Impact of isomeric differences between internal standard and analyte for the LC-MS/MS quantification of 11-Nor-delta9-THC-9-carboxylic acid glucuronide

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1. Overview

Purpose

- Labeled (±)-cis-THC acid glucuronide is more cost-effective to produce than a labeled trans-diastereomer and is explored here as a possible internal standard for (+)-trans-THC acid glucuronide.

Methods

- HPLC-MS/MS for analysis of single component solutions containing internal standards.

Results

- Despite isomeric differences, (±)-cis-THC acid-D_3-glucuronide is shown to be an appropriate internal standard for the LC-MS/MS quantification of (+)-trans-THC acid glucuronide.

2. Introduction

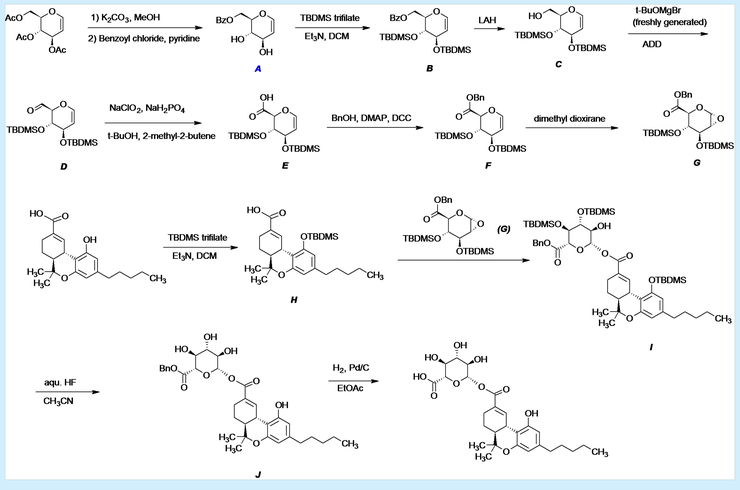
(-)-trans-11-Nor-delta9-tetrahydrocannabinol-9-carboxylic acid(THC acid) and THC acid glucuronide are urinary metabolites of *(-)-trans*-tetrahydrocannabinol (THC), an active constituent of marijuana. Chemical synthesis of THC acid and its glucuronide is complex and low yielding; the materials are not widely available. Development of reference standards focused on the most feasible diastereomers. The *(+)-trans*-isomer was synthesized and developed as a certified reference material for quantification of THC acid glucuronide by LC-MS/MS. (±)-*cis*-THC acid-D_3-glucuronide was developed as an internal standard. This study investigates the feasibility of (±)-*cis*-THC acid-D_3-glucuronide as an internal standard for the LC-MS/MS quantitative analysis of THC acid glucuronide.

The performance of a stable-labeled THC-acid glucuronide internal standard with isomeric differences from the analyte is investigated.

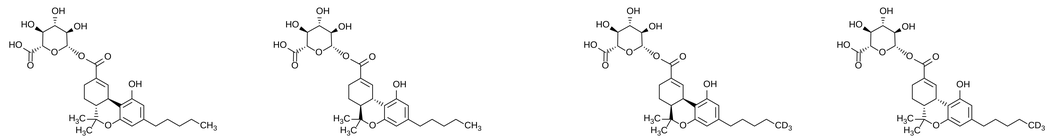
3. Synthesis of (+)-trans-THC-acid glucuronide

The synthesis of the *(+)-trans*-isomer is an 11-step process with an overall yield of 2% and is shown below.

The synthesis of the labeled (±)-cis isomer was also performed using the scheme below.



4. THC-acid glucuronide Diastereomeric Structures



(-)-trans- THC acid glucuronide (endogenous) | *(+)-trans*- THC acid glucuronide | *(-)-cis*- THC acid-D_3-glucuronide | *(+)-cis*- THC acid-D_3-glucuronide

5. Ideal Characteristics of an Internal Standard

An ideal internal standard mimics the properties of the analyte throughout the sample preparation and LC-MS/MS analysis in order to correct for preparation and analytical variability.

A properly selected internal standard paired with a well-developed LC-MS/MS method will improve accuracy or analytical quantification.

