



GERSTEL MultiPurpose Sampler (MPS) equipped with centrifuge, ^mVAP and QuickMix.

Automated Liquid-Liquid Extraction (LLE)

Speeding up the workflow

In addition to SPE workflows, the GERSTEL MultiPurpose Sampler (MPS) can perform fully automated liquid-liquid extraction. Several functionalities can be added as modules.

By Oliver Lerch

An important stepping stone on the path to improved productivity is the automation of manually performed sample preparation steps. Whether these can be transferred to an autosampler of course depends on whether the sampler has the individual capabilities needed to perform each step in the process. Multiple application examples have been reported over the years using the GERSTEL SPE system based on the MultiPurpose Sampler (MPS) [1, 2].

Recently, a similar effort has been under way to automate several aspects of, and complete workflows for, liquid-liquid extractions (LLE).

Application examples include determination of tetrahydrocannabinol (THC), the active compound in cannabis, and the cannabinoids cannabidiol (CBD) and cannabidiol (CBD) all in human hair [3].

In addition, an article on the determination of THC and its metabolites 11-hydroxy-THC (THC-OH) and 11-nor-9-carboxy-THC (THC-COOH) in blood serum has been published [4]. The GERSTEL GC/MS-solutions configured for those applications are being used successfully by Forensic Toxicology Institutes. In addition to these more special analyses, a much wider array of applications require liquid-liquid extraction. All steps required in such a process can be automated using the GERSTEL MPS.

Key elements in such systems are the CF 200 centrifuge, the MultiPosition Evaporation Station (^mVAP) and ^{quick}Mix. The only step that must be performed manually is the addition of the liquid or solid sample into a vial and placing the vial in the MPS sample tray. All other steps are performed automatically. The analytical method and sample sequence is simply set up with a few mouse-clicks using GERSTEL MAESTRO software.

The MPS adds internal standards to the sample followed by extraction buffer and extraction solvent. The extraction is performed in a few minutes while the MPS agitates the

sample vigorously in the ^{quick}Mix ensuring thorough mixing. The phases can then be separated efficiently using the CF 200 centrifuge, or alternatively, a more powerful Sigma® centrifuge. The MPS aspirates the extract and transfers it to a clean vial. As needed an additional extraction step is performed using a new volume of clean solvent. Depending on the analytical workflow requirements, the MPS can inject an aliquot of the resulting extract into the analysis instrument or evaporate it to dryness in the ^mVAP. If evaporated to dryness, the residue can then be taken up in an adequate solvent with the option of adding a derivatization reagent. The steps to include in the final workflow are totally up to the user. These examples show how the MPS

can be used as a highly flexible and rugged tool for automated liquid-liquid extraction processes. An additional application example based on veterinary samples can be found online [5].

References

- [1] www.gerstel.com/en/apps-spe.htm
- [2] www.gerstel.com/en/Solutions-Articles-SPE.htm
- [3] S. Heinel, O. Lerch, F. Erdmann, Journal of Analytical Toxicology 40 (2016) 498–503
- [4] K. Purschke, S. Heinel, O. Lerch, F. Erdmann & F. Veit, Anal Bioanal Chem, <https://doi.org/10.1007/s00216-016-9537-5>
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Dear Reader

Over the past several years, GERSTEL has worked with users in forensic toxicology laboratories to fully automate existing manual or semi-automated analysis methods for the determination of drugs and metabolites in body fluids and tissue. In several labs, the focus was on the determination of THC and its metabolites. Many projects resulted not only in methods that were of practical use, but also in scientific publications. This newsletter offers you an overview of completed projects and a preview of ongoing projects. In addition, we offer references to journal articles and recommended reading for Forensic Toxicology and the related Doping analysis. A few further techniques are revealed that may be of interest to you. We hope that this will stir your interest and we would welcome the chance to learn more about your automation and sample preparation needs.



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Suggested reading

DETERMINATION OF THC AND ITS METABOLITES 11-HYDROXY-THC (THC-OH) AND 11-NOR-9-CARBOXY-THC (THC-COOH) IN BLOOD SERUM, K. Purschke, S. Heinel, O. Lerch, F. Erdmann, F. Veit, Anal Bioanal Chem, Link: <https://doi.org/10.1007/s00216-016-9537-5>

OPEN ACCESS ARTICLE!

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 cannabinalol (CBN) and cannabidiol (CBD) in human hair.

