

Automated determination of THC, CBN and CBD in human hair

In a joint research project with the Institute of Forensic Medicine at the University of Giessen, Germany, GERSTEL has successfully automated a validated manual analysis method for determining Δ^9 -tetrahydrocannabinol (THC) and the cannabinoids cannabinal (CBN) and cannabidiol (CBD) in human hair.

By Oliver Lerch

From a forensic toxicology point of view, human hair samples are extremely well suited for determining a person's consumption pattern of medication or drugs of abuse over a period of time. A human hair grows between 0.25 and 0.4 millimeters per day, in other words between 7.5 and 12 millimeters per month. Once consumed, drugs, active ingredients from medications as well as the metabolites formed, are distributed throughout the body and also incorporated into human hair as it grows. In cases of one-time or short-term drug use, the position of these substances within each hair stays fixed as the hair grows. In other words, prior drug use is no longer detectable after the hair has fallen out or you've gone for a haircut and the hairdresser has thrown away the evidence. Hair analysis can be used to detect drug abuse and to determine, which drugs have been consumed over which time period. By the same token, hair analysis can be used to confirm drug abstinence.

Hair today, gone tomorrow: How to determine cannabis consumption over time

Detecting prior cannabis consumption through hair analysis is a complex and labor-intensive process. The residual substances stored in hair must first be released from the matrix and converted into a form that is suitable for GC/MS analysis. At the Institute of Forensic Medicine at the University of Giessen, the steps required to prepare human hair samples are performed manually. Δ^9 -tetrahydrocannabinol (THC), the active compound in cannabis, and the cannabinoids cannabinal (CBN) and cannabidiol (CBD), are then determined by GC/MS. GERSTEL's goal in working with the Institute of Forensic Medicine was to assess the potential for automation of the analysis processes used and to develop a solution based on a GC/MS system with integrated automated sample preparation and sample introduction.

The manual procedure is the starting point

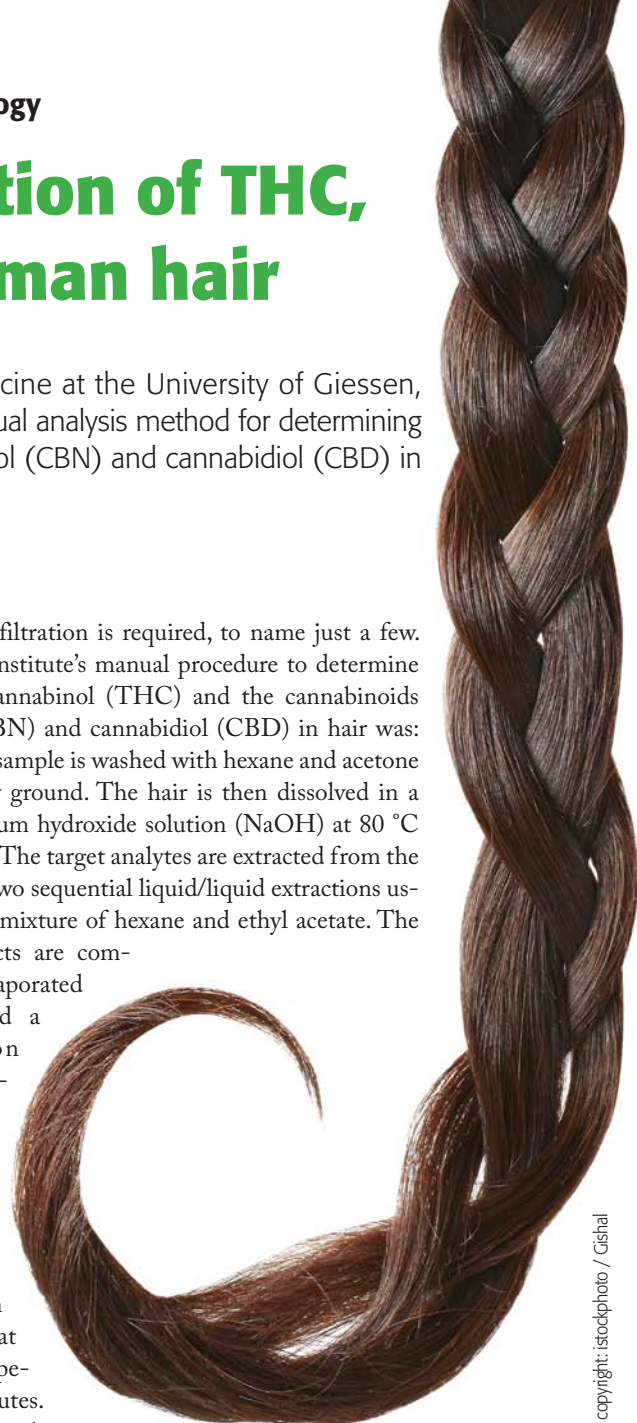
Determining whether an existing manual method of analysis is suitable for automation requires thorough evaluation of the entire process. All aspects of the method need to be examined such as sample throughput required, reagent type, volumes of liquids used, and whether heat-

