K26 The Detection of THC and THCA in Whole Blood Using Two-Dimensional Gas Chromatography and EI-MS

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After attending this presentation, attendees will learn about an improved method for measuring THC and THCA in blood by GCMS. The new method includes the easy use of 2-dimensional chromatography to greatly reduce matrix interference and improve the limit-of-detection.

This presentation will impact the forensic community and/or humanity by demonstrating an easy and inexpensive improvement for the determination of cannabinoids in whole blood by GCMS .

A method is described for the simultaneous analysis of THC and its carboxylic acid metabolite, THCA as their TMS derivatives using 2-dimensional chromatography and EI-MS detection, (2-D GCMS). The addition of a Deans switch to a standard GC oven allows the use of two chromatographic columns of differing stationary phase to greatly reduce matrix interference. The Limit of Quantitation (LOQ) for THC and THCA was determined to be 1.0 ng/ml. The between-run precision at 1.0 ng/ml (N=25) was 7.7 and 11.1 % for THC and THCA, respectively. The method is linear from 1 to 100 ng/mL.

Sample Preparation: Internal standard was added (10 ng of THC-d3 and THCA-d3 in methanol) to 1.0 mL of whole blood. To each sample 2.0 mL of cold (-20^{O} C) acetonitrile was added and immediately vortexed for 30 seconds before proceeding to the next sample. (The cold acetonitrile produces a more finely divided protein precipitate than did room-temp acetonitrile.) The samples were briefly centrifuged, the supernate was decanted to a clean tube and the solids were discarded. 2.0 ml of DI water was added to each sample before it was poured onto the SPE column (*Cerex polychrome THC* columns from *SPEware*). The columns were washed with a 1.0 ml mix of water/acetonitrile/NH_4OH (85/15/1, prepared daily) and dried for 10 minutes by a flow of nitrogen. The THC was eluted into a tube with 2.0 ml of ethyl acetate followed by 10 minutes of drying. The THCA was eluted into the same tube with 2.0 ml of a hexane/ethyl acetate/acetic acid mix (90/10/3, prepared daily). All flows through the column were ~1 drop/sec controlled by positive pressure. The samples were evaporated to dryness at 50^{O} C under a stream of nitrogen. 50 uL BSTFA+1%TMCS and 50 uL ethyl acetate were added to each tube, the tube was vortexed and the contents were transferred to a GCMS vial. The vials were crimp-capped and heated at 70^{O} C for 20 minutes.

Instrumentation: GCMS-FID: The gas chromatograph was an Agilent 6890 equipped with a FID and a Deans switch. The Deans switch, from Agilent Technologies, consists of a second EPC (electronic pressure control) module, a solenoid switch that is outside the oven and a manifold inside the oven to connect the GC columns. The Mass Spectrum detector was an Agilent 5973. The carrier gas was helium. The injection port temperature was 250^{O} C and the transferline was at 300^{O} C. The MSD was operated at 200 volts above the tune. The SIM ions were: m/z 386.3, 371.3, 303.3 for THC, m/z 389.3, 374.3 for THC-d3, *m/z* 371.3, 473.3, 488.3 for THCA and m/z 374.3, 476.3 for THCA-d3. The dwell time for all ions was 25 milliseconds and "high" resolution was selected. The ion mass was determined by scanning each SIM ion in 0.1 *m/z* units. The oven program was: initial temp 120^{O}C, increased at 20^{O}/min to 200^{O}C, further increased at 10^{O}/min to 250^{O} C, increased again at 25^{O}/min to 300^{O} C and held for 0.5 min. The injection port liner was a deactivated 4mm splitless gooseneck with glass wool from Restek. The injection volume was 2 uL.

Deans Switch Parameters: Column 1 was a RTX-200 (20m, 1.18mm id, 0.20 um df). Column 2 was a DB-17 (15m, 0.25 mm id, 0.25 df). The pressure for the injection port and the Deans switch were calculated using the Deans Switch Calculator software (Agilent Technologies) to achieve 1 ml/min flow through the primary column and 2.0 ml flow through the secondary column at an oven temperature of 200^{O} C. The injection port was set for constant pressure at 41.06 psi and the Deans switch pressure was 16.98 psi. The post-run program was set for 1 minute with the oven temp at 320^{O}C and the inlet pressure at 1 psi. With the inlet pressure at 1 psi in the post-run, the carrier gas flow through the primary column is reversed. This ability to back-flush the primary column is an advantage of the Deans switch system and reduces the maintenance frequency of the primary column. The secondary column stays remarkably clean because only a small fraction of the injection volume for each chromatographic run flows through it.

Marijuana, Blood, GCMS

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