By Oliver Lerch

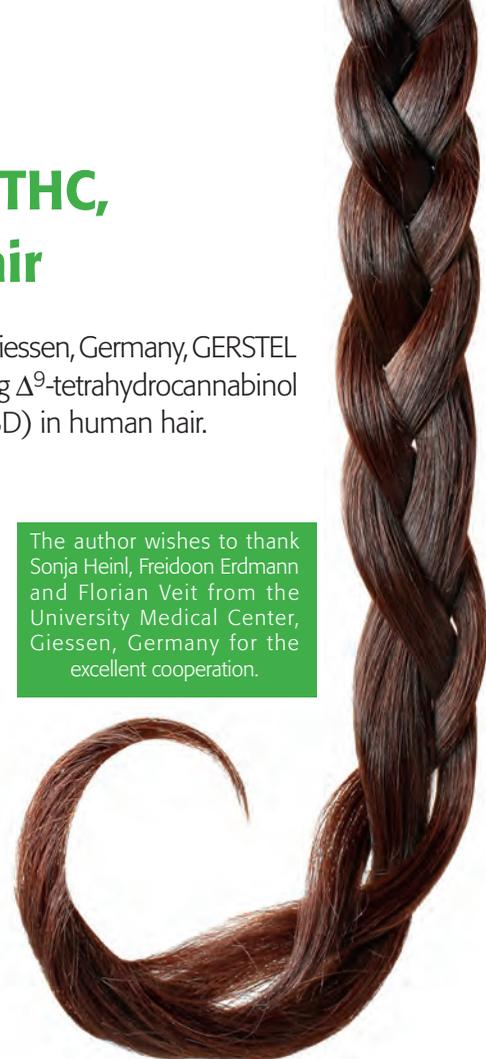
From a forensic toxicology point of view, human hair samples are extremely well suited for determining a person's consumption pattern of medication or drugs of abuse over a period of time. A human hair grows between 0.25 and 0.4 millimeters per day, in other words between 7.5 and 12 millimeters per month. 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.

New Article THC-COOH and THC-OH in hair

In addition to the method presented here, the Institute of Legal Medicine in Cologne, Germany and GERSTEL have jointly developed an automated and highly sensitive method for the determination of THC-COOH, THC-OH, THC, CBN and CBD in human hair. All analytes are extracted and detected in a single analytical run on a GC-QQQ system with electron impact (EI) ionization. LODs for THC-COOH and THC-OH are at 0.2 pg/mg. An article has been accepted for publication in Drug Testing and Analysis. Oliver Lerch, on behalf of GERSTEL, wishes to thank Tobias Kieliba, Hille Andresen-Streichert and Justus Beike for the excellent cooperation. <https://doi.org/10.1002/dta.2490>

The author wishes to thank Sonja Heinl, Freidoon Erdmann and Florian Veit from the University Medical Center, Giessen, Germany for the excellent cooperation.



More information*

AppNote 199

Automated Hydrolysis, Extraction and Determination of Opioids in Urine using a Novel Robotic Autosampler and LC-MS/MS Platform

AppNote 192

Drugs of Abuse in Oral Fluids: Automated SPE Extraction and LC/MS/MS Determination using a Robotic Autosampler

AppNote-2014-07

Comprehensive Automation of SPE-GC/MS based Analysis of Serum and other Matrices for Opioids, Cocaine and Metabolites

AppNote-2014-02

Fully Automated SPE-GC/MS Determination of Δ^9 -Tetrahydrocannabinol (THC) and its Metabolites in Serum Samples

AppNote-2014-01

Automated Hydrolysis, DPX Extraction and LC/MS/MS Analysis of Pain Management Drugs from Urine

AppNote-2013-02

A High Throughput Automated Sample Preparation and Analysis Workflow for Comprehensive Toxicology Urine Screenings using LC/MS/MS

AppNote-2013-01

Determination of Barbiturates and 11-Nor-9-carboxy-9-THC in Urine using Automated Disposable Pipette Extraction (DPX) and LC/MS/MS

AppNote-2012-08

Rapid Automated Extraction and Confirmation of Buprenorphine and Norbuprenorphine in Urine by DPX-LC/MS/MS. Comparison with "Dilute and Shoot" results

AppNote-2012-04

Automated Extraction of Vitamin D Metabolites from Serum

AppNote-2012-01

Rapid Cleanup and Comprehensive Screening of Pain Management Drugs in Urine using Automated Disposable Pipette Extraction and LC-MS/MS

AppNote-2006-11

Performance Evaluation of a Thermal Desorption System for Detection of Basic Drugs in Forensic Samples by GC/MS

