

Drug screening

Joint Efforts – efficient automated THC determination

In cooperation with the Department of Forensic Medicine at the University Medical Center Schleswig-Holstein in Kiel, Germany, GERSTEL has helped to develop a fully 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. Results show the method to be equivalent to manual sample preparation, but with improvements in reproducibility and efficiency while reducing sample and solvent volumes.

Cannabis is among 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 need to be analyzed for these compounds and the analysis method is a routine part of the forensic-toxicological tool-kit. 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 offender.

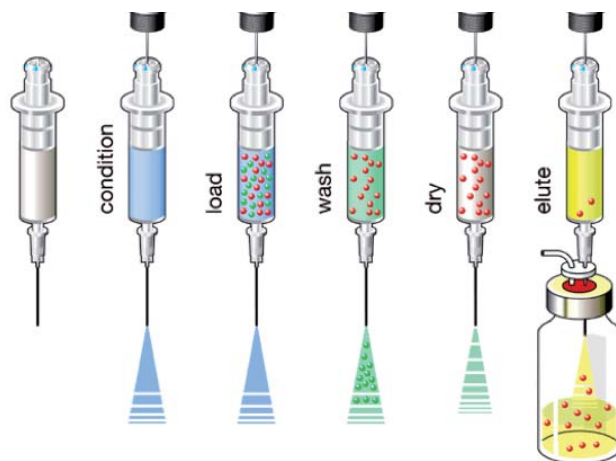
Establishing the consumption pattern

A decision to permanently withdraw a driver's license is not taken lightly; it obviously has to be based on sound evidence: How much is present, how much was consumed – and is it a matter of habitual use or maybe even addiction. The facts are established in the toxicology laboratory based on the metabolism of THC: A high concentration 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 un-

der the influence of alcohol. The presence of THC-OH in serum points to a recent intake of THC. The presence of 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,



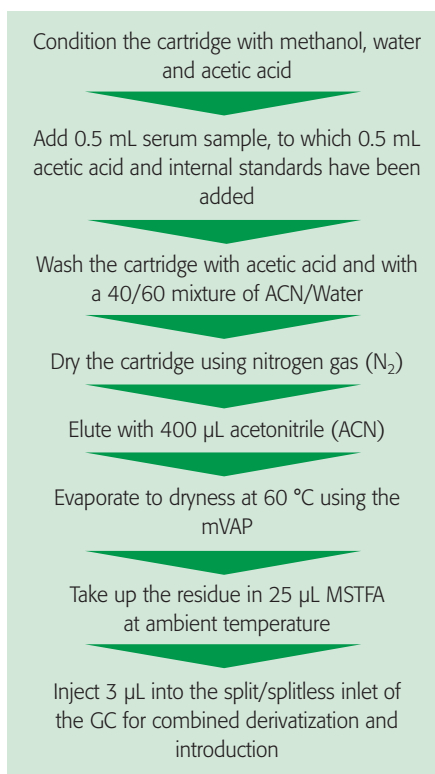
Schematic of the automated solid phase extraction process as performed by the GERSTEL SPE.

Germany have until now determined THC, THCOH 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 is then performed using an ASPEC system (Gilson) using a reversed phase cartridge (C18ec, 3mL, 200 mg, UCT). The eluate is then concentrated by evaporation and N-methyl-N-(trimethylsilyl)-trifluoroacetamide (MSTFA) added to the residue as a 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). This method provides reliable data, but its 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, Germany, GERSTEL has now helped to fully automate the existing SPE-GC/MS method and realized further optimization potential in the process.

Developing a fully 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. The use of the MPS also brings other benefits 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 above. 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 hand-

ling steps, including adding acetic acid and internal standards, evaporative concentra-



tion of the eluate at 60 °C, and finally adding derivatization reagent and injecting into the GC/MS system.

Details - from automated SPE to sample introduction

The GERSTEL SPE system based on the MultiPurpose Sampler (MPS) uses standard SPE cartridges that are modified and fitted with an adapter so that they can be transported by the MPS. Cartridges prepared for use with the GERSTEL SPE system are readily available from multiple sources. A method to method comparison was performed using cartridges from Macherey & Nagel (C18ec, 3 mL, 200 mg) similar to the previously used UCT product. The comparison brought similar results for the two types of cartridges. In order to reach the secondary goal of a reduction in solvent consumption, a smaller cartridge, requiring less sample and solvent volume was tested (M&N-C18ec-cartridges 1 mL, 100mg) using the automated SPE method shown in the box to the left.

