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FH HES Universities of Applied Sciences

Fachhochschulen – Hautes Ecoles Spécialisées

Analytical Platforms at Swiss Universities of Applied Sciences

Christian Berchtold[a], Jean-Pascal Bourgeois[b], Verena Christen[a], Michal Dabros[b], Caspar Demuth[d], Anika Hoffmann[c], Franka Kalman[c], Susanne Kern[d], Nadia Marcon[c], Olivier Nicolet[b], Marc E. Pfeifer[c], Umberto Piantini[c], Denis Prim[c], Cyril Portmann[b], Samuel Roth[b], Jean-Manuel Segura[c], Olivier Vorlet[b], Chahan Yeretzian[d], Mathieu Zollinger[c], and Götz Schlotterbeck*[a]

*Correspondence: Prof. Dr. G. Schlotterbeck[a], E-mail: goetz.schlotterbeck@fhnw.ch
[a]FHNW Fachhochschule Nordwestschweiz, Hochschule für Life Sciences, Institut für Chemie und Bioanalytik, Hofackerstrasse 30, CH-4132 Muttenz;
[b]HES-SO Haute école spécialisée de Suisse occidentale, Haute école d'ingénierie et d'architecture Fribourg, Institute of Chemical Technologies, Boulevard de Pérolles 80, CH-1700 Fribourg;
[c]HES-SO Haute école spécialisée de Suisse occidentale, HES-SO Valais-Wallis, Institute of Life Technologies, Route du Rawyl 64, CH-1950 Sion 2;
[d]ZHAW Zürcher Hochschule für Angewandte Wissenschaften, Life Sciences and Facility Management, Institute of Chemistry and Biotechnology, CH-8820 Wädenswil

Abstract: Numerous projects and industrial and academic collaborations benefit from state-of-the-art facilities and expertise in analytical chemistry available at the Swiss Universities of Applied Sciences. This review summarizes areas of expertise in analytical sciences at the University of Applied Sciences and Arts Northwestern Switzerland (FHNW), the University of Applied Sciences and Arts Western Switzerland (HES-SO), and the Zurich University of Applied Sciences (ZHAW). We briefly discuss selected projects in different fields of analytical sciences

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Introduction

Analytical platforms are highly interdisciplinary and are of great importance to support all fields of research. The technology and instrumentation, based on sophisticated and expensive infrastructure, is as important as the experts who know how to use, improve and optimize the techniques as well as to support the interpretation of results. In the focus of the Universities of Applied Science for education and research, it is important to provide state-of-the-art technology to train students in applied research and to support their own research projects. The main difference to classical universities is the reduced need for cutting-edge instrumentation for fundamental research (like ultra-high field NMR spectrometers or ultra-high resolution mass spectrometers). Robust state-of-the-art technologies for business oriented and applied research are needed instead. However, the analytical platforms at the Universities of Applied Sciences in Switzerland are extremely diverse according to their focus, purpose and needs. The purely supporting platforms such as NMR- and mass spec. services, which are run by dedicated experts within the institutions as a routine service, is one common approach. Another setting is the distribution of instruments within each institute as dedicated support for experts in other fields. This means for example that researchers in organic chemistry run their own instruments (*e.g.* HPLC, MS, NMR) to follow reactions and perform quality control. Sometimes such platforms are partially organized as open-access facilities, where a dedicated team maintains instruments and each scientist uses these open-access systems on a need basis.

Finally, there are independent multidisciplinary analytical platforms that run their own instruments, perform their own projects for method and instrument development and support other teams inside and outside the institution with analytical expertise and instrumentation. The techniques used in all these fields where analytical platforms play a key role, as well as how they are organized within the different institutions, is as diverse as the different research and education models. This article provides an overview of analytical platforms, projects infrastructure and focus of the Universities of Applied Science across Switzerland.

FHNW Muttenz

The School of Life Sciences conducts research and education along the entire healthcare and value creation chain. The spectrum ranges from the development of medical products and drugs, technologies and production processes through to their production and market launch. An additional focus is the development of resource-saving technologies and environmental procedures. The research is organized within four institutes and each runs its own infrastructure for analytical support, development and training.

The institute for Medical Engineering and Medical Informatics (IM^2) needs mostly surface analytical tools (*e.g.* REM, ultrasound, X-ray diffraction) for the development of functional materials, implant design and Biofabrication (3D-Bioprinting).

The Institute of Pharma technology (IPT) conducts research into new technologies and methods for pharmaceutical products. The fields are bioprocess technology, drug delivery and PK/PD, formulation and devices for active chemical and biological substances, oral formulations of chemical drugs and production processes and techniques. Among other technologies, LC-MS and capillary electrophoresis are used for quality control processes and to investigate effects of drugs and formulations on organisms within cell models. Neither IM^2 nor IPT runs a dedicated analytical platform. Their instruments are specifically located among the teams who need them as tools and use the other platforms of the other institutes in collaboration.

The Institute for Ecopreneurship (IEC) for environment and resources performs research and education in the fields of environmental and water technologies, environmental biotechnology, ecotoxicology and sustainable resources management. This institute provide the main platform for inorganic, elemental analysis (*e.g.* LC-QQQ-ICP-MS, laser ablation, ICP-OES, µXRF, XRF, SEM, EVO SEM). For organic analysis an additional platform is present for direct support (*e.g.* LC, LC-OCD, LC-MSn - QqQ and Ion Trap, GC-MS, MALDI-TOF). The radioisotope analysis platform is also integrated within a specialized radiology laboratory (^{14}C and ^{3}H, liquid scintillation, autoradiography, HPLC with liquid scintillation detector, sample Oxidizer). Molecular biology (quantitative PCR, next generation sequencing platforms, electrophoresis), which also supports other institutes, is also located at the IEC.

