

DETERMINATION OF NATURAL AND SYNTHETIC STEROIDS IN ANIMAL URINE SAMPLES

TWO SOLID-PHASE EXTRACTIONS IN ONE METHOD: ROBOTIC SYSTEM SIGNIFICANTLY REDUCES PROCESSING TIME

The Chemical and Veterinary Investigations Office Münsterland-Emscher-Lippe (CVUA-MEL), a governmental laboratory in Germany, has so far carried out all steps required for sample preparation for the analysis of steroids in animal urine manually. This is a lengthy and very time-consuming activity as two solid phase extractions have to be carried out successively. In order to lighten the workload of the laboratory staff and to be more time-efficient, the CVUA-MEL has recently implemented the LCTech GmbH modular robotic system FREESTYLE for automated sample preparation. In conjunction with the MACHEREY-NAGEL's CHROMABOND SPE columns, good quality and reproducible results are obtained and, moreover, a high sample throughput can be achieved. The system operates under a positive pressure of up to 4 bar, and makes it possible to process samples with different viscosities and reliably excludes cross-contamination.

The National Residue Control Plan (NRKP) for food of animal origin is a program for preventative consumer health protection, which has been implemented across the EU since 1989 applying uniform standards. Within the framework of this program, live livestock is also investigated for residues of unwanted substances. In this context, the CVUA-MEL regularly checks animal urine samples for steroids. "The use of these substances as growth promoters is prohibited in the EU. Synthetic steroids must therefore be detectable in urine even at very low concentrations," explains Dr. Thorsten Bernsmann, Head of Division at CVUA-MEL. "Minimum detection limits exist for individual substances of this substance group, i.e. a so-called "recommended concentration" of 1 µg/l urine has been set". In order to achieve this very low detection limit, it is necessary to carry out sample preparation before analysis.

Laborious Manual Clean-up

The sample preparation for the determination of steroids in animal urine is complex, since two solid phase extractions have to be carried out successively: The urine samples are first centrifuged to separate solid impurities. The pH is then adjusted to a value of about 5 using a buffer in order to be able to hydrolyse the samples enzymatically with glucuronidase or arylsulfatase.

By hydrolysis, for example, sugar bound steroids are converted into their free form so that they can be detected during the final measurement. "The first clean-up of the hydrolysed sample extract, which is a retentive solid phase extraction, is carried out with a C18-SPE column. A wash with a 5% methanol solution and elution with pure methanol follows, discarding the first millilitre. The second purification is carried out using an NH₂-SPE

column. This is a non-retentive solid phase extraction," explains Dr. Bernsmann. The eluates are collected, evaporated, re-dissolved with solvent and subsequently measured.

"Up to the beginning of 2015, we carried out the described clean-up procedure manually at the CVUA-MEL, which was very time-consuming: Around 24 samples keep one employee busy for a whole working day," explains Dr. Bernsmann. The samples often behave differently on the SPE columns, because they have, for example, different viscosities. "During manual processing, the lab staff are able to regulate the negative pressure, however, they have to pay attention to each sample individually, since several samples are processed at the same time," says the scientist. "While one sample runs slowly through the SPE column and requires some negative pressure, the other runs smoothly, and this column must be prevented from running dry".

FREESTYLE Software Allows for Method Set-up with Just a Few Mouse Clicks

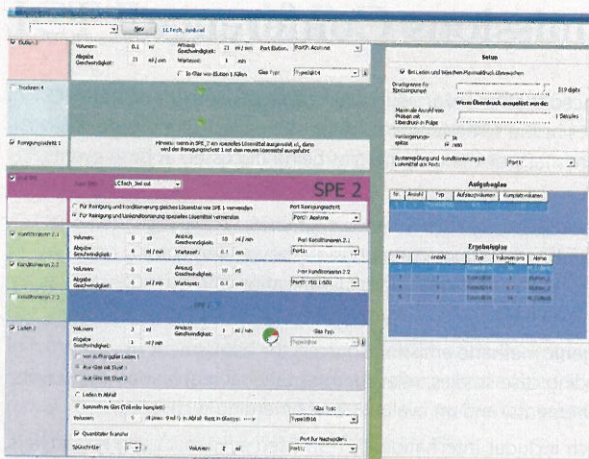
Since February 2015, the CVUA-MEL changed their most complex, so far manually performed methods over to automated sample preparation using the LCTech FREESTYLE system. One of the recently automated methods is the analysis of urine samples for steroids. The software of the modular robotic system was specifically adapted for this special type of sample preparation: "The special feature was the integration of two successive solid phase extractions using different column formats into one single method ("Dual SPE")," explains Sebastian Wierer, process developer at LCTech. For this purpose, we have developed a

special system software expansion, which allows for the combined processing of two columns within one method." Owing to this modification, the processing method for the "Dual SPE" can be easily set up in the software: In a special software window, all parameters required for the processing steps are stored.



