## K17 A Two-Dimensional-Cryofocusing GC/EI-MS Method for Determination of $\Delta^9$-tetrahydrocannabinol, 11-Hydroxy-$\Delta^9$-tetrahydrocannabinol, and 11-Nor-9- carboxy-$\Delta^9$-tetrahydrocannabinol in Human Urine

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The objective of this presentation is to provide a detailed description of a GC/MS procedure for the simultaneous quantification of THC, 11-OH- THC, and THCCOOH in human urine. The method utilizes two-dimen- sional chromatography and cryofocusing to enhance resolution and improve sensitivity.

The presentation will impact the forensic science community by allowing participants to develop and apply two-dimensional chromatography to the quantification of THC and major metabolites. The method may be a useful analytical procedure in forensic toxicology applications.

A sensitive and specific two-dimensional (2D) gas chromatography/ electron impact-mass spectrometry (GC/EI-MS) method for simultaneous quantification of $\Delta^9$-tetrahydrocannabinol (THC), 11-hydroxy-$\Delta^9$-tetrahydrocannabinol (11-OH-THC), and 11-nor-$\Delta^9$-tetrahydrocannabinol- 9-carboxylic acid (THCCOOH) in human urine was developed and validated. The method employs 2D capillary GC and cryofocusing for enhanced resolution and sensitivity. GC separation of trimethylsilyl derivatives of analytes was accomplished with two capillary columns in series coupled via a pneumatic Deans switch system. Detection and quantification were accomplished with a bench-top single quadrupole mass spectrometer operated in electron impact-selected ion monitoring mode.

To ensure complete hydrolysis of conjugates and capture of total analyte content, urine specimens were hydrolyzed by two methods in series. Two mL urine fortified with THC-$d_3$, 11-OH-THC-$d_3$, and THCCOOH-$d_3$ was hydrolyzed with 5000 units/mL of *Escherichia coli* b-glucuronidase (pH 6.8) for 16 h at 37°C in a shaking water bath followed by a second hydrolysis uti- lizing 10N NaOH at 60°C for 20 min. Specimens were adjusted to pH 5-6.5 with concentrated glacial acetic acid. Two mL of acetonitrile were added to precipitate protein followed by 2 mL 2N sodium acetate buffer (pH 4.0). Specimens were centrifuged and supernatants applied to conditioned solid phase extraction (SPE) columns. SPE columns (Clean Screen ZSTHC020, United Chemical Technologies) were washed with 3 mL deionized water, 2 mL 0.1N hydrochloric acid /acetonitrile (70:30 v/v), and dried by full vacuum for 10 min. After priming the sorbent bed with 0.2 mL hexane, analytes were eluted with 5 mL elution solvent (hexane:ethyl acetate 80:20 v/v) into tubes containing 0.5 mL ethanol and dried under nitrogen. Extracts were recon- stituted with 25 µL acetonitrile, transferred to autosampler vials, and 20 µL BSTFA was added. Vials were capped and derivatized at 85°C for 30 min.

2D chromatographic separation was achieved with a primary DB-1MS capillary column (15 m x 0.25 mm i.d., 0.25 µm film; Agilent Technologies) and a secondary ZB-50 capillary column (30 m x 0.32 mm i.d., 0.25 µm film; Phenomenex). One µL derivatized extract was introduced in "cuts" of the analyte elution bands to the secondary GC column for further chromatographic resolution. The secondary column was inserted through the cryogenic trap and the effluent end interfaced to the MSD for detection and quantification. Three analytes were quantified simultaneously with 2.5 to 300 ng/mL dynamic ranges for THC and THCCOOH and 2.5 – 150 ng/mL for 11-OH-THC. Calibration curves exhibited coefficients of determination ($r^2$) of 0.99 or greater ($n$ = 12). Accuracy ranged from 87.6% to 102.1% for all analytes. Intra- and inter-assay precision, as percent relative standard deviation, were less than 8.6% for all analytes. Extraction efficiencies were 34.6 – 38.9% for THC, 44.0 – 52.8% for 11-OH-THC, and 39.3 – 54.9% for THCCOOH.

The combination of 2D-GC and cryogenic focusing achieved improved resolution of analyte from complex matrix components. The result was a rugged, flexible method with enhanced resolution power and lower detection and quantification limits compared to single dimensional chromatography. Focusing of the analyte band at the head of the secondary column markedly enhanced the chromatographic signal-to-noise (S/N), improving sensitivity. The method employs a rapid SPE and utilizes readily available single quadrupole GC/MS instrumentation. Acceptable assay characteristics and enhanced analytical sensitivity with improved S/N and detection limits were achieved. This method was applied to the analysis of urine specimens collected from individuals participating in controlled cannabis administration and monitored withdrawal studies, and may be a useful analytical procedure in forensic toxicology applications.

**GC/MS, THC, Two-Dimensional Chromatography**

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