A4 Reaction, and the Validation of HPLC Method for the Quantitative Determination of Cannabinoids

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After attending this presentation, attendees will gain a better understanding into stability of cannabinoids in cannabis and the quantitative determination of cannabinoids by HPLC.

This presentation will impact the forensic science community by enriching attendee knowledge in the chemistry of cannabinoids.

Cannabis refers to any part of a plant of the genus Cannabis. It is used for fiber (hemp), for medicinal purposes, and as a recreational drug. The three main forms of cannabis products are the herb (marijuana), resin (hashish), and oil (hash oil). Tetrahydrocannabinol (THC) is the main psychoactive ingredient in cannabis and is known to degrade into cannabinol through oxidation over time. The extent of degradation could be co-related to the age of the seized exhibits. The stability of cannabinoids in cannabis stored under different conditions over a period of time was investigated. The influence of temperature and exposure to light, on the stability of the exhibits was investigated. The rate of degradation of THC in cannabis was evaluated by Gas Chromatography/Flame Ionization Detection (GC/FID). The results indicated that the degree of degradation is highest in exhibits exposed to sunlight under ambient temperature while those kept in the freezer showed the highest stability. In addition, the stability study of a THC standard solution was conducted in parallel to evaluate the influence of matrix on the degradation process.

Total THC content often represents the maximum potency of the smoked cannabis and is of great importance to legal systems. Measurement of total THC content comprises the sum of free Δ^9-Tetrahydrocannabinol (Δ^9-THC) and its precursor Δ^9- Tetrahydrocannabinolic acid A (Δ^9-THCA-A). The influence of extraction solvent on the ratio of Δ^9-THC and Δ^9-THCA-A extracted was studied. Since cannabinoids are easily soluble in most organic solvents, such as methanol, petroleum ether, n-hexane, toluene, chloroform and methanol:chloroform combinations, these solvents are suitable for extraction. However, non-polar solvents such as n-hexane and petroleum ether will only extract the free cannabinoids quantitatively, while other solvents extract both cannabinoids and its acids. Therefore, for total THC determinations, the choice of extraction solvent plays an important role in determining the amount of the different types of cannabinoids extracted.

GC/FID is can be used for the quantitative analysis of cannabis samples. Δ^9-THCA-A converts to the psychoactive Δ^9-THC when heated. During the GC analysis, thermal conversion occurs as the acidic forms of the cannabinoids are decarboxylated into the neutral counterparts. Therefore, the sum of free Δ^9-THC and Δ^9-THC generated from the decarboxylation of Δ^9-THCA will be measured in the GC. Previous studies have shown that thermal conversion of Δ^9-THCA-A to Δ^9-THC in GC is only partial, yielding about 70% at the maximum. Decarboxylation reaction can be conducted prior to GC analysis. The temperature and

duration of heating parameters influencing the extent of decarboxylation and establish the optimum condition for the reaction were investigated.

When the free Δ^9-THC and Δ^9-THCA-A needs to be determined independently, derivatisation (trimethylsilyl derivatives) has to be performed prior to GC analysis. Alternatively, the High Performance Liquid Chromatography (HPLC) method has to be applied. Analysis of

Δ^9-THCA-A by GC involves derivatizing the carboxyl and phenol functions of the molecule, while HPLC does not cause any decomposition as the method does not involve any input of heat.

The application of a HPLC method for the direct quantitative determination of the cannabinoids will be discussed. The major advantage that HPLC has over GC is in the direct analysis of the thermally labile carboxylic acid derivatives of the cannabinoids. HPLC method will be validated and the ratio of Δ^9-THCA-A and Δ^9-THC in local cannabis exhibits will be investigated. Reversed-phase columns and solvent programmed gradient systems are required for the separation of cannabinoids and their acids.

Tetrahydrocannbinol, Degradation, Cannabinoids

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