In order to lighten the work load of the lab staff and to reduce the effort involved in two successive solid phase extractions, the CVUA-MEL has recently introduced automated sample preparation using the modular FREESTYLE robotic system by LCTech GmbH for the detection of steroids in animal urine samples.

Source: LCTech GmbH



LC Tech expanded the software of the robotic system, enabling the automated processing of two solid phase extractions in succession using two different column formats within one method. Method set-up for the so-called "Dual SPE" is easy just by clicking on the required parameters.

Source: LC Tech GmbH

By clicking on various on-screen buttons, processing steps are selected, parameters such as flow rate and pressure can be entered, thus defining the method," says Wierer. As soon as the method is saved, it can be called up and utilised by any employee for their laboratory routine at any time.

Effective and Fast Clean-up by Combining Columns and Plungers

As SPE columns, the CVUA-MEL uses MACHERY-NAGEL'S CHROMABOND models to effectively and quickly purify the samples. "The columns can be used for both the systematic method development in sample preparation or the standard routine clean-up of a more regular volume of samples using the FREESTYLE," explains Dr. Hans Rainer Wollseifen, application specialist at MACHERY-NAGEL GmbH & Co. KG. The base materials ensure an efficient enrichment and ideal flow behaviour. The optimised pore structure and the highly specific surface area are, amongst other things, responsible for a low solvent consumption. "In order to increase the flow rates and minimize dead volumes in the columns, we have developed special plungers that are inserted into the CHROMABOND columns," says Wierer. "Without the plungers and at higher flow rates, one would often have to wait until the column bed supernatant has drained off. Now, however, all conditioning, washing and elution solutions can be passed over the column at a constant pressure".

More Consistent Measuring Signals with Less Effort

For automated sample preparation, the already hydrolysed samples and the plunger-equipped SPE columns as well as two elution vials are simply put into the device. The first elution vial receives the sample after the first solid phase extraction. The container into which the sample is eluted after the second solid phase extraction is shaped in such a way that it can then be placed into the TurboVap for evaporation.

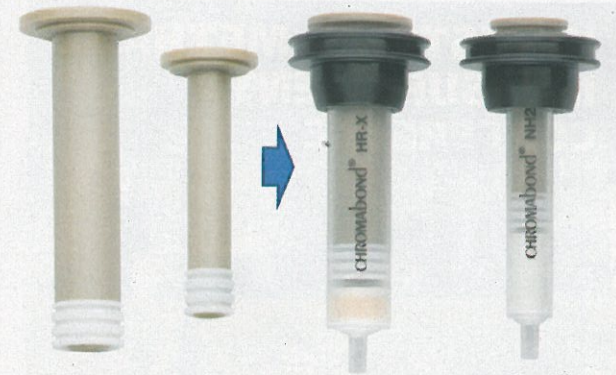
Then the sequence is started. "The device carries out the two clean-up steps automatically in the manner described earlier, after which the samples will only need evaporating and re-dissolving in another solvent," explains Dr. Bernsmann. For example, 18 samples can be processed in one run, while the respective staff have the freedom to carry out other tasks.

The automated clean-up of sample extracts significantly saves time: "Loading the FREESTYLE and starting the sequence takes only a fraction of the time required for manual processing of the samples," says Dr. Bernsmann. "The sensitivity of the process is just as good as that of the manual preparation". The consistency of the measuring signals has even improved since all solutions are passed over the column at a constant pressure. In contrast to manual processing, the standard deviation in the results has decreased significantly, which means that the system works far more reproducible and more stable than manual processing. In addition, the different viscosities of the samples hardly play a role anymore.

Processing with up to 4 Bar Pressure

"This is, because the FREESTYLE system operates under a pressure of up to 4 bar. Only when this relatively high value, or another pressure value previously defined in the method, is exceeded, the device will interrupt sample processing. The interrupted sample is marked in the sample list, the system will clean itself, and then continues processing with the next sample," explains Wierer. The sequence as such is not terminated, and the unprocessed sample is clearly recognizable. With two special features, namely the plungers and the positive pressure of up to 4 bar, many samples, which may lead to blockages on other systems, can be reliably passed through the columns. Thus, unlike other robotic systems, the FREESTYLE is also particularly suitable for difficult matrices containing suspended particles or filaments. For example, in addition to urine, this system can also be used for river water with sediments.

