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Confirmation of 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid in Urine by Ion Trap GC-MS/MS

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Introduction

IOC and WADA include cannabinoids into the class of substances prohibited under certain circumstances. A concentration in urine of the main metabolite of tetrahydrocannabinol, 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (THC-9-COOH), higher than 15 ng/ml is considered doping. Before attempting quantitation a confirmation protocol is needed. Current methods of detection and confirmation involve enzymatic hydrolysis of the metabolite glucuronide, extraction with organic solvent, silylation, and GC-MS analysis of the trimethylsilyl derivatives in SIM mode [1]. For confirmation purposes a comparison, among the suspicious urine sample and a positive control urine monitored ions relative abundance, is made. The relative abundance difference of any of the ions shall be within established limits to confirm the presence of the metabolite [2]. For profiling cannabinoids, there are other methods in the literature [3].

An alternative approach for confirmation could be the comparison, between suspicious and a positive sample, of the product ions spectra of some significant ion (molecular ion or base peak) of any THC-9-COOH derivative, obtained by gas chromatography-tandem mass spectrometry in an ion trap. With this aim, the bis-trimethylsilyl (TMS) and bis-*t*-butyl-dimethylsilyl (TBDMS) derivatives of THC-9-COOH were prepared and studied by ion trap GC-MS/MS. The preliminary results of this alternative procedure, which shows encouraging results, are presented hereby.

Experimental.

Methyltestosterone (30 µl, 10 mg/ml) was used as ISTD. Spiked urine samples (THC-9-COOH 1 µg/ml in methanol) and true positive samples were treated following the protocol for anabolic steroids [4], including the preparation of TMS derivatives. TBDMS derivatives were prepared by a slight modification of Mawhinney protocol [5]: TBDMSTFA 100 µl, 55 °C, 30 min. GC-MS and GC-MS/MS spectra were obtained with a Varian CP-3800 gas chromatograph (CP-8400 Autosampler) coupled to a Varian Saturn 2000 Ion Trap mass spectrometer. Column: Chrompack CP-Sil 5 CB (30mx0.25mmx0.25µm), ramped from 190 to 300 °C at 5 °C/min at 1 ml/min constant flow of He.

Results and Discussion

Different ions of both derivatives were chosen as precursors, and CID conditions (excitation mode, excitation time, and collision energies) were adjusted in order to optimize the quality and reproducibility of the product ion spectra, and the overall sensitivity, reproducibility and precision of the method. Spiked urine samples and real positive samples in the range 5 – 100 ng/ml were studied under optimized conditions.

Resonant excitation CID yielded more abundant product ion spectra than non resonant CID for precursor ions of m/z 488 and 572 (TMS and TBDMS-derivatives, respectively), as exemplified in **Figure 1**. Precursor ions m/z 371 and 515 yielded poor product ion spectra in both excitation modes. THC-9-COOH TBDMS-derivative ion trap full scan and MS/MS spectra are presented in **Figure 2**. THC-9-COOH TMS-derivative MS/MS chromatogram and spectrum under optimized conditions are shown in **Figure 3**. For this last derivative, a comparison between a true positive sample and a standard control is shown in **Figure 4**.

TBDMS-derivative was no further studied because sensitivity dropped off when moving from the high to the low ppb region, and MS/MS spectra were no longer reproducible. Much better results were obtained with THC-9-COOH TMS-derivative. Retention times are reproducible and precise: rrt = 0.9741 ± 0.0008 (N = 12, positive and spiked urine samples), and there is good linearity in the range 5–100 ng/ml (R^2 = 0.9933). This method has excellent sensitivity at the cut-off limit (**Figure 2**), being four times more sensitive than current GC-MS SIM

analysis, and shows also cleaner TIC chromatograms. However, additional resonant excitation parameters, such as modulation range, modulation rate and CID bandwith, need to be further optimized to avoid a slight change in main ions relative abundance with concentration.

Note: After this initial communication the method development continued. The afore-mentioned drift in relative abundance with concentration was solved returning to the non resonant CID method, optmising product ion yield by proper choice of the CID excitation storage level. The comparisons thus obtained were reproducible, precise and independent of analyte concentration. A complete presentation of this enhanced method will be published elsewhere [6].

