TIAFT 2019

GERSTEL Newsletter

GERSTEL GmbH & Co. KG · Eberhard-Gerstel-Platz 1 · 45473 Mülheim an der Ruhr, Germany · Phone +49 208 765 03-0 · E-mail: gerstel@gerstel.com

57th Annual Meeting of the International Association of Forensic Toxicologists

Automation

More efficiency and flexibility in the forensic toxicology laboratory

Automating key processes and routines can increase productivity and performance of your analytical laboratory in a sustainable manner.

Oliver Lerch, Ph.D.
Application Specialist
GERSTEL Germany

Automating sample preparation and introduction depends to a large extent on the right choice of autosampler: The MultiPurpose Sampler (GERSTEL MPS) is an innovative X-Y-Z robot that can be used for many sample preparation and introduction tasks for GC/MS and LC/MS. The MPS can be operated integrated with the analytical instrument or as an independent workstation and can be individually configured.

Examples of MPS-based application solutions in the field of forensic-toxicologicy are, among others, the determination of tetrahydrocannabiol (THC), cannabinol (CBN) and cannabidiol (CBD) as well as the THC metabolites 11-Hydroxy-THC (THC-OH) and 11-Nor-9-carboxy-THC (THC-COOH) in serum and hair (cf. references on page 3). The MPS automates extraction and purification techniques such as liquid-liquid extraction and solid phase extraction (SPE) and adds internal standard to the sample. The available SPE techniques include standard SPE, based on standard dimension cartridges, µ-scale SPE as well as online SPE (GERSTEL SPE^{xos}) with exchangeable cartridges that are directly coupled to the LC/MS system for quantitative analyte transfer and optimized sensitivity.

The MPS offers mechanical mixing and separation techniques based on mechanical agitation, ultrasonication, filtration, and centrifugation. Solutions and liquid extracts can be automatically weighed (weighing option), kept at defined temperature, agitated (^{quick}MIX), evaporated (^{m}VAP) and derivatized. The MPS produces dilution series and calibration standards both accurately and reproducibly. The MPS also automates a wide range of standard techniques, such as Solid Phase Micro Extraction (SPME), Static Headspace (HS) and Dynamic Headspace (DHS), Stir Bar Sorptive Extraction (SBSE, GERSTEL Twister) as well as thermal desorption and pyrolysis. A wide range of sample vessels for a variety of different tasks and applications are available. In addition to standard trays with optional temperature control, space-saving cool stacks with trays in drawers allow a maximum number of samples to be stored protected from heat and light.

Biomarkers of alcohol consumption

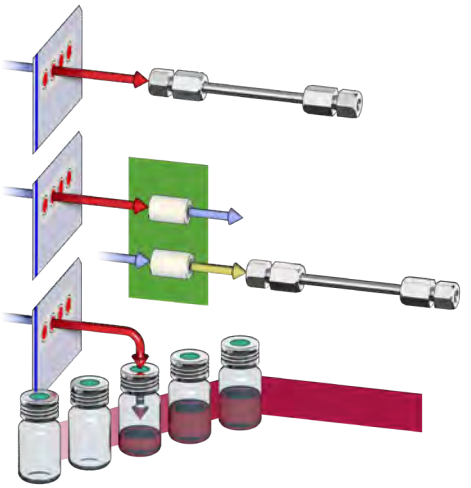
Fully automated detection of phosphatidylethanol (PEth) from dried blood spots (DBS)

By measuring the concentration of alcohol in breath or blood it may be possible to determine whether someone has just or recently consumed alcohol. However, these methods are unable to determine whether someone has abstained from drinking alcohol for a longer period of time, as may be required, for example, in connection with drivers´ aptitude tests or detox therapy. In those cases, other methods, including the analysis of specific alcohol biomarkers, are required.

Phosphatidylethanol (PEth) is a promising candidate because it can be detected at sufficient levels in the blood for a long period of time after alcohol has been consumed. However, enzymatic activity in liquid blood samples can cause PEth concentrations to change during storage and transport, PEth analysis from dried blood spots (DBS) is therefore recommended.