The Institute for Chemistry and Bioanalytics (ICB) provides research in synthesis and medical chemistry, nanomaterials and surfaces molecular nanotechnology, process engineering and technology, *in vitro* diagnostics, DNA and RNA diagnostics, protein and tissue engineering, cell biology and toxicology as well as instrumental analytics.

A large analytical platform for nanomaterials and surfaces molecular nanotechnology such as a variety of microscopes (*e.g.* TEM, SEM, EDX, AFM, infrared- and Raman imaging, LEXT), spectroscopy and other technologies (*e.g.* µCT) is located in the group of Prof. Dr. Uwe Pieles and were described already last year in an article about material sciences.[1]

DNA and RNA diagnostics, cell biology and toxicology focus on molecular techniques such as qPCR or NGS. This platform is highly cooperative and overlaps with the platform at the Institute of Ecopreneurship (IEC).

For the development of point of care devices (for *in vitro* diagnostics), surface plasmon resonance and bio-layer interferometry play a key role as analytical tools. Therefore, a dedicated platform for the analysis of protein–ligand interactions exists as well.

The group of Prof. Dr. Götz Schlotterbeck represents the main analytical research and runs the instrumental analytical infrastructure of HPLC, GC, LC-MS, GC-MS and NMR (400 MHz NMR, LC/GC single quads, LC/GC triple quad, GC-TOF, LC-Q-TOF, LC - Ion Trap and OrbiTrap). These techniques are not only support for the other groups within the institute and FHNW (*e.g.* for medical chemistry or chemical engineering), also analytical research and training for diagnostics, food and environmental analytics, structure elucidation, metabolomics and proteomics is done there. The following exemplary projects show some typical collaborations between external partners and several institutes in analytical chemistry and are exemplary for the research performed at FHNW.

Targeted, Correlative Single-cell Proteomics and Metabolomics

The characterization of the metabolite and protein expression on a single-cell level are important to understand the underlying mechanisms of cellular processes. Picking, lyzing and spotting of the content of single cells on the surface of microscope slides was introduced by the team of Prof. Dr. Thomas Braun at Uni Basel (C-Cina, Biozentrum).[2,3] A self-made interface based on a TLC-MS interface (CAMAG, Muttenz, BL) was used to extract, transfer and detect a few metabolites (glutamic acid, glutamine and dopamine) by LC-MS down to single cell level. The proteins remaining on the glass slide were antibody stained (thanks for support of Dr. Gregor Dernick, F. Hoffmann-La Roche). The antibody staining is still possible after the metabolite extraction and analysis by LC-MS from the same cell thus enabling a correlative analysis.

The results of this fundamental proof of concept study are shown in Fig. 1. This project was supported by SNF project 200021_162521 (for the cell picker development) and nano argovia A.9.12 (for mass spectrometry interface and Roche collaboration on visual proteomics) as well as an internal FHNW collaboration of ICB and IPT.

Automated Dried Blood Spot Analysis for Newborn Screening

All approx. 80'000 newborns in Switzerland are screened for a list of serious genetic and metabolic disorders. Early diagnosis of those conditions can help prevent their further development, which untreated can result in brain damage, organ damage, and even death (*www.neoscreening.ch*). The dried blood spots (DBS) are sampled on-site by a nurse and analyzed in a centralized laboratory.[4] To optimize the process and the sample tractability, a fully automated DBS extraction system for online mass

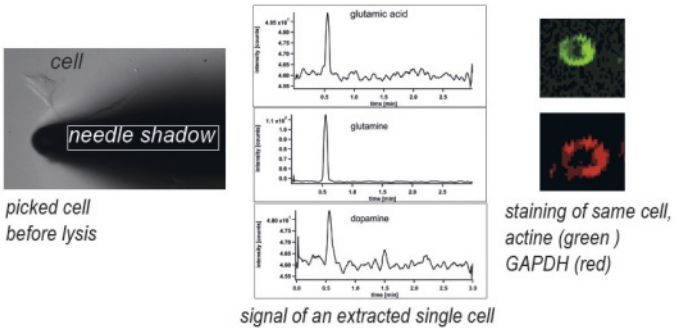

Fig. 1. Left: The visual appearance of the used SH-SY5Y (neuronal cells). In the middle, mass spectra of single cell metabolites by LC-MS (targeted triple quad). Right: The remaining proteins on the microscope slide detected by fluorescent antibodies in an IR-scanner.

spectrometry coupling was developed by CAMAG (*https://dbs.camag.com/dbs-ms-500*; Fig. 2). In a joint effort between the University of Applied Sciences FHNW, the newborn screening laboratory of the children's Hospital Zürich and CAMAG, a new mass spectrometry-based screening method for amino acids, acylcarnitins, and steroids was developed and implemented (CTI Project: 16898.1 PFLS-LS).[5–7]

