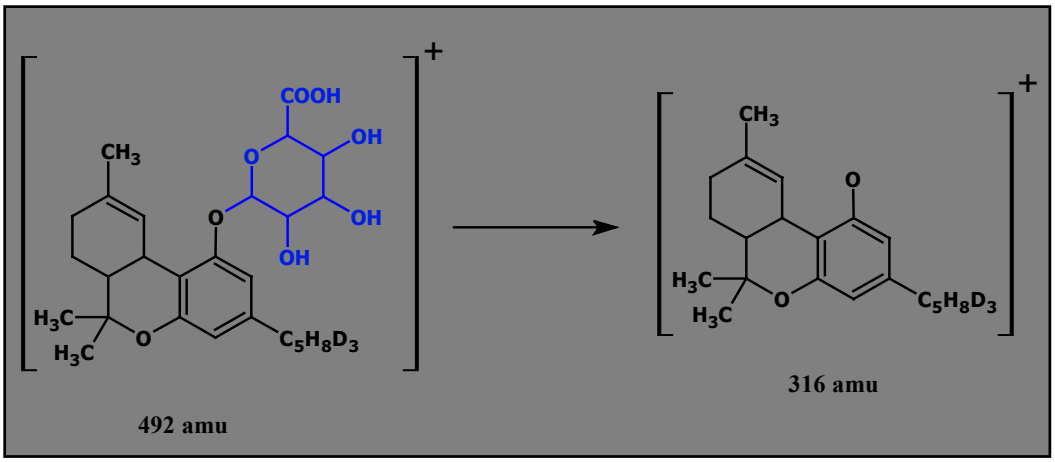

Figure 2. MRM Transition 492 > 316

Results and Discussion

Among the enzymes evaluated, UGT1A7, UGT1A10 and UGT2B7 turned out to form efficiently the THC-glucuronide-D3 within 24h under the conditions used (Figure 3).

UGT1A1, UGT1A3 and UGT1A4 catalysed the formation of THC-glucuronide-D3 too, but to a significantly lower extent.

Only UGT1A6 did not show any activity to convert THC-D3 into its glucuronide.

However, the qualitative study of the behaviour of the enzymes was the purpose of this work rather than determination of exact kinetic parameters.

LC/MS-MS using the transition m/z 492.2 > 316.2 has proven to be both selective and sensitive and therefore ideally suited to detect the product formation from the reaction mixture.

The enzymatic reaction has been demonstrated as a way to synthesize isotopically labelled THC-glucuronide-D3 in solutions that can be used for quantitative analysis by isotope dilution mass spectrometry (IDMS).

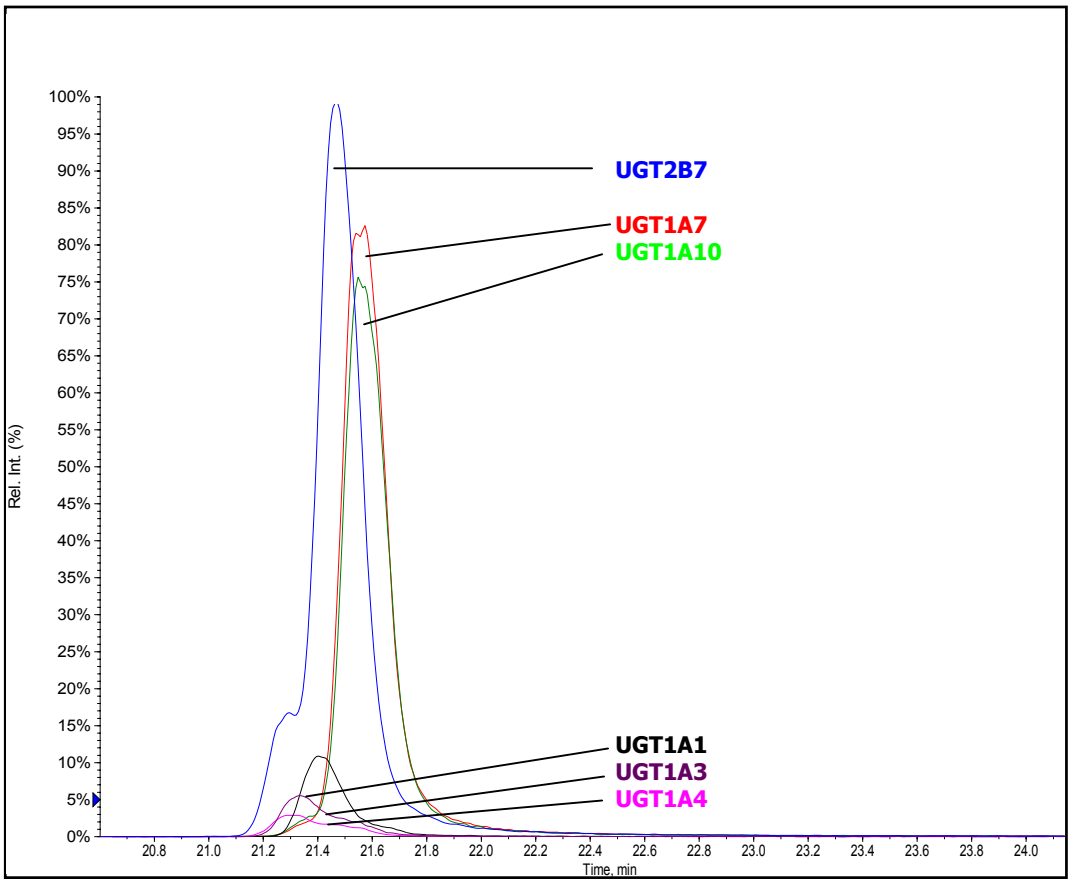

Figure 3. Influence of enzyme on yield of THC-glucuronide

Literature

[1] Harvey, D. J. and W. D. Paton (1984). "Metabolism of the cannabinoids." Rev.Biochem.Toxicol. **6**: 221-264.

[2] Halldin, M. M. and M. Widman (1983). "Glucuronic acid conjugate of delta 1-tetrahydrocannabinol identified in the urine of man." Arzneimittelforschung **33**(1): 177-8.

[3] Skopp, G., L. Pötsch (1999). "Freie und glucuronidierte Cannabinoide im Urin - Untersuchungen zur Einschätzung des Konsumverhaltens." Rechtsmedizin **10**: 21-28.

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