With the GERSTEL DBS A system, PEth can be detected efficiently, accurately and reliably. All steps in the sample handling process, including barcode reading, internal standard addition, extraction of a defined sample area from the DBS card, solid phase extraction (SPE) and online LC-MS/MS detection can be performed in a quick, reliable and safe manner, and fully automated from start to finish. More details on the GERSTEL MPS-DBSA-LC-MS/MS system are available at the **GERSTEL booth No. 62** or send a request to oliver_lerch@gerstel.de.

Simple and exact sampling of a 10 µL volume of whole blood onto Dried Blood Spot (DBS) cards. Image: DBS System



Automated Dried-Blood-Spot-Analysis

What a drop of blood reveals

In addition to precision and sensitivity of the analysis, the sample volume and the time it takes to achieve measurement results all play a role in analyzing blood. Analysis devices that are simple and easy to use do a lot to improve acceptance among users. Dried Blood Spot (DBS) analysis ticks all the boxes with the MPS-based GERSTEL DBS Autosampler (DBS-A).

Just a few drops of blood are enough to perform DBS analysis. The blood samples are applied and stored and analyzed on special cellulose cards that act as sample carriers. The homogeneous distribution of the components and constituents within the drop of blood and on the card make it possible to take and analyze a defined blood volume (2-3 µL) by punching out a disc of a defined diameter (e.g. 3 mm). The disc is extracted with a suitable solvent and centrifuged. The supernatant is cleaned-up or analyzed immediately or after solvent exchange, often by HPLC-MS/MS or GC-MS/MS.

While DBS analysis provides a simple technique for blood testing, automation can increase efficiency and productivity significantly. That's why GERSTEL, in cooperation with the Dutch company Spark Holland, has developed an automated all-in-one solution (DBS-A) that is based on its MultiPurpose Sampler (MPS) and is either coupled to an HPLC-MS/MS system or available as a workstation.

This automated process is currently being used in pharmaceutical and anti-doping laboratories, e.g. at the German Sport University Cologne. It goes without saying that the DBS-A system is an attractive solution for a number of other interesting fields, including forensic toxicology and metabolomics research. The MPS transports the DBS card to a camera, where an integrated image recognition software determines its position and evaluates the quality of the dried blood drop (Dried Blood Spot). The MPS places the card in the desorption interface, where a solvent flows through a defined area of the blood drop (partial or complete sampling) to desorb the analytes (Flow Through Desorption, FTD™). The MPS add an internal standard and perform sample clean-up using a replaceable SPE cartridge as needed.

After transferring the analytes into the HPLC system or collecting extracts using the workstation, the DBS card is released, the connecting tubing is rinsed and a photo of the card after the desorption step is taken for documentation. If the system is directly coupled to the HPLC, the analytes from the desorbed area are quantitative transferred to the HPLC column and on to the mass spectrometer, which guarantees lowest possible limits of detection.

Fully automated, MPS-based DBS-A system.

The DBS-A system is well suited for more than just analyzing blood samples: It is suitable for a multitude of other liquid matrices (e.g. plasma, urine, water, beverages).

Suggested reading

L. Tretzel, A. Thomas, T. Piper, M. Hedeland, H. Geyer, W. Schänzer, and M. Thevis, Fully automated determination of nicotine and its major metabolites in whole blood by means of a DBS online-SPE LC-HR-MS/MS approach for sports drug testing, Journal of Pharmaceutical and Biomedical Analysis 123 (2016) 132–140

Open Access

Suggested reading

L. Tretzel, C. Görgens, H. Geyer, A. Thomas, J. Dib, S. Guddat, V. Pop, W. Schänzer, and M. Thevis, Analyses of Meldonium (Mildronate) from Blood, Dried Blood Spots (DBS), and Urine Suggest Drug Incorporation into Erythrocytes, International Journal of Sports Medicine · DOI 10.1055/s-0036, www.thieme-connect.com/products/ejournals/pdf/10.1055/s-0036-1582317.pdf

References

[1] F. D. Foster, J. R. Stuff, and E. A. Pfannkoch, Automated Desorption, SPE Extraction, and LC-MS/MS Analysis of Dried Blood Spots, GERSTEL AppNote 4/2015 (www.gerstel.com/pdf/p-lc-an-2015-04.pdf)

[2] J. Dib, L. Tretzel, T. Piper, A. Lagojda, D. Kuehne, W. Schänzer, and M. Thevis, Screening for adiponectin receptor agonists and their metabolites in urine and dried blood spots, Clinical Mass Spectrometry 6 (2017) 13–20

Büchi NIR used to determine the DBS hematocrit value before analysis in the DBS-A-LC-MS/MS system.