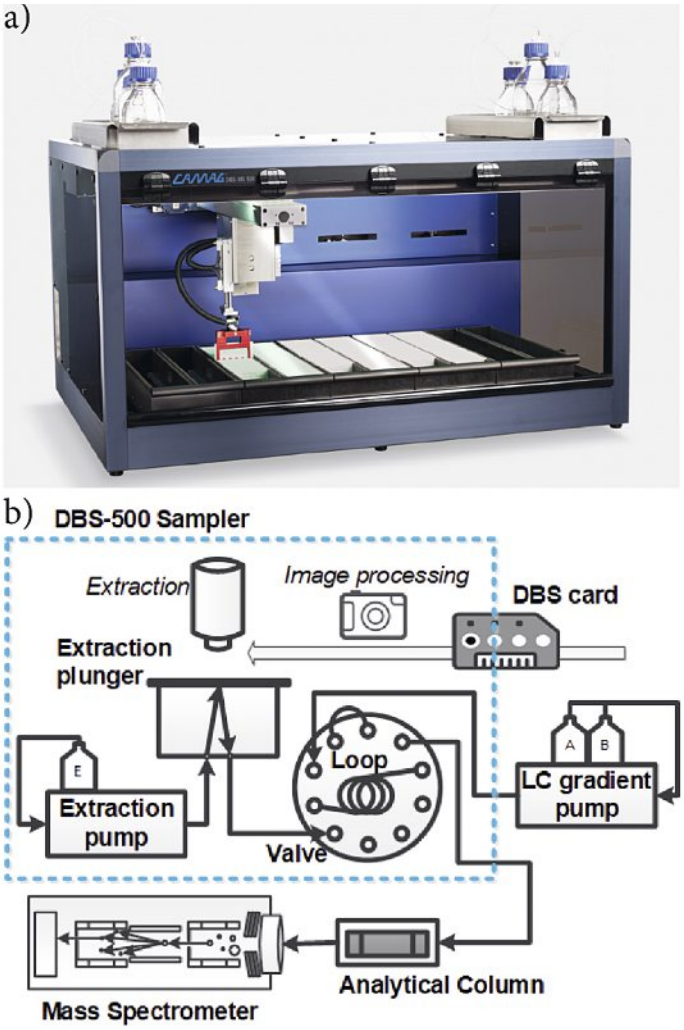

Fig. 2. a) The CAMAG DBS-500 auto-sampler for DBS cards; b) function scheme of the imaging, extraction process and online external analysis using chromatographic separation and mass spectrometric detection (chromatography and mass spectrometry are not part of the system shown in a)

Personalized Life Style Guiding by Minimum Volume Blood Analysis

This CTI project (18365.2 PFLS-LS) was a collaboration of the startup Sanalytica GmbH/Baze (*www.baze.com*), the proteomics laboratory Biognosy AG (*www.biognosys.com*) and the diagnostic laboratory SwissAnalysis AG (*www.swissanalysis.ch*). The main idea was to support a healthy lifestyle with frequent blood testing, using a minimum amount of blood, sampled at home. This frequent testing allows to monitor the effect of a healthy lifestyle and supplementation strategy beyond the classical blood testing at the doctor's surgery. Methods for fatty acids by GC-MS (FHNW ICB), for vitamins and proteins by LC-MS (FHNW ICB and Biognosy AG) as well as trace elements by ICP-MS (FHNW IEC) were developed. All specialized methods focused on small blood volumes and potential automation, which were finally implemented by SwissAnalysis AG.

The TAP device (*www.7sbio.com*) was implemented to collect approximately 100 microliter of blood at home. The samples were shipped (cooled) to the laboratory, prepared and analyzed by the parallel methods. The results are transferred and displayed in an app, including tips for nutrition and supplement (by Baze). The concept is shown in Fig. 3.

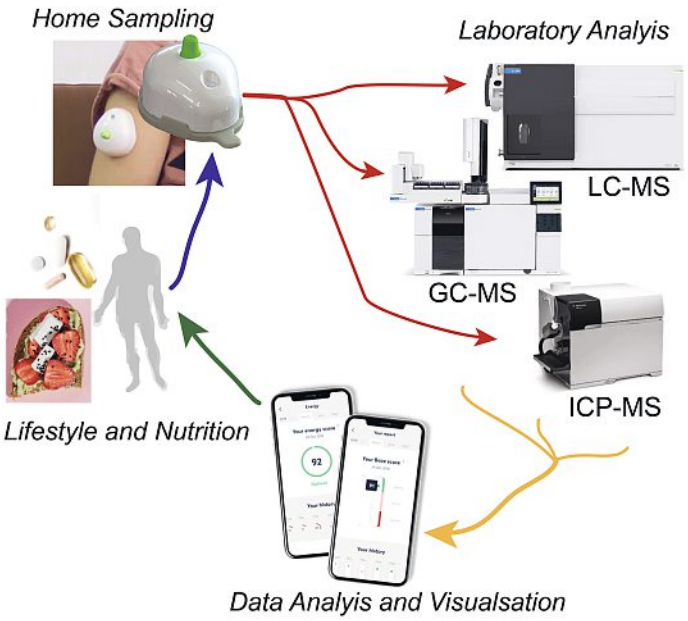

Fig. 3. Concept of home sampling and testing. The TAP device is send to the laboratory for analysis (amino acids, vitamin D/E, and proteins by LC-MS, fatty acids by GC-MS, and trace elements by ICP-MS). The results are displayed online or by app to adjust the diet and supplement strategy.

Gene Expression Analysis in Honey Bees after Exposure to Pesticides

Honey bees, wild bees, and other insects are important pollinators and thus contribute to securing our food supply and promote biodiversity. In recent years, there has been a sharp decline in the number of insects and an increased mortality rate among bee colonies.[8] Several factors are responsible for this, including exposure to pesticides.[9] It is known that exposure to pesticides causes sublethal, chronic effects in honey bees in addition to acute toxic effects. For example, memory formation, orientation and flight behavior are negatively affected.[10] To identify the underlying mechanism of action, honeybees are fed in the laboratory of FHNW with sugar syrup containing pesticides and gene expression is analyzed after 1, 2 and 3 days of exposure using quantitative PCR and next-generation sequencing (Fig. 4).[11] In joint projects between the FHNW and Agroscope, the molecular mechanism of action of behavioral changes in honey bees after exposure to pesticides is investigated. The exact exposure concentrations are determined in the sugar syrup by using HLPC coupled with MS.[12] Honey bee research at FHNW was initially a collaboration with the Federal Office for Agriculture and the Federal Office for the Environment. Current running projects are partially granted and in collaboration with Agroscope.

