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Drug screening

Efficient automated THC determination

Intelligently automated sample preparation is used to optimize the determination of THC and its metabolites in serum.

In cooperation with the Department of Forensic Medicine at the University Medical Center Schleswig-Holstein in Kiel, Germany, GERSTEL has helped to develop an automated solution for the determination of Δ^9-tetrahydrocannabinol (THC) and its metabolites 11-hydroxy-Δ^9-tetrahydrocannabinol (THC-OH) and 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH) in serum. The result of the cooperation between toxicologists and GERSTEL application experts is a very promising method.

Cannabis is among to the most popular and most widely used drugs and the active ingredient Δ^9-tetrahydrocannabinol (THC) as well as its metabolites 11-hydroxy-Δ^9-tetrahydrocannabinol (THC-OH) and 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH) are therefore frequently found in serum samples associated with drug-related traffic incidents. This means that a large number of samples needs to be analyzed for these compounds and the analysis is a routine part of the forensic-toxicological arsenal. In Germany, road users found to have more than 1 ng/mL THC in their blood are facing not just a fine, but also the loss of their driver's license. Whether the loss is for a period of time or permanent will depend on the consumption pattern of the delinquent.

Establishing the consumption pattern

A decision to permanently withdraw a driver's license is not taken lightly; it obviously has to be based on solid facts: How much was consumed - and is it a matter of regular consumption or maybe even addiction. The facts are established in the toxicology laboratory based on the metabolism of THC: A high concentration level of THC in blood points to acute intoxication, similar to the blood alcohol levels established by the police through breath- or blood analysis when drivers are suspected of driving under the influence of alcohol. The presence of 11-hydroxy-Δ^9-tetrahydrocannabinol (THC-OH) in serum points to a recent intake of THC. The presence of 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol

(THC-COOH) in serum points to regular THC intake at levels between 75 and 150 ng/mL, and to frequent THC intake when levels are >150 ng/mL THC-COOH in serum. Frequent intake indicates addiction and, in Germany, this would typically lead to permanent withdrawal of the driver's license following a medical-psychological evaluation.

Manual SPE and GC/MS status quo

The toxicologists from the Department of Forensic Medicine at the University Medical Center Schleswig-Holstein in Kiel, Germany have until now determined THC, THC-OH and THC-COOH in serum based on solid phase extraction (SPE) followed by gas chromatography with mass spectrometric detection (GC/MS). The method is as follows: A 1 mL aliquot of Serum is taken and 1 mL acetic acid added along with internal standards in the form of the deuterated analogues of the three analytes. The sample extraction then follows on an ASPEC system (Gilson) using reversed-phase cartridges (C18ec, 3mL, 200 mg, UCT). The eluate is then concentrated by evaporation and N-methyl-N-(trimethylsilyl)-trifluoroacetamide (MSTFA) added to the residue as derivatization reagent. Analyte derivatization takes place in the hot split/splitless inlet of the GC and the derivatized analytes are determined using a GC/MS system from Agilent Technologies based on Single Ion Monitoring (SIM). All in all, the described method is proven and it works well. The main drawbacks are that it is somewhat labor intensive, requiring several manual steps, and that it requires relatively large volumes of solvent and of serum sample. In cooperation with the Dept. of Forensic Medicine in Kiel, GERSTEL has now automated the existing SPE-GC/MS method and realized further optimization potential in the process.

Developing an automated SPE-GC/MS method

The serum samples must be extracted and cleaned using solid phase extraction (SPE) prior to GC/MS analysis. This made the GERSTEL MultiPurpose Sampler (MPS) the obvious choice for the automation since it enables one to one replication of manual SPE process steps on an automated platform. Typically, the MPS brings other positive side effects such as improved precision and reliability of the analysis, especially since the automated SPE method does not rely on the experience of the user and isn't influenced by user-to-user variations. The automated SPE process is illustrated in the figure below. The GERSTEL MAESTRO software enables easy and intuitive set-up of all steps in the process by mouse-click from a drop-down menu. And this applies not only to the SPE process, but equally to all other liquid handling steps including adding acetic acid and internal standards, evaporative concentration of the eluate at 60 °C, and finally adding derivatization reagent and introducing an aliquot to the GC/MS.