Determining DBS hematocrit values automatically

In cooperation with Büchi (www.buchi.com), GERSTEL has implemented automated non-destructive determination of hematocrit values in dried blood spots placed on DBS cards. The automated function was added to the GERSTEL DBS-A, which extracts the sample before analyzing by LC-MS/MS. The hematocrit value is determined by near infrared measurement (NIR) combined with multivariate spectral analysis. The process is fully automated under GERSTEL MAESTRO software, every step is simply and easily set up by mouse-click.

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.

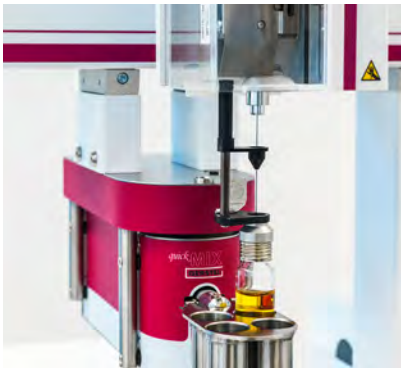
Liquid-liquid extraction is one of the most widely used standard procedures in sample preparation. All relevant steps can be automated with the MPS. The only manual effort required is to load the samples in 2-, 4- or 10 mL vials and place them in the MPS sample trays.

The method is conveniently generated by mouse-click. Your daily analysis sequence table is then set up in just a few steps, again, conveniently by mouse click, and you're ready to go: the MPS adds an internal standard to the sample, along with extraction buffer and solvent as specified by the user. During the extraction step, the MPS provides efficient agitation for optimal results.

Phases can be separated quickly – thanks to the integrated centrifuge. The MPS removes the relevant phase and transfers it to a clean vial; if necessary, a further extraction with fresh solvent can be performed. The MPS can also inject the extract to the analysis system or evaporate it to dryness in the mVAP to reconstitute with an LC-compatible solvent or to add a derivatization reagent.

Whichever steps are to follow, you have the choice: the MPS is a flexible and rugged tool for the automation of liquid-liquid extraction (LLE) not just due to the wide range of options it affords the user, but because it is astoundingly simple to control using the MAESTRO software.

MPS workstation, including integrated centrifuge.



GERSTEL *quick*MIX

GERSTEL Multi-Position Evaporation Station (mVAP)

Suggested reading

[1] Sonja Heinl, Oliver Lerch, and Freidoon Erdmann, **Automated GC-MS Determination of Δ⁹-Tetrahydrocannabinol, Cannabinol and Cannabidiol in Hair**, Journal of Analytical Toxicology (2016) 498-503, DOI 10.1093/jat/bkw047

[2] Kirsten Purschke, Sonja Heinl, Oliver Lerch, Freidoon Erdmann, and Florian Veit, **Development and validation of an automated liquid-liquid extraction GC/MS method for the determination of THC, 11-OH-THC, and free THC-carboxylic acid (THC-COOH) from blood serum**, Anal Bioanal Chem (2016) 4379–4388, DOI 10.1007/s00216-016-9537-5

[3] Oliver Lerch, Oliver Temme, and Thomas Daldrup, **Comprehensive automation of the solid phase extraction gas chromatographic mass spectrometric analysis (SPE-GC/MS) of opioids, cocaine, and metabolites from serum and other matrices**, Anal Bioanal Chem (2014) 4443–4451, DOI 10.1007/s00216-014-7815-7