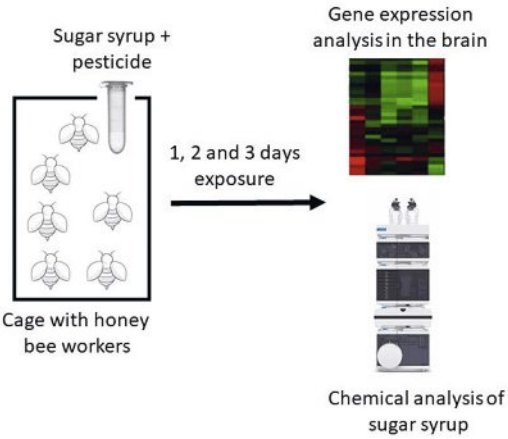

Fig. 4. Graphical overview of honey bee exposure. Honey bee workers are exposed with sugar syrup containing pesticides for 1, 2 and 3 days followed by gene expression analysis in the brain and chemical analysis of exposure solution.

ZHAW Wädenswil

The Institute of Chemistry and Biotechnology (ICBT) was founded in 2016 as a result of a merger of the former Institute of Chemistry and Biological Chemistry and the Institute of Biotechnology. About 180 lecturers, researchers and scientific staff work at the ICBT. Over 240 students are enrolled in the two Bachelor programs, and more than 70 students in the two Master programs. The ICBT is active in teaching and third-party funded applied research to about the same extent. Many of its research projects have a strong international orientation.

The convergence of biological and chemical sciences results in strong synergies between the institute's research groups and a very broad scope in applied research. Our understanding of chemistry and biotechnology is that it involves the linking of discoveries in the natural sciences with technological knowledge, with the aim of applying biological systems to the analysis and manufacture of products.

In any case, a very sound understanding of analytical methods and instrumentation is required in the different ICBT working groups, even if they do not solely focus on the application and optimization of analytical methods. As a brief and non-comprehensive overview, selected examples of research groups who apply advanced analytical tools to pursue their activities in applied research are given below. The Centre for Functional Materials and Nanotechnology employs analytical techniques such as scanning electron microscopy (SEM/EDX), atomic force microscopy and confocal Raman spectroscopy to characterize surfaces and interfaces (for a review, see ref. [1]). In the Centre of Molecular Biology and Microbiology, among other analytical and bioanalytical methods, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI TOF-MS) of the newest generation is used for the identification of microorganisms and the qualitative analysis of (bio)macromolecules. The Centre for Biochemistry and Bioanalytics applies high-resolution LC-MS/MS (ESI-Q-TOF) to generate information about sequences and post-translational modifications of proteins. Mass spectrometry,

capillary electrophoresis and ion chromatography are used to analyze complex glycosylation patterns of recombinant proteins. Ligand–analyte interactions are examined in label-free real-time measurements using surface plasmon resonance spectroscopy. Many of ICBT's research groups are dedicated to chemical and biochemical engineering and bioprocess technology. These groups make use of process analytical methods, such as inline spectroscopy (IR, NIR, Raman, UV/Vis, fluorescence, for details see ref. [13]), inline particle measurements (focused beam reflectance measurement, photon density wave spectroscopy), or analytical methods that are coupled online to bioprocesses, such as flow cytometry and HPLC.

In the following, a brief overview of the research activities and selected projects in the Centre for Analytical Chemistry is given. Here, four different research groups dedicate their work to the development and application of analytical methods. The Analytical Technologies group is engaged in developing and validating analytical methods with a focus on chromatography, mass spectrometry and time-resolved real-time technologies, with a particular emphasis on statistical data analysis and chemometrics. Attached to the Analytical Technologies group, the Coffee Excellence Center is a leading public research group in the field of coffee. Together with worldwide partners, it works on projects along the entire value chain of coffee. The Environmental Analysis group is concerned with identifying and quantifying organic substances and chemical elements in environmental samples and materials at trace levels. In the Measurement and Sensor Technology group, sensors and other analytical methods are developed and applied that are suitable for online monitoring and control of (bio)processes. The Physical Chemistry group focuses on molecular spectroscopy in the infrared (IR, NIR) and UV spectral range and electrochemical methods.

Analytical Technologies

The groups for Analytical Technologies and the Coffee Excellence Center are closely connected while having distinct and complementary orientation. The group for Analytical Technologies is dedicated to developing and refining state-of-the art analytical technologies with a focus on direct-injection real-time mass-spectrometry of mostly volatile organic compounds (VOC). Analytical technologies including couplings to processes and data analysis are at the center of the research effort. In this context, the two key technologies are proton-transfer-reaction time-of-flight mass spectrometry (PTR-ToF-MS),[14,15] and ion-mobility time-of-flight mass spectrometry (IM-ToF-MS).[16] In the following an example of a technological development in the fields of PTR-ToF-MS and IM-ToF-MS are outlined.

Development of Improved Nose-Air Sampling Technology by PTR-ToF-MS

Analyses of mouth and nose-spaces have been used abundantly in research over the past few decades for medical purposes as well as for sensory analysis.[17–19] All of these sampling methods have been using the analysis of the respiratory flow to determine the content of volatiles present in the lungs, the mouth or the nasal cavity. With regard to sensory analysis, this means that the compounds of interest that accumulate in the mouth and nasal cavity become diluted by the respiratory flow, leading to low signal intensities and consequently only a limited number of flavor active compounds can be monitored. Hence, one aspect of the research in our laboratory is to increase the signal intensity when sampling VOCs present in the mouth cavity.