References

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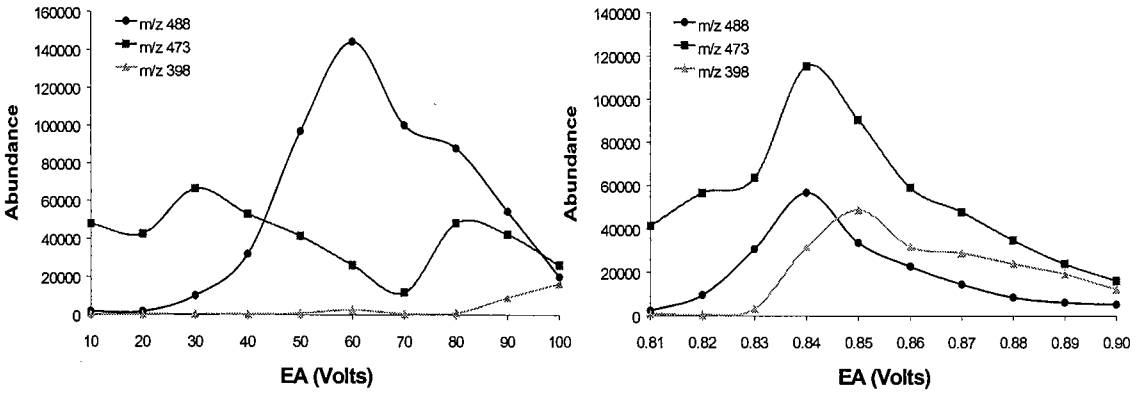

Figure1. Breakdown curves for non resonant (**left**) and resonant (**right**) excitation of m/z 488.