Internal Standard Factors to Consider	Ideal Characteristic	(±)-cis-THC acid-D3-glucuronide as ISTD for (+)-trans-THC acid glucuronide
Structure compared to analyte	As similar as possible e.g. stable-isotope labeled ISTD	Cis isomer; mixture of diastereomers
Retention time	Co-elutes with analyte	Co-elutes under isocratic conditions
Mass difference	Not isobaric with analyte, minimum of 3 Da difference for stable-isotope labeled ISTDs	3 Da difference
Label placement (stability)	Not at chemically labile positions	Stable position on hydrocarbon chain
Label placement proximity to fragmentation	Distant proximity to fragmentation	Labels are distant from the fragmentation
Label retention after fragmentation	Labels retained after fragmentation	Labels retained after fragmentation

5. LC-MS/MS Method

Experimental design: Utilize *(±)-cis*-THC acid-D_3-glucuronide as an internal standard for *(+)-trans*-THC acid glucuronide across a range of concentrations diluted from a certified standard. Assess linearity over range of concentrations and %RSD of replicate injections.

(+)-trans-THC acid glucuronide 1.0 mg/mL solution standard was diluted to prepare solutions with concentrations of 50, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, and 5000 ng/mL.

(±)-cis-THC acid-D_3-glucuronide was used as the internal standard at a concentration of 500 ng/mL.

The samples with concentrations of 50 ng/mL to 5000 ng/mL were analyzed in triplicate.

- LCMS system: Agilent 1100 HPLC with 6410 tandem MS system
- Column: Phenomenex Kinetex 2.6 µm, 100 Å, C18, 2.1x50mm column
- Mobile Phase: 5 mM Ammonium Formate:Acetonitrile, isocratic 65:35 at 0.4 mL/min

LC-MS/MS monitored transitions

Compound Name	Precursor Ion	Product Ion	Fragmentor	Collision Energy
(+)-trans- THC acid glucuronide	521.2	345.2	120	13
	521.2	327.1	120	28
	521.2	299.1	120	38
(±)-cis- THC acid-D3 glucuronide	524.3	348.2	120	9
	524.3	330.1	120	25
	524.3	302.1	120	35

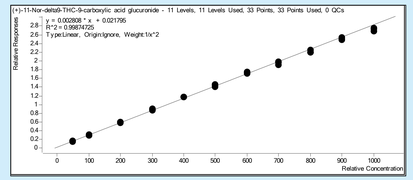
LC-MS/MS transition fragmentation

(+)-trans-THC acid glucuronide	*(+)-cis*-THC acid-D_3-glucuronide
(521.2 → 345.2)	(524.3 → 348.2)
(521.2 → 327.1)	(524.3 → 330.1)
(521.2 → 299.1)	(524.3 → 302.1)

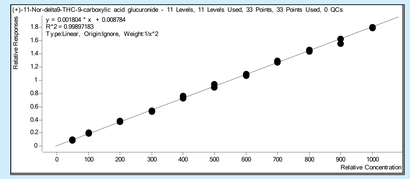
6. (±)-cis-THC-acid-d_3- glucuronide as an internal standard for (+)-trans-THC-acid glucuronide

A linear response is observed from 50 ng/mL to 1000 ng/mL for three monitored mass transitions.

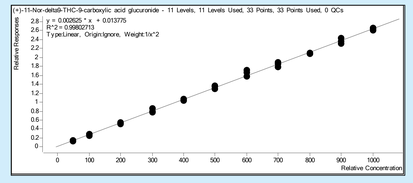
(521.2→345.2) fitted curve from 50 ng/mL to 1000 ng/mL



(521.2→327.1) fitted curve from 50 ng/mL to 1000 ng/mL



(521.2→299.1) fitted curve from 50 ng/mL to 1000 ng/mL



%RSDs of calculated concentrations

	% RSD calc. concentration 521.2→345.2	% RSD calc. concentration 521.2→327.1	% RSD calc. concentration 521.2→299.1
50 ng/mL	2.339	4.747	6.098
100 ng/mL	5.266	3.452	5.658
200 ng/mL	1.901	1.699	3.219
300 ng/mL	1.289	1.262	5.237
400 ng/mL	0.273	1.885	1.678
500 ng/mL	1.940	3.404	2.220
600 ng/mL	0.841	1.044	4.234
700 ng/mL	1.765	1.194	2.942
800 ng/mL	1.684	0.817	0.631
900 ng/mL	0.956	2.507	2.864
1000 ng/mL	1.493	0.675	1.233

- The %RSD of the calculated concentrations at each level are all below 6.1%.
- The response of the 5000 ng/mL sample was found to be low, possibly due to detector saturation.

7. Conclusions

Despite isomeric differences, (±)-cis-THC acid-D_3-glucuronide is shown to be an appropriate internal standard for the LC-MS/MS quantification of (+)-trans-THC acid glucuronide under the listed experimental conditions. The impact of isomeric differences between internal standard and analyte on LC-MS/MS quantification is compound-specific and must be assessed prior to analysis.