To this end, we have tested, compared and optimized three different sampling methods: (i) Nose-space sampling of the exhalation stream (with nose-piece); (ii) mouth-space sampling of the exhalation stream, using a Buffered End-Tidal breath sampling inlet (BET) by Ionicon; (iii) direct mouth-space: drawing sample gas directly from the mouth cavity while breathing through the nose - mouthpiece from BET (non-re-breathing mouthpieces) coupled to a sampling and dilution lance.[20] A schematic of this setup is shown in Fig. 5.

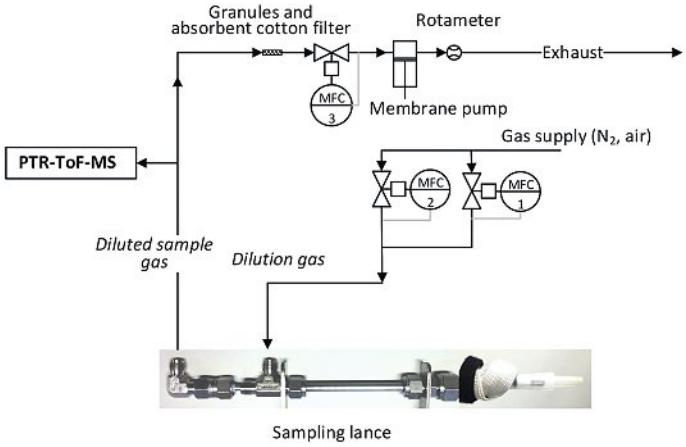

Fig. 5. Sampling setup with direct mouth-space sampling setup.

The measurements of seven different coffees have revealed the same order of magnitude in differences between the three sampling methods. Fig. 6 shows two series of graphs. The series to the left depicts the intensities for m/z 153.055 and m/z 153.091, tentatively identified as vanillin and ethylguaiacol respectively, which represent signals with the lowest intensity that were still distinguishable from the background. The series to the right shows the intensities of m/z 73.065, m/z 87.080 and m/z 101.0S60, tentatively identified as 2-methylpropanal, 2-/3-methylbutanal and 2,3-pentadione respectively, which represent signals with high intensities.

In summary, the signal area of our new direct mouth-space sampling method was at least a factor of 5 higher in intensity, compared to the indirect mouth-space sampling and a factor of at least 20 higher compared to nose-space sampling.

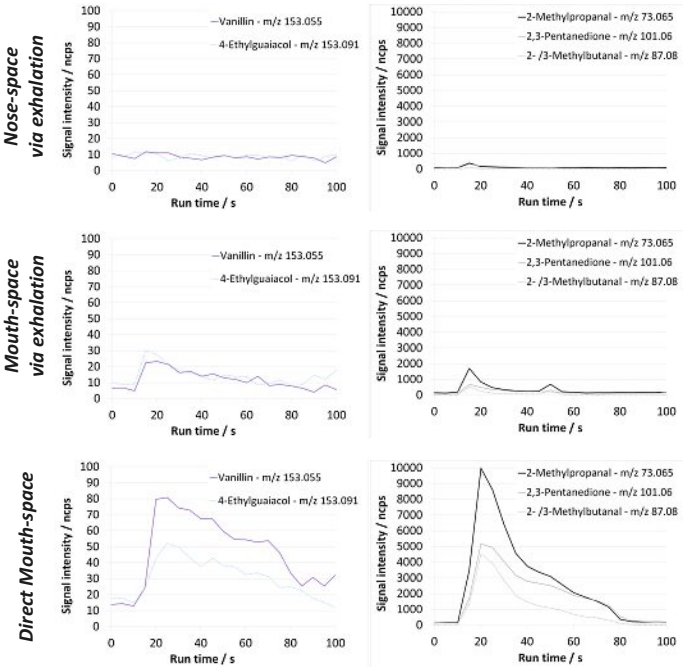

Fig. 6. Comparison of signal intensities of low intensity compounds (to the left) and high intensity compounds (to the right) for three different sampling methods: Upmost panels: nose-space; Middle panels: BET mouth-space; lowest panels: direct mouth-space.

The results from the comparison of the three different sampling methods have shown a massive improvement in signal intensity of the newly developed direct mouth-space sampling compared to the other two (conventional) sampling methods. The improvement in signal intensity can be directly linked to the fact that this approach uses a direct sampling of the volatiles present in the mouth-cavity instead of indirectly sampling these in the exhalation stream. It therefore enables the sensitivity of the measurement to be increased under otherwise equal conditions such as time resolution and a given measurement setup. Thereby, the direct mouth-space method enables a more sensitive dynamic measurement of volatiles during the aftertaste or lingering phase following the ingestion of food or drinks.

Online Analysis of Coffee Roasting with Ion Mobility Spectrometry–Mass Spectrometry (IMS–ToF-MS)

Online analysis of coffee roasting was performed using ion mobility spectrometry–mass spectrometry (IMS–MS) with corona discharge ionization. This is the first time that the formation of volatile organic compounds (VOCs) during coffee roasting was monitored using ion mobility spectrometry, in positive and in negative ion mode.[16] The temporal evolution of more than 150 VOCs was monitored during the roasting of Brazilian *Coffea arabica*. Mass-selective ion mobility spectrometry allowed a separation of isobaric and isomeric compounds. In positive ion mode, isomers of alkyl pyrazines were found to exhibit distinct time-intensity profiles during roasting, providing a unique insight into the complex chemistry of this important class of aroma active compounds. Negative ion mode gave access to species poorly detectable by other online methods, such as acids. In this study, the release of fatty acids during coffee roasting was investigated in detail. These increase in the early phase of the roasting process, followed by a decrease at the later phase, as other VOCs start to be formed.