[4] L. Tretzel, C. Görgens, H. Geyer, A. Thomas, J. Dib, S. Guddat, V. Pop, W. Schänzer and M. Thevis, **Analyses of Meldonium (Mildronate) from Blood, Dried Blood Spots (DBS), and Urine Suggest Drug Incorporation into Erythrocytes**, Int J Sports Med, DOI 10.1055/s-0036-1582317

[5] Laura Tretzel, Andreas Thomas, Thomas Piper, Mikael Hedeland, Hans Geyer, Wilhelm Schänzer, and Mario Thevis, **Fully automated determination of nicotine and its major metabolites in whole blood by means of a DBS online-SPE LC-HR-MS/MS approach for sports drug testing**, J Pharmaceut Biomed 123 (2016) 132–140, DOI 10.1016/j.jpba.2016.02.009

[6] Josef Dib, Laura Tretzel, Thomas Piper, Andreas Lagojda, Dirk Kuehne, Wilhelm Schänzer, and Mario Thevis, **Screening for adiponectin receptor agonists and their metabolites in urine and dried blood spots**, Clinical Mass Spectrometry 6 (2017) 13–20, DOI 10.1016/j.clinms.2017.10.002

[7] Tobias Kieliba, Oliver Lerch, Hilke Andresen-Streichert, Markus A. Rothschild, and Justus Beike, **Simultaneous quantification of THC-COOH, OH-THC, and further cannabinoids in human hair by gas chromatography-tandem mass spectrometry with electron ionization applying automated sample preparation**, Drug Test Anal (2018) 1–12, DOI 10.1002/dta.2490

[8] Joseph A. Crifasi, Michael F. Bruder, Christopher W. Long, and Kimberly Janssen, **Performance Evaluation of a Thermal Desorption System (TDS) for Detection of Basic Drugs in Forensic Samples by GC-MS**, J Anal Toxicol 30 (2006) 581–592, DOI 10.1093/jat/30.8.581

Your contacts

Oliver Lerch
Application Specialist
GERSTEL

Nathan Hawkins
Account Manager
Anatune

Alice Pannérec
Business Development Manager
DBS System

Christophe Tabacchi
Chief Sales & Marketing Officer
DBS System

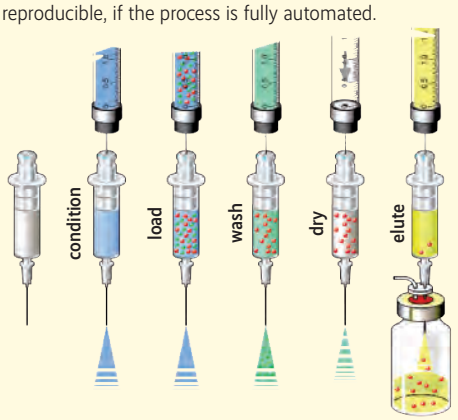
Automated extraction techniques

Step by step

Every GC(GC/MS)- and LC(LC/MS) application in forensic toxicology requires a particular, unique sample preparation. In order to unlock the secrets of a sample, a great deal of preparatory work is typically required. First of all, the sample must be converted into an analyzable form. That entails removal of interfering matrix components and/or analyte concentration. The extraction technique used depends not least on the sample, the analytes and the task at hand. On this page, we present two different Solid Phase Extraction (SPE) processes that are automated with GERSTEL technology.

Solid Phase Extraction (SPE)

Solid phase extraction (SPE) is probably one of the most versatile and most frequently used extraction techniques in the field of chemical analysis. SPE is used to eliminate matrix components on the one hand and to extract and enrich analytes on the other. SPE is labor-intensive and time-consuming when carried out by hand, especially when recovery and repeatability leave something to be desired. In contrast, SPE is more relaxed, time-saving, precise and reproducible, if the process is fully automated.