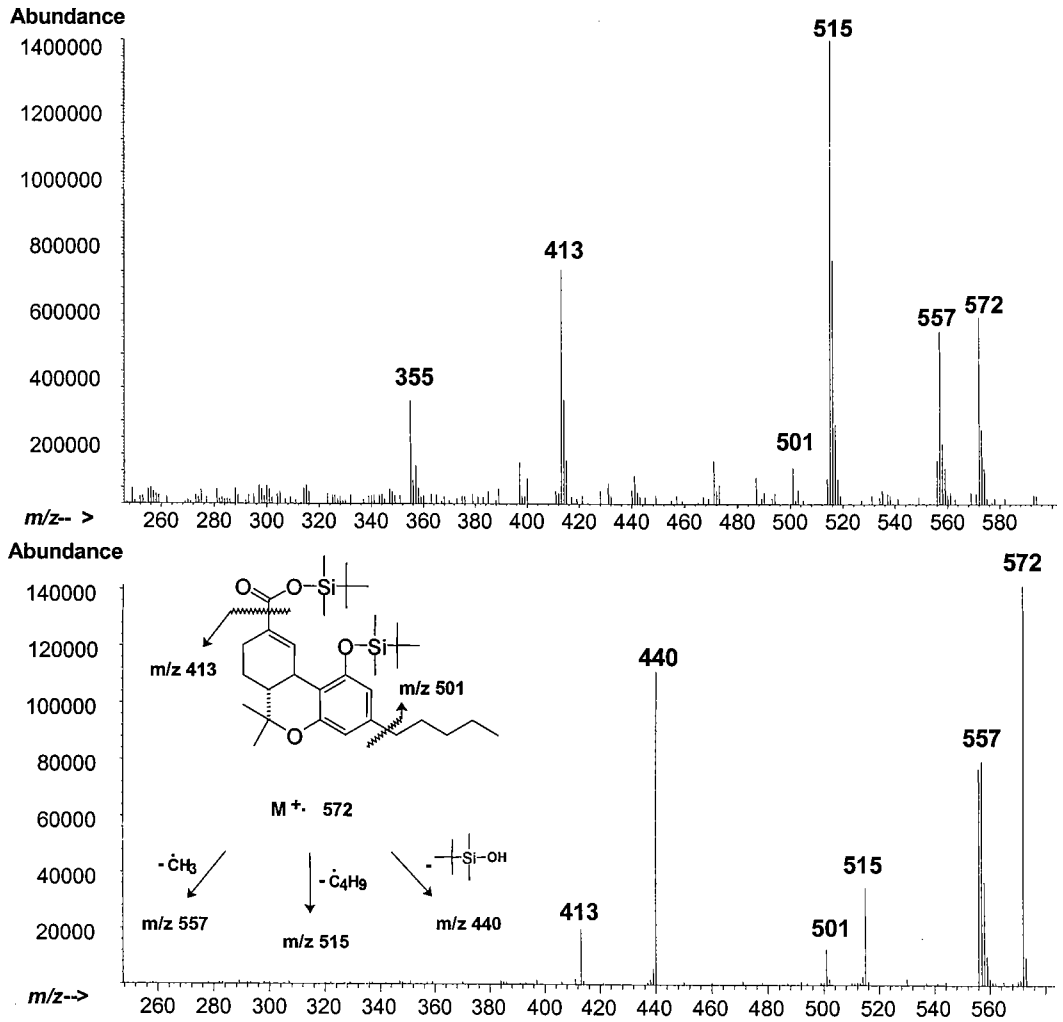

Figure 2. THC-9-COOH TBDMS-derivative Ion Trap full scan spectrum (**top**), and MS/MS spectrum (**bottom**). Resonant excitation of *m/z* 572 at 0.82 V collision energy for 20 ms.

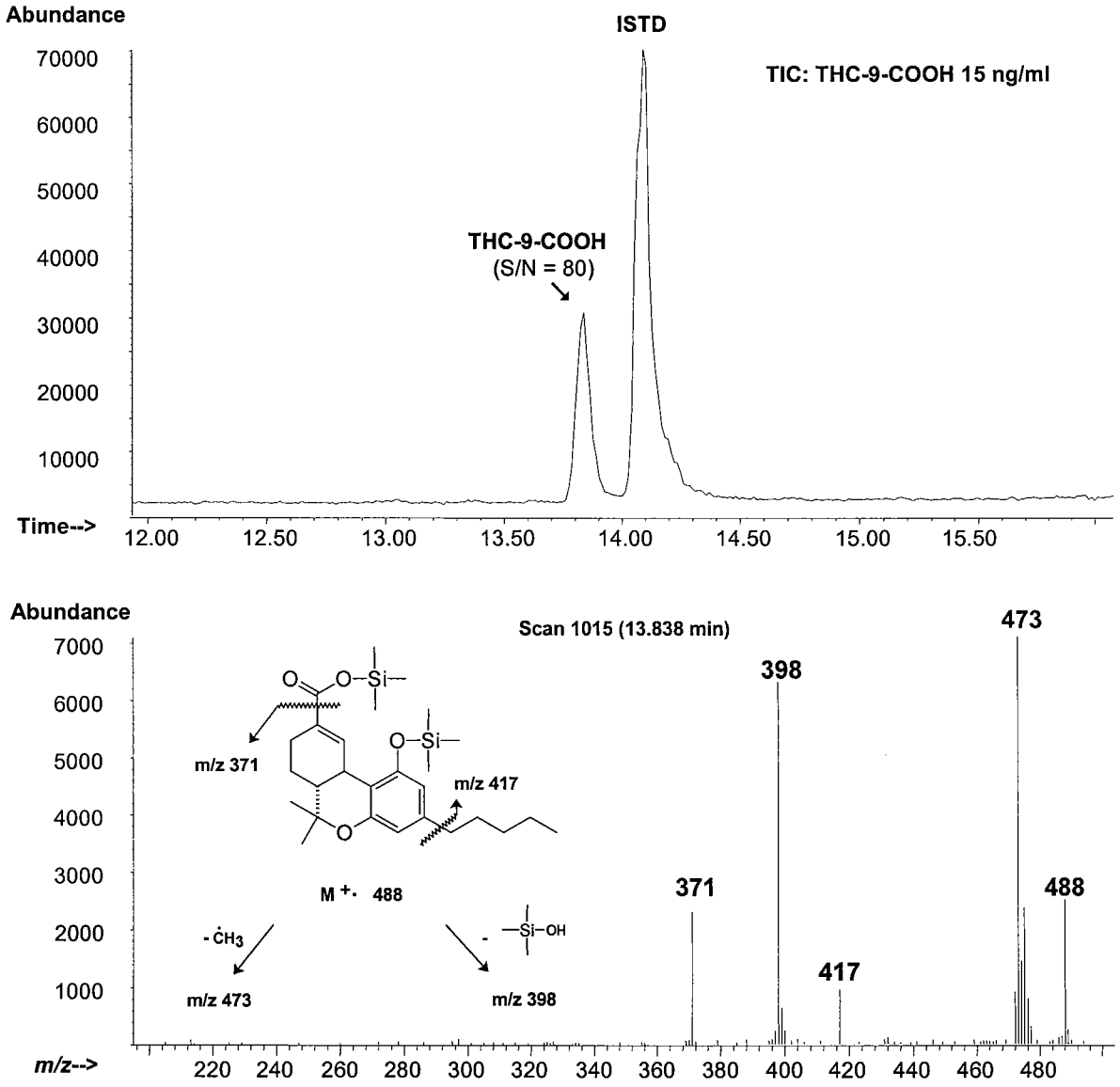

Figure 3. THC-9-COOH spiked urine TIC MS/MS chromatogram (**top**) and MS/MS spectrum (**bottom**). Resonant excitation of *m/z* 488, at 0.85 V collision energy for 20 ms (ISTD: nonresonant excitation of *m/z* 356 → 341,251; at 85 V collision energy for 20 ms)