Normalized ion mobility spectra of some fatty acids are shown in Fig. 7. For fatty acids with the same number of carbon atoms, the IMS peaks shift to shorter drift times with increasing number of double bonds, indicating a smaller collision cross section for the unsaturated fatty acids. In contrast, prolonging the chain length of the fatty acid increases the drift time and correspondingly the collision cross section.

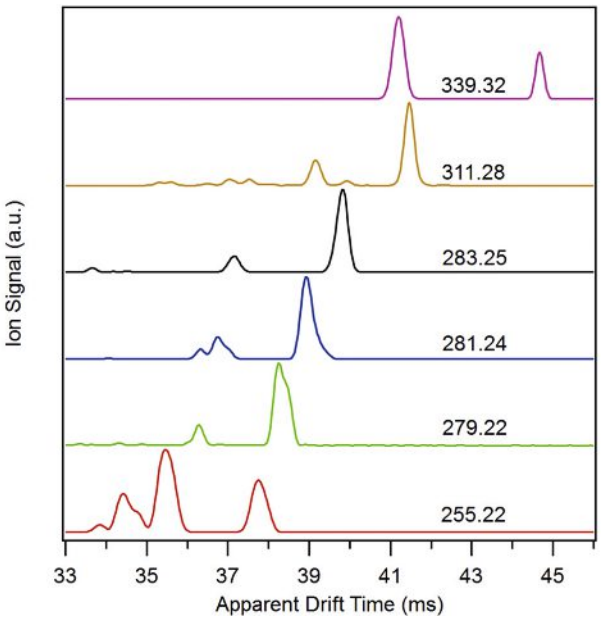

Fig. 7. IMS spectra of mass peaks corresponding to free fatty acids show multiple isomers/isobars. From bottom to top: c16:0 (m/z 255.22), c18:2 (m/z 279.22), c18:1 (m/z 281.24), c18:0 (m/z 283.25), c20:0 (m/z 311.28), c22:0 (m/z 339.32).

Within this study it was shown that corona discharge coupled to IMS–MS is a powerful tool for online analysis of the temporal evolution of volatile organic compounds, demonstrated here during coffee roasting. The method exhibits two main advantages: First, the ion mobility dimension separates based on collision cross section, which often resolves isomers and isobars that cannot be separated by MS alone. This was demonstrated for alkyl pyrazines, which make different coffee aroma contributions depending on their alkyl chain lengths and positions, as well as for free fatty acids as discussed here (Fig. 7). The ion mobility spectra showed that multiple isomers often contribute to single mass peaks. This is a huge step ahead in comparison to prior direct inlet online monitoring methods which are unable to separate isomers. Second, the corona ion source provides easy and straightforward access to both positive and negative ions, while prior online methods are restricted to positive ions. This allows the routine observation of a much larger range of chemical species.

Coffee Excellence Center

While the Coffee Excellence Center builds on the expertise and infrastructure on the analytical technology group, its mission is to advance our understanding of coffee, secure its future and support a sustainable grow of the coffee sector along the whole value chain through research, knowledge building and outreach. From an educational perspective, its goal is to demonstrate the application side of analytical technologies in one specific field of research.

Hence, our research includes investigation of coffee along the whole chain from the seed to the cup. In Fig. 8, PTR-ToF-MS profiles of green coffee, roasted whole bean coffee, roast and ground (R&G) coffee and finally of a coffee brew are shown, all plotted on an identical intensity scale. PTR-ToF-MS is an emerging analytical technique that has first been applied by Yeretzian and co-workers (since 1997) to coffee aroma analysis and is today an established technique in the field.[14] While green coffee shows a distinctively different profile of much lower intensities than roasted coffee, grinding the roasted beans leads to a strong increase of the volatile intensities.

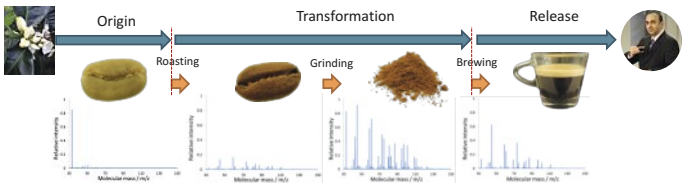

Fig. 8. PTR-ToF-MS mass spectra profiles of coffee as green whole beans, roasted whole beans, roast and ground beans and coffee brew. The volatiles of ground coffee clearly show the strongest overall intensities.

The past 10 years have proven that the Coffee Excellence Center is responding to a strong need of a growing and flourishing coffee industry. Today the Coffee Excellence Center is working in collaborative projects with most large and multinational companies. In this process, we also take the role of consultants and partners in helping formulate and crystalize their strategies and pipelines. Our involvement and deep knowledge of large number of key actors of the sectors puts us in a unique position, overseeing and understanding trends and opportunities.

Environmental

One of the main research activities of the Environmental group lies in the field of elemental analysis and speciation. Among other analytical methods, inductively coupled plasma-optical emission spectrometry (ICP-OES), atomic absorption

spectroscopy (AAS), and wavelength-dispersive X-ray fluorescence (WD-XRF) are used. A project to prevent fuel abuse and unauthorized burning of wood was conducted with the regional governments of central Switzerland and the two federal offices of Energy (BFE) and of the Environment (BAFU). Based on the project results, benchmarks to limit heavy metals in wood incineration ash were established. A long-term project with the «Spiez Laboratory» focuses on the monitoring of military legacies, *i.e.* sites contaminated with toxic heavy metals in Switzerland or conflict areas in other parts of the world.