Details - from automated SPE to sample introduction

The GERSTEL SPE system based on the MultiPurpose Sampler (MPS) requires standard-dimension SPE cartridges that are cut at the top and mounted with a transport adapter for use by the MPS. Finished cartridges are readily available from multiple sources. Method to method comparison was performed using cartridges similar to the previously used UCT product in the form of Macherey & Nagel (M&N), C18, 3 mL, 200 mg cartridges. The comparison brought similar results for the two types of cartridges. In order to reach the secondary goal of a reduction in solvent consumption, the informed assumption was pursued that the same promising results could be achieved with finer dimensioned cartridges based on the same packing material. To this end, M&N-C18ec-cartridges 1mL, 100mg were used together with the following automated SPE method:

Condition the cartridge with methanol, water and acetic acid

↓

Add 0.5 mL serum sample, to which 0.5 mL acetic acid and internal standards have been added

↓

Wash the cartridge with acetic acid and with a 40/60 mixture of ACN/Water

↓

Dry the cartridge using nitrogen gas (N2)

↓

Elute with 400 µL acetonitrile (ACN)

↓

Evaporate to dryness at 60 °C using the mVAP

↓

Take up the residue in 25 µL MSTFA at ambient temperature

↓

Inject 3 µL into the split/splitless inlet of the GC for combined derivatization and introduction

The separation was performed on a capillary column (VF-1 ms 25 m x 0.2 mm x 0.33 µm, Agilent Technologies) using a GC 6890 and MSD 5973 in single ion monitoring mode (SIM), both from Agilent Technologies.

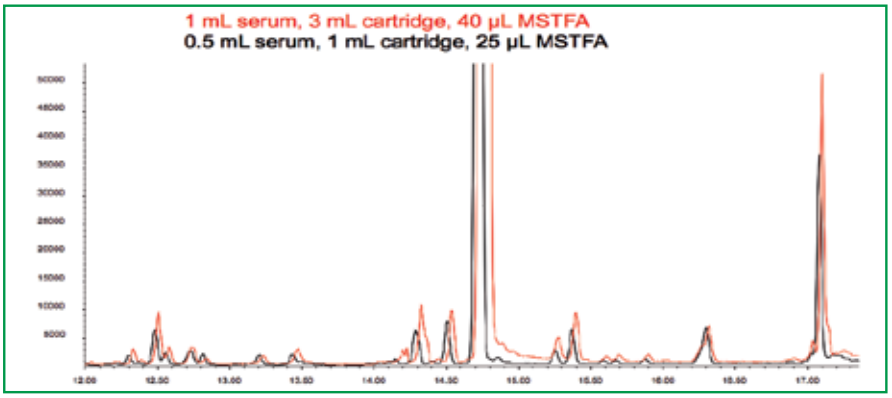
Standard Solid Phase Extraction (SPE) cartridge and same cartridge in GERSTEL format for automation. Cartridges in this format are readily available from several vendors: Agilent, Sigma-Aldrich, Macherey&Nagel and others on request.

Schematic of GERSTEL automated Solid Phase Extraction (SPE)

System employed for THC analysis. From left: Solvent Filling Station (SFS 2) supplying large volumes of solvents, SPE station, eluate and sample vials, tray for SPE cartridges, multi Position Evaporation Station (mVAP) for eluate evaporation

Analyte	Limit of Detection [ng/mL]	Limit of Quantification [ng/mL]	Repeatability [%]			Inter-day Repeatability [%]			Extraction Efficiency [%]		
			1.2 ng/mL	5.5 ng/mL	25 ng/mL	1.2 ng/mL	5.5 ng/mL	25 ng/mL	1.2 ng/mL	5.5 ng/mL	45 ng/mL
THC	0.3	0.7	5.2	7.2	3.8	7.8	7.2	6.7	75	74	73
THC-OH	0.3	0.9	3.5	10	3.5	16.3	10	6.9	93	82	88
THC-COOH	<1	9	3.7	6.6	3.3	8.6	7.1	7.1	83	79	85

Validation data according to GTFCh.