ing, mixing or filtration is required, to name just a few. The Forensic Institute's manual procedure to determine Δ^9 -tetrahydrocannabinol (THC) and the cannabinoids cannabinal (CBN) and cannabidiol (CBD) in hair was: A 100 mg hair sample is washed with hexane and acetone and then finely ground. The hair is then dissolved in a one molar sodium hydroxide solution (NaOH) at 80 °C for 25 minutes. The target analytes are extracted from the hair matrix in two sequential liquid/liquid extractions using a 9/1 (v/v) mixture of hexane and ethyl acetate. The resulting extracts are combined and evaporated to dryness and a derivatization reagent consisting of BSTFA and TMCS is added to the residue at a ratio of 99/1 (v/v); derivatization is performed at 110 °C over a period of 20 minutes.

The resulting solution is again evaporated, the residue taken up in ethyl acetate and an aliquot injected into a GC/MS system for analysis in single ion monitoring (SIM) mode.

Automated hair analysis

The efforts to fully automate the manual method used for determining THC, CBN and CBD in hair were successful. The primary focus of the project was automating the sample preparation. For this task, the dual-head version of the GERSTEL MultiPurpose Sampler (MPS) was selected. The Dual Head version allows two different syringes to be used simultaneously, allowing the MPS to handle different



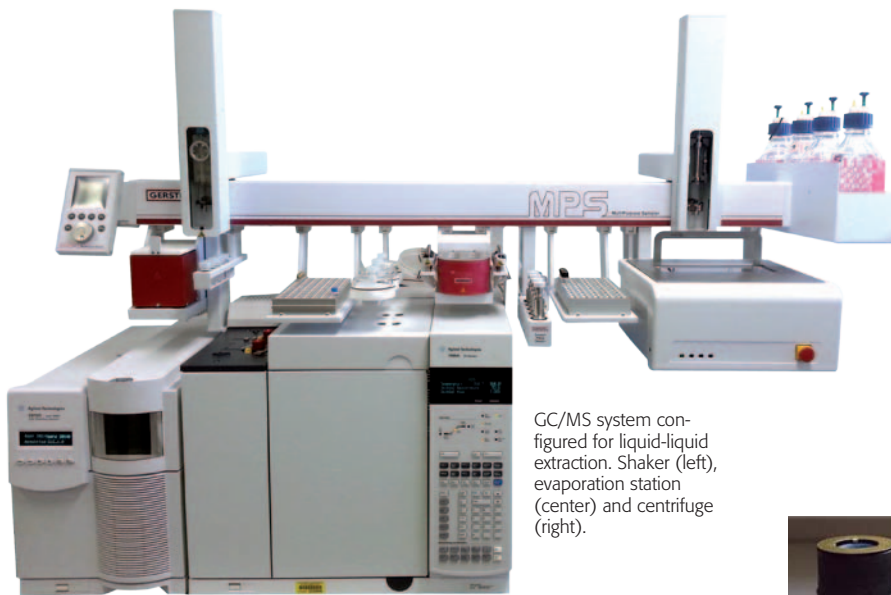
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Suggested reading

Determination of THC and its metabolites 11-hydroxy-THC (THCOH) and 11-nor-9-carboxy-THC (THC-COOH) in blood serum

K. Purschke, S. Heinel, O.Lerch, F. Erdmann, F. Veit, Anal Bioanal Chem, DOI 10.1007/s00216-016-9537-5, <http://link.springer.com/article/10.1007/s00216-016-9537-5>

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GC/MS system configured for liquid-liquid extraction. Shaker (left), evaporation station (center) and centrifuge (right).

Using the GERSTEL method, the extraction of THC, CBN and CBD from hair is fully automated and is accelerated using the GERSTEL *quickMIX* module mounted on the MPS.