* <http://www.gerstel.com/en/apps-forensics.htm>

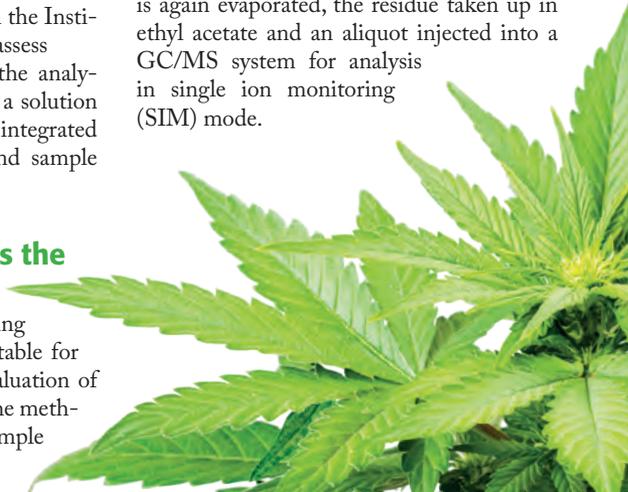
Hair today, gone tomorrow: How to determine cannabis consumption over time

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 cannabinalol (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 heating, 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 cannabinalol (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 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:

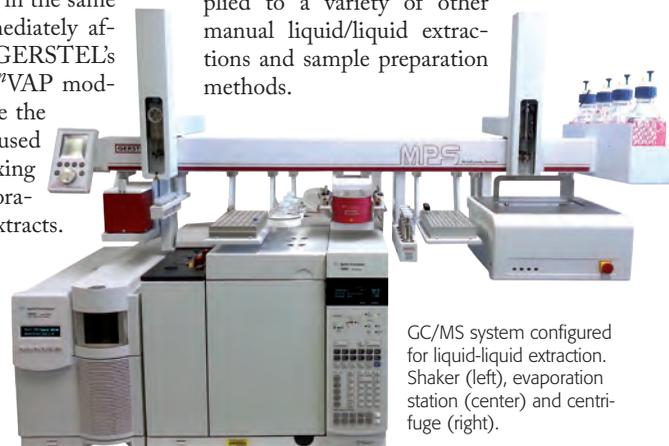
- A 100 mg sample of ground hair (plus ISTD) was placed in 1 molar NaOH and incubated at 85 °C for 13 minutes until dissolved
- Two extractions with hexane/ethyl acetate (9/1, v/v) were performed for 4 minutes each at 200 rpm in the GERSTEL *quickMIX*
- Phase separation by centrifugation at 4500 rpm 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 samples was validated in accordance with GTFCh guidelines. The limit of determination for THC was 0.01 ng/mg; the extraction efficiency (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.



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

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Suggested reading

O. Lerch, O. Temme, T. Daldrop:
COMPREHENSIVE AUTOMATION OF THE
SOLID PHASE EXTRACTION GASCHRO-
MATOGRAPHIC MASS SPECTROMETRIC ANALYSIS
(SPE-GC/MS) OF OPIOIDS, COCAINE, AND ME-
TABOLITES FROM SERUM AND OTHER MATRICES,
Anal. Bioanal. Chem. 406 (2014) 4443,
[https://dx.doi.
org/10.1007%2Fs00216-014-7815-7](https://dx.doi.org/10.1007%2Fs00216-014-7815-7)

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.

GERSTEL Company Description



GERSTEL develops and produces automated sample preparation and sample introduction accessories for GC, GC/MS, LC, and LC/MS. GERSTEL technology enhances productivity and significantly improves detection limits. GERSTEL is recognized by Agilent Technologies as one of their Premier Solution Partners. GERSTEL solutions can also be integrated into other leading manufacturer's systems.

Markets Served

- Forensic toxicology
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Major Products and Services

MAESTRO Software: Operates GERSTEL modules and systems independently or as integrated solutions with Agilent ChemStation/MassHunter, LECO ChromaTOF®, AB SCIEX Analyst®, Thermo Scientific Xcalibur™, and others. One sequence table and, depending on the system, one method can run the complete system including GC/MS or LC/MS.

MultiPurpose Sampler MPS: GC, GC/MS, LC, and LC/MS autosampler and sample preparation robot. Performs the sample preparation techniques listed below and more.

Online SPE system: (SPE^{XOS}): Performs online SPE with automated cartridge exchange

Automated SPE: Independent of LC/MS or GC/MS or combined with sample introduction. Based on standard cartridges.