Analysis of THC and metabolites from serum with automated sample preparation. Extraction of 0.5 mL serum with 1 mL 100 mg C18ec SPE cartridge and 1 mL serum with 3 mL 200 mg C18ec cartridge. Reconstitution in 25 µL MSTFA or 40µL MSTFA respectively. Same performance is achieved, but the 1 mL cartridge requires less sample, less solvent, less elution volume, less time for evaporation, less MSTFA volume, less time for reconstitution, and therefore results in higher throughput.

Efficient LC-MS/MS drug screening

American LC/MS experts have developed an efficient high throughput automated extraction of small volumes of urine samples (< 500 µL) used in the determination of for example pain management drugs by LC-MS/MS. Disposable pipette extraction (DPX) was used in a novel manner to extract pain management drugs for comprehensive screening. Extracts were automatically diluted and injected into the LC-MS/MS system. Sample preparation was performed "just-in-time", the cycle time averaged 7 min per sample. Validation results show that the automated DPX-LC-MS/MS screening method provides adequate sensitivity for more than 65 analytes and internal standards. Lower limits of quantitation (LLOQ) ranged between 0.5 – 50 ng/mL and % RSDs were below 10 % in most cases.

Read more

Rapid Cleanup and Comprehensive Screening of Pain Management Drugs in Urine using Automated Disposable Pipette Extraction and LC-MS/MS, GERSTEL AppNote AN-2012-01

Successful automation of a manual method

Any method has to prove its worth in practical use under real world conditions. In the case of the work described here, the manual SPE method was successfully transferred to an automated system that included directly coupled GC/MS determination of THC and its metabolites THC-OH and THC-COOH. The scientists at the Dept. of Forensic Medicine in Kiel and the GERSTEL application experts were able to demonstrate that the stated goals were reached. This included reaching a reduction of the sample (serum) volume used; transfer of the method to smaller SPE cartridges; and, finally, a reduction of the solvent volume used per sample. This resulted not only in a reduction of cost, but also in shorter analysis cycles and higher throughput, leading to improved laboratory efficiency. The limits of determination for THC and THC-OH in serum were < 1 ng/mL and the calibration curves were linear over a wide concentration range. The average reproducibility was below 5 % for all three compounds with day-to-day repeatability averaging below 9 %. Extraction recovery ranged from 73 to 93 %, which was deemed satisfactory, and no sample to sample carry-over was seen. Apart from these points that are of course highly relevant when a method is developed, further criteria must be met by instrument manufacturers when developing methods in the field of forensic science: Firstly, the validated SPE-GC/MS method for the determination of THC, THC-OH and THC-COOH must meet the requirements of forensic toxicological work in practice. Secondly, further forensic toxicological institutes have announced their interest in automating this method, and thirdly, the SPE-GC/MS system can be used not just for the determination of the compounds mentioned in this work and for the SPE technique, but also for a long list of other techniques and compounds that are relevant to the toxicologist. Examples are the use of dispersive SPE in the form of Disposable Pipette Extraction (DPX) or of Stir Bar Sorptive Extraction (SBSE) for the determination of drugs and metabolites in body fluids and in tissue.

