Quantitative analysis of 11-nor-delta^9-tetrahydrocannabinol-9-carboxy acid in urine by GC-MS/MS : selecting the most suitable precursor ion

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Background :

Cannabis, obtained from the plant species *Cannabis sativa,* is the most widely used illicit drug in the world. The major urinary metabolite is 11-nor-delta^9-tetrahydrocannabinol-9-carboxy acid (THC-COOH) and is excreted as the glucuronic acid conjugate. In a clinical setting, THC-COOH concentrations found in urine samples can vary widely and thus one prefers a quantitative confirmation method with a broad linear range in order to avoid re-assays and dilution issues. Often precursor ions are chosen solely based on relative abundancy. In this study we took a different approach after examining the effect of the precursor ion on the characteristics of a GC- ion trap MS/MS method.

Methods and Instrumentation :

Sample preparation :

- alkaline hydrolysis
- anion exchange SPE procedure
- derivatisation with BSTFA/TMCS
- THC-COOH-d9 as internal standard

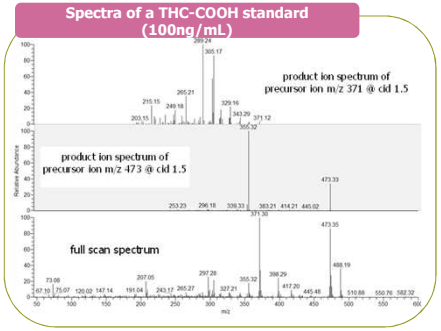
Instrumentation :

- Trace GC Ultra (Thermo Scientific)
- ITQ 900 iontrap MS (Thermo Scientific)
- capillary column : Rxi-5Sil, 30m x 0.25mm i.d., 0.25µm
- helium at 1.3mL/min
- injection volume : 2µL splitless

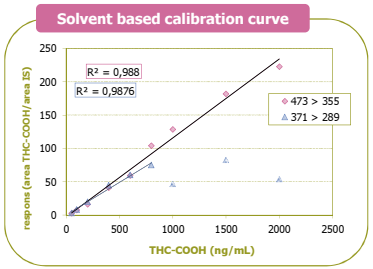
Results :

The full scan spectrum of THC-COOH shows 2 predominant ions : m/z 371 and 473 with ion 371 being the most abundant. The collision induced dissociation (CID) was optimized for both precursor ions and the corresponding product ions were selected. This optimisation resulted in 2 potential MS/MS transitions for THC-COOH : 371 > 289 (+305) and 473 >355. The corresponding transitions for the internal standard were 380 > 292 (+314) and 479 > 361.

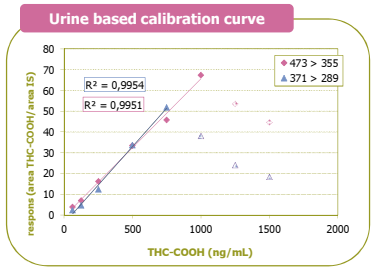
Spectra of a THC-COOH standard (100ng/mL)



Solvent based calibration curve



Urine based calibration curve



Both solvent and urine based calibration curves were recorded with both MS/MS transitions. For the solvent based curve the linear dynamic range was 0-2000 ng/mL and 0-800 ng/mL for transition 473 >355 and 371 > 289, respectively. Similar results were obtained with transition 371 > 305. In the urine based calibration curves the difference between the linear dynamic range obtained with both transitions was less pronounced : 0-1000 ng/mL versus 0-800 ng/mL. This can probably be explained by the presence of ions derived from the urine matrix.
Ten urine based calibration curves ranging from 0 to 500 ng/mL were recorded with both MS/MS methods. In each analytical run two levels of internal quality control (iQC) samples were analysed. Results of the analysis of the iQC samples are summarized in the table and point out that, aside from the linear dynamic range, precision and trueness are very significantly affected by the choice of the MS/MS transition.

Results of the analysis of the iQC samples

	iQC low level target conc = 18.5 ng/mL		iQC high level target conc = 150.0 ng/mL	
	371 > 289	473 > 355	371 > 289	473 > 355
mean (ng/mL)	13.4	19.0	121.8	151.3
CV (%)	12.7	7.6	24.4	8.9
% deviation from target concentration	-27.5	+2.5	-18.8	0.9

Conclusions :

Our study shows that, aside from its abundance, the fragmentation pattern of the precursor ion determines the characteristics of the assay.

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