K5 Quantitative Analysis of 11-Nor-9-Carboxy-Tetrahydrocannabinol in Hair by Column Switching LC/ESI/MS3

Meejung Park, 139 Jiyangno, Yangchoen-gu, Narcotic Analysis Division, National Forensic Service, Seoul 158-707, SOUTH KOREA; Jihyun Kim, MSc, Jiyangno, Yangcheon-gu, Seoul 158-707, SOUTH KOREA; Yuran Park, MSc, Jiyangno, Yangcheon-gu, Seoul 158-707, SOUTH KOREA; Eunmi Kim, PhD, 139 Jiyangno Yangcheon-gu, Seoul , SOUTH KOREA; and Hee-Sun Chung, PhD, 1402-703 Mokdong Apt, Shunjung-dong, Yangchon-ku, Seoul 158-774, SOUTH KOREA*

After attending this presentation, attendees will better understand the column switching technique of High-Performance Liquid Chromatography (HPLC) as well as Liquid Chromatography/Electrospray Ionization/Tandem Mass Spectrometry^3 (LC/ESI/MS^3) to decrease background hair matrix to identify and quantify 11-nor-9-carboxytetrahydrocannabinol (THCCOOH), a metabolite of tetrahydrocannabinol (THC) in hair samples.

This presentation will impact the forensic science community by providing a new sensitive and selective method of hair analysis by the column-switching technique of HPLC and LC/ESI/MS^3. This method can be used as an alternative method for Gas Chromatography-Mass Spectrometry (GC/MS) analysis of cannabis in hair without derivatization.

Hair analysis has been regarded as an alternative method for urine analysis in forensic and criminal cases. Cannabis (marijuana) is one of the most widely used drugs in the world and it has been controlled in South Korea since 1976. Identification of THCCOOH in hair can be an important proof of cannabis use because it can exclude the possibility of passive cannabis smoke exposure. This study describes a quantitative method of THCCOOH in hair using simple Liquid-Liquid Extraction (LLE) and selective column-switching LC/ESI/MS^3.

The column-switching valve was placed in the column compartment. A pre-column (C_{18}, 2.0 × 30mm, 3.0μm) was used in the modified gradient method. For the column-switching system, the trap column (C_{18}, 1.0 × 30mm, 2.6μm) and the analytical column (C_{18}, 2.1 x 75mm, 2.7μm) were used. The valve switch from precolumn to trap column was set from 3.0 to 4.0min. The sample extract was injected on the precolumn which was flushed with 2mM ammonium formate/0.2 % formic acid in water and 2mM ammonium formate/0.2 % formic acid in acetonitrile at a flow rate of 0.5ml/min. THCCOOH appeared around 3.5min in this precolumn. From 3.0 to 4.0min, the analytes were flushed onto the trap column with 0.5mM ammonium formate in water and 0.5mM ammonium formate in acetonitrile. After 4.0min, the valve was switched to the original position and the analytes in the trap column were eluted onto the analytical column. Resolution occurred in this column by increasing the ratio of organic solvent and finally eluted into the ESI/MS^3 system. The internal standard was THCCOOH-d_3.

In the MS^3 experiment, THCCOOH ionized best in negative ESI mode and an (M-H^{-}) ion was observed at m/z 343, which was fragmented into the second precursor ion at m/z 299.2. This second precursor ion was trapped and accumulated in the Linear Ion Trap (LIT) using a fixed LIT fill time of 250ms and excitation time of 20ms. The resulting MS^3 spectrum showed an intense peak at m/z 245.1; therefore, for the quantification of THCCOOH, the MS^3 ion transition monitored was m/z 343.2 → 299.1 → 245.2 (343.1/299.2/245.1) with the LIT set to perform a mass scan centered at m/z 245.1. For the internal standard (THCCOOH-d_3), the MS^3 ion transition monitored was 346.2 → 302.2 → 246.1 (346.1/302.2/246.1) in the same method.

Chromatographic separation was completed within 12min. No interferences were detected in 10 blank hair samples. The correlation coefficients (r^2) of calibration curves were larger than 0.9997 with mean slope of 0.0202 and the mean intercept of 0.0017, using a weighing factor 1/x. In the intra- and inter-assay precision and accuracy study, Coefficient of Variation (CV) (%) and bias (%) were below 10. The limit of detection was 0.08pg/mg and the limit of quantification was 0.1pg/mg. The mean values of matrix effect at 10pg/20mg and 50pg/20mg were 82.8 and 70.6%, respectively. The CVs of the matrix effect, a measure of the relative matrix effect for an analyte, at each concentration were 9.5 and 6.1%, respectively, which showed no significant variation among hair samples from different individuals. The mean values of recovery were 91.7 and 74.0% and those of process efficiency were 80.6 and 66.3% at 10 and 50pg/20mg hair, respectively.

The range of concentrations of THCCOOH from 94 authentic human hair samples was 0.1 ~ 15.7pg/mg. This method was successfully applied in the analysis of authentic human hair samples. The

 ** Presenting Author*

developed method will be very useful for quantification of THCCOOH in hair in both legal and public health aspects.

Cannabis, Hair, LC/MS/MS

 ** Presenting Author*