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Sample vial after hair digestion, extraction and centrifugation. A clear phase separation between the upper organic extract phase and lower aqueous phase (dissolved hair) is achieved.

solvents, different volumes and make injections into an analytical system quickly and efficiently by eliminating the need to stop the sample preparation process in order to change syringes. The MPS can be configured with a wide range of optional modules to meet almost any need, including a solvent evaporation module (*mVAP*) for sample concentration, a centrifuge, and a vortex shaker to name a few. The automated procedure involves placing a vial containing a ground hair sample and internal standard in the MPS sample tray, after which all subsequent steps in the method are performed by the MultiPurpose Sampler under MAESTRO software control.

The following steps were performed:

- A 100 mg sample of ground hair (plus ISTD) was placed in 1 molar NaOH and incubated at 85 °C for 13 minutes until dissolved
- Two extractions with hexane/ethyl acetate (9/1, v/v) were performed for 4 minutes each at 200 rpm in the GERSTEL *quickMIX*
- Phase separation by centrifugation at 4500 rpm for 3 minutes
- Extracts evaporated to dryness at 65 °C (*mVAP*)
- Introduction of MSTFA / ethyl acetate (GERSTEL *quickMIX*)
- Injection of 2 µL (inlet derivatization)
- GC separation was performed using a DB-5MS 30 m x 0.25 mm x 0.25 µm capillary column (Agilent Technologies) with mass spectrometric detection (MSD) in single ion mode (SIM).

Results

Any method must prove its worth in practical use. The automated method for determination of THC, CBN and CBD in hair using the MPS to prepare and inject the sam-

ples was validated in accordance with GT-FCh guidelines. The limit of determination for THC was 0.01 ng/mg; the extraction effi-

ciency (at 0.02 ng/mg) was 102 % and the precision (at 0.02 ng/mg) was 4.2 %.

Conclusion and outlook

In close cooperation with the Institute of Forensic Medicine at the University Medical Center, Giessen, GERSTEL successfully automated the manual method previously used at the institute for determination of THC, CBN and CBD in human hair. The required 0.02 ng/mg limit of detection for THC was achieved. As planned, the manual sample preparation steps were automated using a GERSTEL MultiPurpose Sampler (MPS) under MAESTRO software control. The PrepAhead feature optimizes throughput by preparing the next sample in parallel during the ongoing GC/MS analysis. This ensures that each sample is treated in the same manner and injected immediately after it has been prepared. GERSTEL's *quickMIX*, centrifuge and *mVAP* modules were able to reproduce the manual steps previously used in the method, from mixing and extraction to evaporative concentration of the extracts. It was found that derivatization of the analytes was best performed in the hot GC inlet. In addition to the determination of THC, CBN and CBD in human hair, the steps automated in this method can be applied to a variety of other manual liquid/liquid extractions and sample preparation methods.

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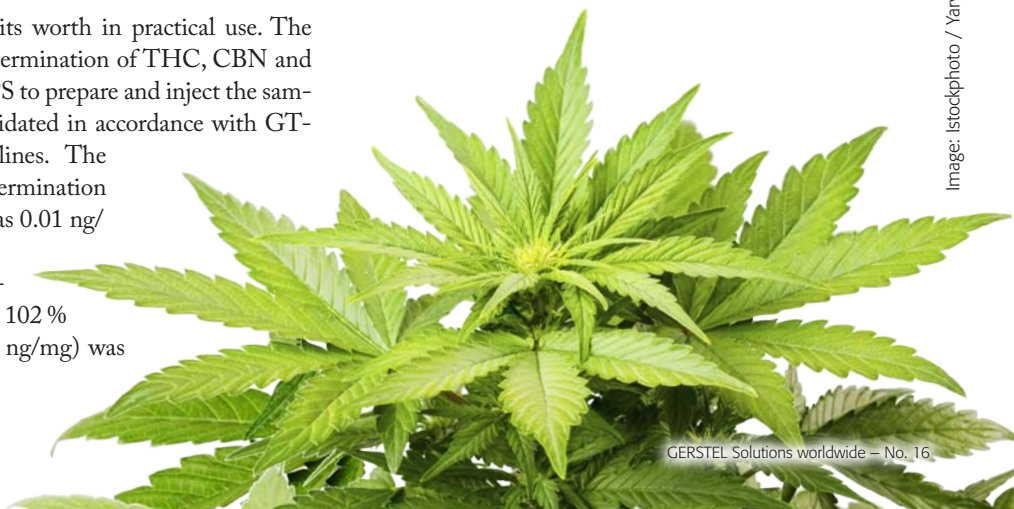


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