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.

Successful automation of all sample preparation steps

Any method has to prove its worth in practical use under real world conditions. In the case of the work described here, the 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 automated method was equivalent to the previously used method with the

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	25 ng/mL
THC	0.3	0.7	5.2	7.2	3.8	7.8	7.2	6.7	75	74	70
THC-OH	0.3	0.9	3.5	10	3.5	16.3	10	6.9	93	82	86
THC-COOH	<1	9	3.7	6.6	3.3	8.6	7.1	7.1	83	79	87

Validation data according to the German Society of Toxicological and Forensic Chemistry (GTFCh).



Standard solid phase extraction cartridge (B) and same cartridge in GERSTEL format (A) for automation. Cartridges in this format are readily available from several vendors: Macherey & Nagel, Sigma-Aldrich, Agilent Technologies, and others on request.



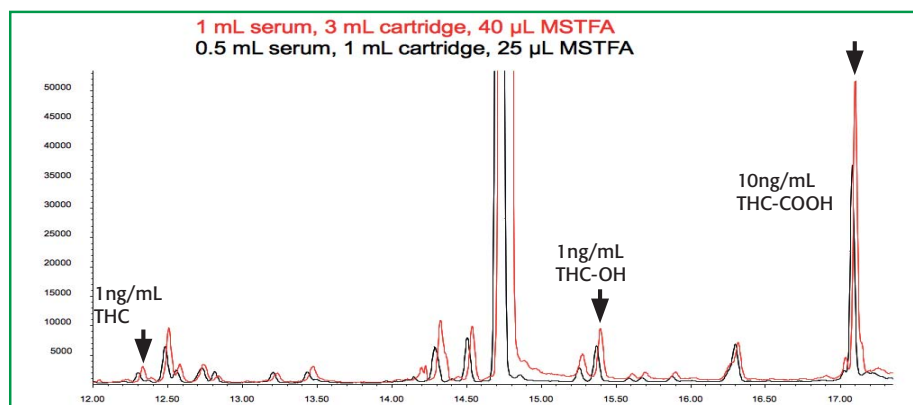
MPS-GC/MSD system used for THC analysis. From the right: Four-position solvent reservoir; multi-Position Evaporation Station (M²VAP) for eluate evaporation with the associated vacuum pump placed below; dual Solvent Filling Stations (SFS) supplying large volumes of solvents; SPE station; sample vial tray; SPE cartridge tray; eluate vial tray; Solvent Wash Station for syringe rinsing; Heated Agitator; second solvent reservoir.

added benefits of sample volume reduction (serum); transfer of the method to smaller SPE cartridges; and, finally, a reduction of the solvent volume used per analysis.

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 repeatability 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.

These criteria of course are highly relevant when a method is developed; however, other factors need to be considered by instrument manufacturers when developing methods in the field of forensic science: First, 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. Second, other forensic toxicological institutes must have expressed their interest in automating this method, and third, the SPE-GC/MS system must be versatile so it 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.



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 40 µL 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 DPX-LC-MS/MS drug screening

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 can be used to determine 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.

Suggested reading and additional information: www.gerstel.com

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

GERSTEL AppNote 02/2013: A High Throughput Automated Sample Preparation and Analysis Workflow for Comprehensive Toxicology Urine Screenings using LC/MS/MS

GERSTEL AppNote 01/2013: Determination of Barbiturates and 11-Nor-9-carboxy-Δ⁹-THC in Urine using Automated Disposable Pipette Extraction (DPX) and LC/MS/MS

GERSTEL AppNote 08/2012: Rapid Automated Extraction and Confirmation of Buprenorphine and Norbuprenorphine in Urine by DPX-LC/MS/MS

GERSTEL AppNote 04/2012: Automated Extraction of Vitamin D Metabolites from Serum

Author

Oliver Lerch, Ph.D. GERSTEL GmbH & Co. KG
oliver_lerch@gerstel.de

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