In a collaboration with Empa, organic pollutants that are used as flame-retardants in polystyrene, were investigated. These include hexabromocyclodecanes (HBCDs) and chlorinated paraffins (CPs), as well as their transformation and degradation products. Due to their high bioaccumulation potential and suspected cancerogenic properties, these substances are regulated under the Stockholm Convention list of persistent organic pollutants. However, longer chain chlorinated paraffins are used as substitutes with yet unknown transformation products. Considering the various structural isomers of the chloroparaffins, the challenging quantitative determination of these compounds was successfully achieved using LC-MS/MS and LC-QTOF-MS.[21]

Measurement and Sensor Technology

In the Measurement and Sensor Technology group, sensors and related online measurement techniques are developed, optimized and applied to various processes, with a special focus on parameters relevant in bioprocess monitoring and control. In these processes, specific requirements have to be taken into account, such as long-term stability, robustness in cleaning and sterilizing procedures, or biocompatibility of materials used. For the biotechnological cultivation of cells and microorganisms, single-use systems have been increasingly used in recent years. As this offers new challenges and perspectives in sensor technology, single-use sensors are among the relevant research topics of the working group. In the following, selected projects are presented. For confidentiality reasons, no details can be given, since most of these projects were carried out in collaboration with industrial partners.

For parallel and rapid bioprocess development, small bioreactors are increasingly being used, but these offer only limited installation space for sensors. Therefore, robust sensors for critical process parameters (such as the pH value and the concentration of dissolved oxygen, dO) are required that meet the size constraints of these bioreactors, while still adhering to standardized dimensions of sensor ports. In a project in collaboration with the Bern University of Applied Sciences and an industrial partner, a sensor that combines pH and dO measurement in a slim design was developed and evaluated. Whereas the pH value was measured potentiometrically with a classic glass electrode, an optical measuring method was used to measure the concentration of dissolved oxygen. The optical sensor was based on an immobilized dye that changes its fluorescence properties due to selective quenching upon interaction with molecular oxygen. In the course of the project, new features of the sensor were realized, such as a special glass membrane, a light guide, an immobilization technique for the optical sensor material, and the measurement electronics. The built prototypes were evaluated in bioprocesses and compared with other sensor systems. The innovative pH/DO sensor proved to combine the advantages of the potentiometric pH electrodes (high measuring range and robustness) with the benefits of optical dO measurements (high stability and low maintenance requirements).

The concentration of viable biomass is one of the most relevant parameters in bioprocess control. For example, online measurement of biomass concentration or viable cell density allows to control the culture conditions or to determine the moment of induction of the production of a recombinant protein. Although offline methods to measure biomass concentration exist, they are time-consuming, may be inaccurate and bear the risk of contamination. As an alternative, dielectric spectroscopy has proved to be a valuable tool for real time and *in situ* measurements of biomass concentration. However, this measurement technique is still relatively new, and therefore, the prospects and limits of new applications in bioprocesses have yet to be investigated and understood. In a collaboration with an industrial partner, a sensor based on dielectric spectroscopy was applied to BHK cells grown on polymeric microcarriers. Cultivations on microcarriers are especially critical in this respect, as they do not allow the use of optical measurement techniques due to the light-scattering properties of the beads. The investigations showed a very high correlation between online and offline measurements, therefore proving that it is possible to analyze viable cell density of mammalian cells on microcarriers in real-time.[22]

In another project in collaboration with the Institute for Bioprocessing and Analytical Measurement Techniques (Heiligenstadt, Germany), dielectric spectroscopy was used to monitor the growth of hairy root cultures (Fig. 9). Up to now, the growth of these plant organ cultures can only be monitored by applying time-consuming and destructive methods, such as the determination of the dry cell weight. The measurements proved that dielectric spectroscopy can be successfully applied not only to cell suspensions, but also to organ cultures.[23]

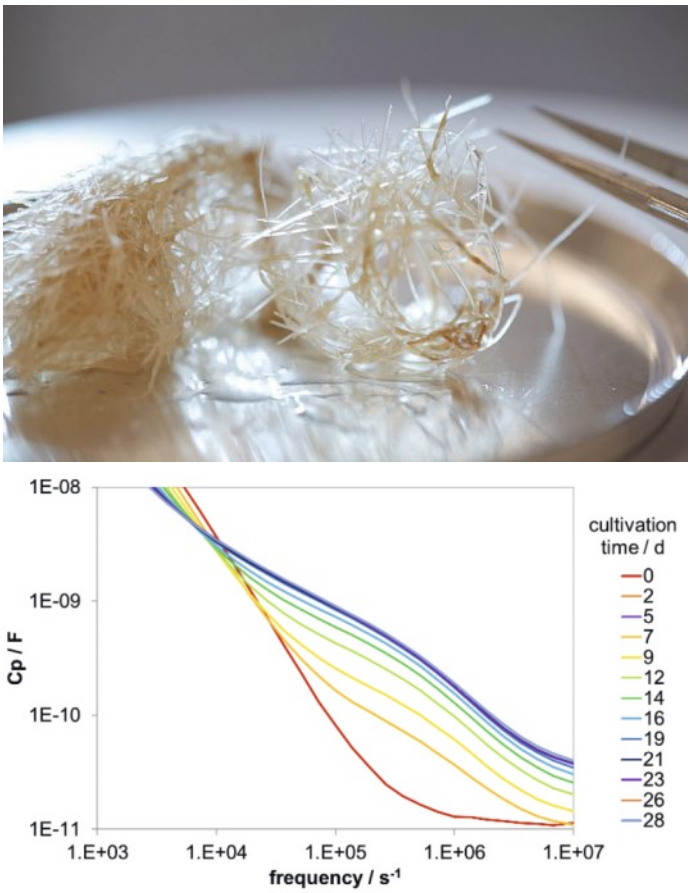

Fig. 9. As the spatial distribution of Hairy Root biomass is not homogeneous, online measurement of these cultures is challenging. Nevertheless, the sensor signal (a characteristic frequency dependent drop of capacitance C_p) can be correlated to the cultivation time.