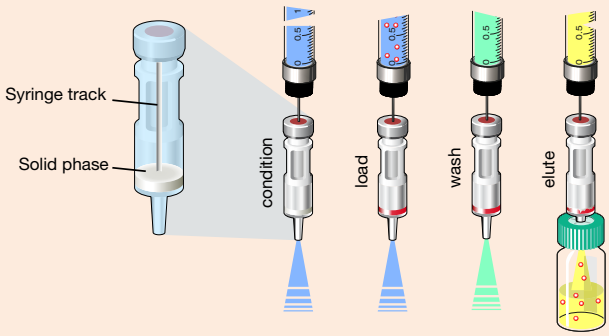


1. Standard 1, 3 or 6 mL SPE cartridges are available equipped with transport adapters for easiest possible automation using the GERSTEL SPE. An added benefit is that the GERSTEL SPE cartridges have practically no dead volume.
2. During conditioning cartridges are prepared for sample loading. Activation steps can be performed by the GERSTEL SPE, in many cases without adding to the overall analysis time.
3. Loading the sample with defined volumes at constant flow rates leads to highly reproducible results even if there are variations in packing density or amount of the solid phase material.
4. Rinsing steps can be performed in order to remove interferences or to introduce a solvent that matches the elution solvent. Up to 12 different solvents can be used, providing unlimited flexibility and enabling effortless implementation of highly complex rinse cycles.
5. Drying the cartridge prior to elution can be helpful in some cases. The GERSTEL SPE lets you automate this intermediate step by mouse click.
6. Retained compounds are eluted from the SPE cartridge into a closed vial via a disposable syringe needle. This eliminates the risk of cross contamination. The obtained eluate can be concentrated by solvent evaporation directly in the collection vial in the mVAP module.

The MPS performs automated SPE while enabling additional sample preparation steps, e.g. adding standards, derivatization, etc. Prep sequences can be easily set up at the click of a mouse using the intuitive MAESTRO software.

SmartSPE / µSPE

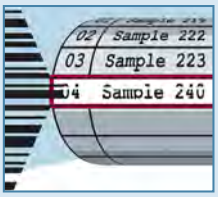
SmartSPE or µSPE is a patented microscale solid phase extraction (SBE) technigue. SmartSPE / µSPE allows significantly smaller volumes to be handled, which reduces solvent consumption and the time and effort involved in sample preparation. Since SmartSPE / µSPE uses conventional SPE sorbents, existing methods can typically be adapted to the micro-scale approach and implemented.



After conditioning the sorbent, the cartridge is loaded with sample. If necessary, the sorbent bed can be washed to remove matrix components before elution is carried out in the final step using a suitable solvent. Compared to conventional SPE, this process requires significantly less solvent due to the lower quantity of sorbent (10 - 45 mg).

The SmartSPE / µSPE is fully supported by the GERSTEL-MultiPurpose Sampler (MPS). Its automation with the MPS not only offers the user the benefits and added value described above. It is also fully integrated with the MAESTRO PrepAhead functionality and Agilent Technologies' MassHunter and ChemStation software. Other GERSTEL modules and sample preparation steps can be used in conjunction with SmartSPE / µSPE. Your daily sequence table is set up and started using just a few mouse-clicks – including both sample preparation steps and GC/MS- or LC/MS analysis.

Sequence by Barcode



New automation efficiency and QC options have been integrated into the MAESTRO software using the GERSTEL Sample ID (SID) barcode reader. In addition to sample logging and sample ID transfer to the data file and file name, SID enables automated analysis by predefined methods as well as automated generation of sequence tables. Single sample mode or Batch Analysis Mode (BAM) is selected by mouse-click. Trigger vials can be introduced at user defined intervals, for example, to activate solvent injection(s) for system background checks followed by one or more check standard runs as part of the QC routine operation. Trigger functions are performed without taking up multiple sample positions in the tray enabling a higher overall analysis throughput while ensuring adherence to set quality control requirements. Multiple options are available for sample verification and the handling of deviations and the software can be locked to rule out unauthorized changes, for example, in a production QC environment. Data import and export functions enable LIMS or data base synchronization.

Contact
GERSTEL GmbH & Co. KG
Eberhard-Gerstel-Platz 1 · 45473 Mülheim an der Ruhr, Germany
Phone +49 208 76503-0
E-mail: gerstel@gerstel.com

Editor: Guido Deussing
Layout: PauraDesign