Twister® Stir Bar Sorptive Extraction (SBSE): Ultra trace-level determination of organic compounds in liquid matrices. Up to 1000 times more sensitive than SPME.

Cooled Injection System (CIS): Universal PTV inlet for optimized GC performance. High temperature version up to 650 °C, cryostatic cooling.

Automated Liner EXchange (ALEX): For samples with a heavy matrix load.

Further techniques: Centrifugation, Solvent Evaporation, Weighing, Filtration, Vortexing, and Bar Code Reading

What a drop of blood reveals

In blood analysis, precision, sensitivity and the required sample amount are important factors – along with the analysis time. In all these areas, Dried Blood Spot (DBS) sampling and analysis is a convincing alternative – especially when automated with the GERSTEL MultiPurpose Sampler (MPS).

By Oliver Lerch

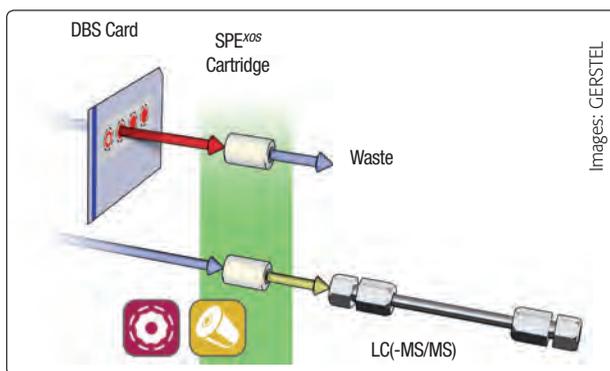
Since the 1960s, Dried Blood Spot (DBS) sampling has been used. Initially, especially neonatal screening for metabolic disorders was performed based on the DBS technique. In recent years, especially since more powerful GC/MS and LC/MS system became generally available, DBS has become useful in pharmaceutical research [1], forensic toxicology and doping analysis [2-4] as well as veterinary analysis [5].

Just a few drops of blood taken from a fingertip are sufficient to perform DBS analysis. The drops are placed onto small circular fields on special cards used for DBS work. As soon as the drops have dried, the cards are used to store and transport the samples, which are extracted directly from the card using dedicated sampling equipment.

A DBS sample typically contains between 15 and 30 μ Ls of blood evenly distributed across the spot. This means that a representative sample – and a defined amount of blood – can be taken by simply punching out a small area of the blood spot. Traditionally, a small, well defined disc of a few millimeters across has been punched out from the blood spot and transferred to a vial or micro-titer plate in which it was extracted using a suitable solvent. The resulting extract was centrifuged and the supernatant cleaned or analyzed directly following solvent exchange. Most often, LC-MS/MS or GC-MS/MS are the analysis techniques used. The described procedure is used for manual sample preparation based on DBS cards.

Automation improves productivity

In order to improve throughput and simplify the routine analysis workflow, the DBS technique must be properly automated. In cooperation with Spark Holland B.V., GERSTEL has developed an integrated system based on the Spark DBS system, with fully automated sampling from up to 240 DBS cards. Automation is performed using the GERSTEL MultiPurpose Sampler (MPS) under MAESTRO software control. The system can operate as stand-alone workstation preparing samples for LC/MS analysis – or integrated with the LC-MS/MS system. Slightly simplified, the DBS-MPS-LC-MS/MS System* works as follows: The MPS transports a DBS card to a camera. An image recognition software evaluates the dried blood spot. The card is loaded into the desorption interface and clamped into position. A desorption eluent then flows through a defined area of the blood spot desorbing the analytes based on the patented Flow Through Desorption technique (FTD™); an internal standard can be added to the desorption eluent for quality control purposes if required.



Schematic diagram of DBS flow-through desorption (FTD™) and SPE clean-up. FTD is patented by, and is a registered Trade Mark of, Spark Holland B.V.

An eluate clean-up step can further be performed based on an on-line SPE module which can be integrated into the overall analysis system. The online SPE system (GERSTEL-SPE^{xos}) is based on replaceable SPE cartridges, ensuring minimal sample to sample carry over while efficiently transferring the analytes to the LC-MS/MS system with best possible recovery for best possible LODs. Finally, the card is released and the associated tubing thoroughly rinsed with solvent. The card is again photographed to enable full documentation of the sample area,

which has been desorbed.

Conclusion: The fully automated DBSA system enables fast and reliable determination of analyte concentrations with excellent LODs.

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MPS-based GERSTEL DBSA Autosampler (to the left) with SPE^{xos}-Module (right hand side) for direct connection to the LC-MS/MS system (not shown).