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Automated Pyrolysis Option

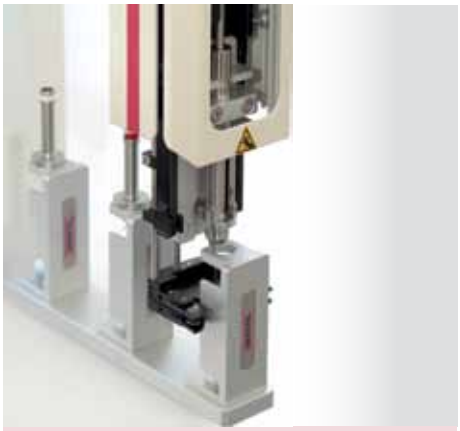
The GERSTEL PYRO module for the GERSTEL Thermal Desorption Unit (TDU) is available for automated pyrolysis of up to 14, 98, or 196 samples in combination with the GERSTEL MultiPurpose Sampler (MPS). PYRO enables pyrolysis of solids and liquids at up to 1000 °C. PYRO performs the following techniques: Standard pulsed pyrolysis; fractionated pyrolysis; and solvent venting combined with pyrolysis. Thermal desorption prior to pyrolysis enables the determination of volatile compounds and provides a clean pyrolysis chromatogram that is easy to interpret. Solvent venting can remove water, for example, from a polymer solution prior to Pyrolysis. PYRO is controlled using the MAESTRO software or integrated with the GC/MS software.

Multi-Position Evaporation Station (mVAP)

A six-position evaporation station (mVAP) is available for the GERSTEL MultiPurpose Sampler (MPS). Samples are concentrated at user-defined temperature and vacuum, enabling significantly improved limits of detection. Solvent exchange to a GC- or HPLC compatible solvent can be performed for improved chromatography. mVAP can be used in combination with SPE, Dispersive SPE (DPX) or liquid/liquid extraction to evaporate solvent from extracts combined with injection to GC/MS or LC/MS. Every step is controlled by mouse-click using the MAESTRO PrepBuilder. Just one method and one sequence table is needed for the entire process including GC/MS or LC/MS analysis.

Vortex / Shaker option for the MPS

The novel Vortex and shaker option (mVORX) for the GERSTEL MultiPurpose Sampler (MPS) is now available. The GERSTEL mVORX performs vortexing and agitation at up to 3,000 rpm, speeding up sample preparation steps such as liquid-liquid extraction, dissolution, and homogenization. The mVORX performs efficient simultaneous vortex mixing of up to eight samples depending on the vial size. The mVORX orbital motion is provided by a precise linear, direct drive motor for finely-tuned operation. Mixing movements are restricted to the horizontal plane, allowing even the most sensitive samples to be mixed efficiently without over-agitation or wetting of the vial cap. Automated operation under MAESTRO software control enables reliable and flexible sample processing, the PrepAhead functionality ensures best possible productivity and throughput.



SPME Fiber EXchange

The SPME Multi-Fiber EXchange (MFX) enables the GERSTEL Multi-Purpose Sampler (MPS) to replace SPME fibers at user-defined intervals. MFX trays are available for three or 25 fibers. Conditioned SPME fibers are kept in sealed compartments ready for use; a bake out station or the GC inlet can be specified for conditioning. Pre- or post-extraction derivatization can be specified, and the MPS 007 agitator supports shaking or stirring of the sample for longest possible fiber-life. **NEW:** Passive Air-Sampling devices for SPME fibers.

New connection technology for multi-dimensional GC/MS

The GERSTEL µFlowManager provides easy and rugged column connections for multi-dimensional GC/MS analysis of complex samples. The system is based on deactivated low thermal mass connectors. Metal ferrules enable easy set-up, ultra-low air background and best GC/MS system stability. Using EPC, either pressure or flow programming can be used. MAESTRO software and set-up tools enable easy method development and fully optimized separation. System features:

- Venting, backflush and multi-dimensional GC/MS by mouse-click
- Leak-free, low-dead-volume connections
- Metal ferrules, ultra low air background
- Rugged and reliable GC/MS analysis
- Deactivated, inert surfaces
- Correct results, no memory effects or discrimination

For more information, please visit the Da Vinci Europe booth.