In addition to the constant pressure, which leads to reproducible results, the switch to automated sample preparation has also other advantages. "As with the manual method, the LC Tech

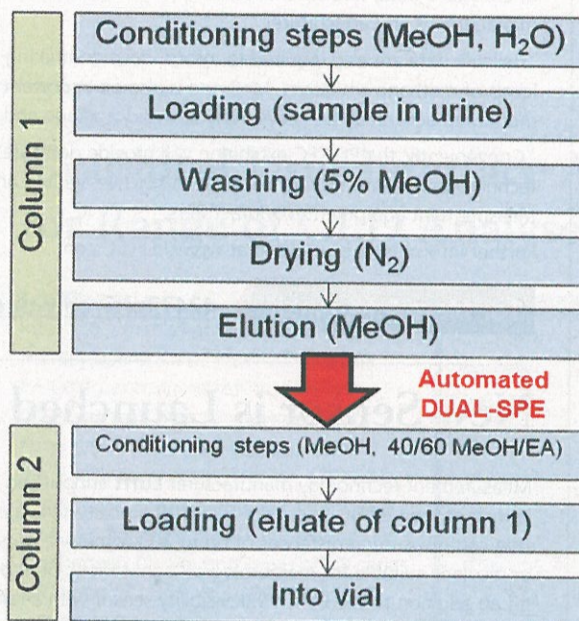


The CVUA-MEL uses for clean-up the MACHERY-NAGEL CHROMABOND columns, which are particularly suitable for the fast and effective sample clean-up in the robotic device. In order to minimize dead volumes in the columns and to increase the flow rates, LC Tech has developed special plungers that are inserted into the columns.

robotic system will process approximately 50 samples per week. However, the use of the FREESTYLE system significantly reduces man hours," explains Dr. Bernsmann. "In fact, the robotic system actually increases our efficiency, as the CVUA-MEL staff can carry out other important tasks that cannot be automated during the sample clean-up. Even the previously manually performed, time-consuming SPE method development can now be carried out automatically overnight. Unattended sample processing is also possible over the weekend, so that the system can be used very efficiently. In addition, the results show that the system processes absolutely cross-contamination free," sums up the scientist.



"The FREESTYLE system operates with a pressure of up to 4 bar. As a result, many samples, which lead to blockages on other systems, reliably pass through the columns. This makes the FREESTYLE particularly suitable for difficult matrices with suspended particles or filaments – apart from urine, also river water with sediments could be tested," explains Sebastian Wierer, process developer at LC Tech.



*Column 1: CHROMABOND™ HR-X, 6 mL
*Column 2: CHROMABOND™ NH₂, 3 mL

The urine samples together with the SPE columns and two elution vials are simply placed into the FREESTYLE, and then the sequence can be started. The first clean-up of the sample extract is carried out with a C18-SPE column. Washing is done with a 5% methanol solution and pure methanol is used for elution. The second clean-up is carried out using an NH₂-SPE column. These clean-up steps are performed automatically, and then the samples only need evaporating and re-dissolving in another solvent before the measurement can take place.

The Chemical and Veterinary Investigation Office Münsterland – Emscher – Lippe (CVUA-MEL) with two sites in Münster and Recklinghausen is a public institution. Institutional authority lies with the counties and cities of the administrative district of Münster and the state of North-Rhine-Westphalia. Their duties include the maintenance of animal health and welfare, and the protection of consumers against fraud, deception and exposure to health hazards.

MACHERY-NAGEL GmbH & Co. KG was founded in 1911 in Düren as a manufacturer of special filtration papers. In 1952, the company launched the first products for paper chromatography and, from the end of the 1950s, developed into a specialist in analytical chemistry and pioneer in thin-layer chromatography. In 1970, the product area chromatography was expanded by column chromatography. Today, MACHERY-NAGEL is Europe's leading manufacturer of SPE products. The DIN EN ISO 9001 certified company places high value on the quality and purity of the sorbents and plastics used, and is as a manufacturer of sorbents, particularly attentive to high reproducibility, freedom from blank values and ideal flow characteristics of the cartridges. The family business has branches in Switzerland, France and the USA, and supplies customers in over 150 countries worldwide.

The **LC Tech GmbH** has headquarters in the Bavarian Obertaufkirchen, where products and methods for the preparation and analysis of food, feed, forensic and environmental samples have been developed and distributed since 1998. The product range includes semi- and fully automated sample preparation systems and consumables for the analysis of contaminants and residues. The company, which has over 40 employees, is represented through an extensive distributor network worldwide and acts as exclusive distributor for PICKERING Laboratories in Europe, Africa and the Arab countries. Numerous public and private laboratories in food and feed analysis, pharmaceuticals and research are among the customers of LC Tech.

Author Contact Details

Sebastian Wierer, LC Tech GmbH • Daimlerstr. 4, D-84419 Obertaufkirchen • Tel: +49 8082 2717-0 • Email: info@LC Tech.de • Web: www.LC Tech.de

Dr. Hans Rainer Wollseifen, MACHERY-NAGEL GmbH • Neumann-Neander Str. 6-8, D-52355 Düren • Tel: +49 2421 969-0 • Email: sales@mn-net.com • Web: www.mn-net.com

Read, Print, Share or Comment on this Article at: Envirotech-Online.com/Articles