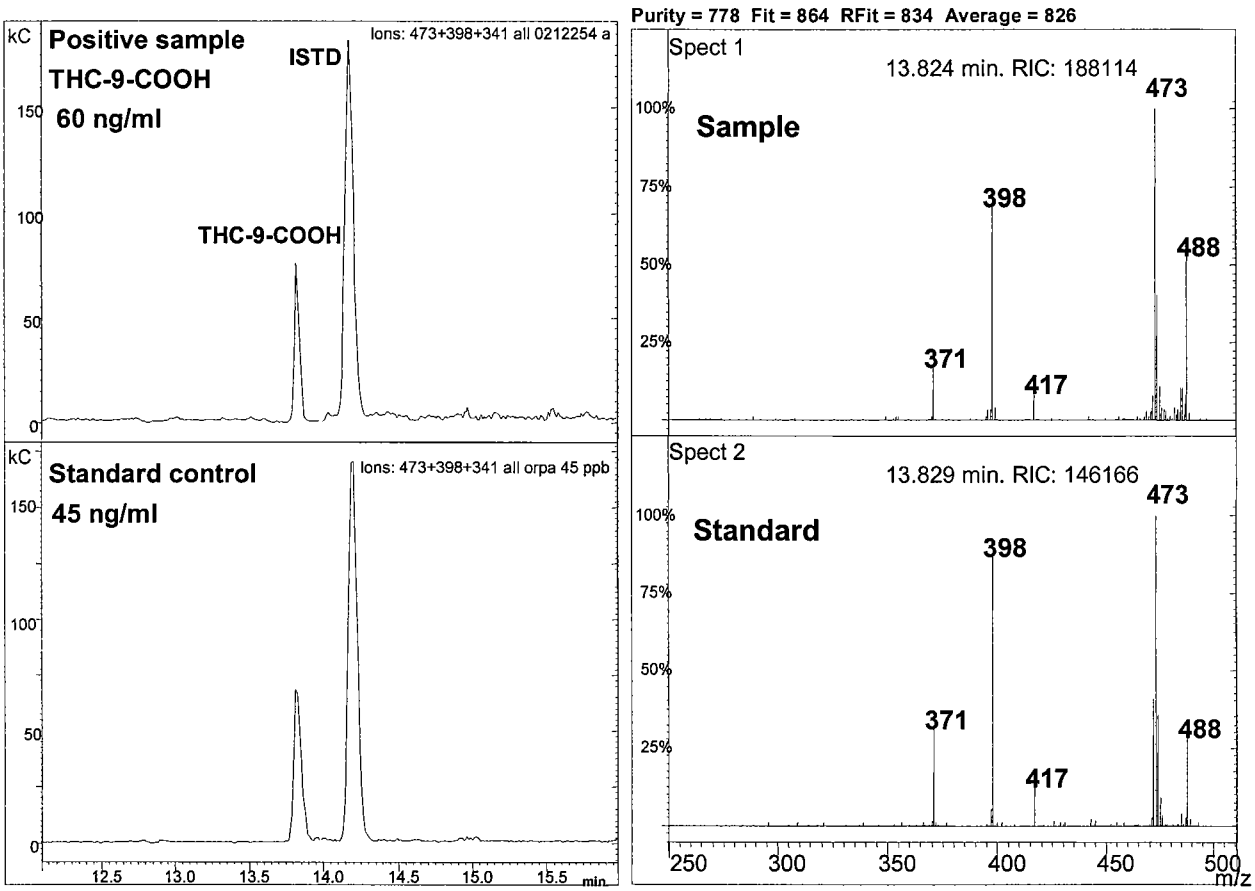

Figure 4. Identification of a THC-9-COOH positive sample by MS/MS. Reconstructed ion (m/z 473, 398, 341) MS/MS chromatograms (**left**), and MS/MS spectra (**right**)