In a project in collaboration with the Swiss Federal Institute of Metrology, a new activity scale for sodium, potassium, magnesium, calcium and chloride ions was proposed. This will allow ion activity measurements of these physiologically relevant ions with high

comparability and traceability, independent from the measurement systems utilized. This is one important prerequisite for safe and efficient measurements of these ions in clinical laboratories based on ion-selective sensors and other methods. To prove this concept, the Measurement and Sensor Technology group successfully participated in an interlaboratory comparison, in collaboration with several leading European metrology institutes (Fig. 10).[24]

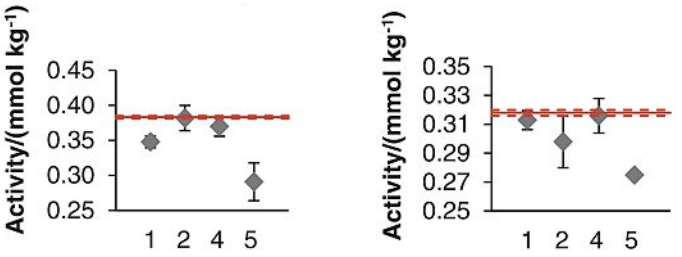

Fig. 10. Selected results of an interlaboratory comparison based on measurements of ion activities of Mg^{2+} and Ca^{2+} ions. Reference values are displayed in red color, activity values measured by ZHAW are depicted in column 4.

HEIA-Fribourg

The analytical platform of the Institute of Chemical Technology (ChemTech) at HEIA-Fribourg is formed by several professors working in different fields of chemical characterization, and includes an Analytical Laboratory Service. Its main purpose is to provide strong support to the ChemTech Institute and to the training of our students. The laboratories at HEIA-Fribourg are well equipped with all the essential instrumentation in analytical chemistry (LC, GC, MS, ICP-OES, NMR…), as well as physical chemistry (DSC, TGA, Raman spectroscopy, rheology, Particle Size Distribution...).

Analytical Laboratory Service

From the start of its activity, the main objective of the Analytical Laboratory Service at HEIA-Fribourg was to make the institute's analytical infrastructure available to the largest possible number of partners and to develop new collaborations with various local economic players.

With more than 15 years of experience, the Service offers its expertise and key skills to support the Institute's strategic axe of material characterization technology. In particular, it is active in the fields of NMR spectroscopy, gas and liquid chromatography coupled with mass spectrometry, dynamic scanning calorimetry, EDX analysis with a scanning electron microscope and Raman spectroscopy (Fig. 11)).

Fig. 11. The Analytical Laboratory Service.

The Analytical Laboratory Service is facing increasingly more diversified and sophisticated demands, coming primarily from internal R&D projects of the Swiss Universities of Applied Sciences, but also from external, national industry partners.

The Service has built and maintained long-term, strong relationships with its key clients. For example, the Laboratory is performing a part of the quality control process for a designer and manufacturer of hyaluronic acid dermal fillers and skincare products by measuring density and refractive index. Metalor Technologies SA – a leading participant in the field of precious metals and advanced materials (*www.metalor.com*) - is a partner with whom the Laboratory works together on HPLC reaction monitoring. As a last example, the Service collaborated with the R&D laboratory of a well-known watchmaker on molecular structure determination by NMR.

Analytical Chemistry R&D

The ChemTech Institute participates in several projects with other Universities of Applied Sciences, especially in the environmental chemistry field. A recent project, named 'Xyloclean' and carried out with Prof. R. Röthlisberger from HEIG-VD, aimed to minimize the polluting emissions from wood combustion. For this purpose, the residual HAP were quantified in the emitted particulate matter using GC-MS and a specific extraction technique.

The Institute is now involved in a new project called 'Conforto', in collaboration with HEPIA-GE and Prof. B. Oertli. This project deals with a multi-disciplinary approach to design a new concept of urban water basins with multiple ecosystem services (biodiversity protection, flood regulation, refreshing effect…). The role of the Institute's analytical platform will be to support the evaluation of the impact of specific urban pollutants and to study the potential use of these basins for water treatment. The carbon-trapping capacity of these ponds will also be assessed by looking analytically at the CO_2 exchanges.

In the field of instrumental chemistry, ChemTech is active in the conception, development and production of affordable instruments. This field of research started about ten years ago with the construction of a budget capillary electrophoresis (ECB) designed for the detection of counterfeit medicines.[25]

Building on this experience, the team is now developing a new generation of capillary electrophoresis instrument based on the open-source hardware principle, and looking for new applications for this technology.

Finally, a budget Raman instrument (RAB) was also developed with the idea to offer a portable screening capability to support the identification of counterfeit drugs directly in the field.[26]

Physical Chemistry R&D

Over the past 3 years, a special effort has been made at ChemTech to develop skills in the field of powder characterization. For this purpose, various student projects have been carried out in order to study the behavior of certain food powders. In particular, the water absorption process and its effect on the physical properties of the powder has been investigated. In addition, phenomena such as oxidation and degassing in roast & ground coffee have also been studied. The recent acquisition of an instrument to measure the kinetics of solid sample water intake should allow the Institute to increase our skills further in this area.

PAT & bio-PAT

The ChemTech Institute at HEIA-FR is active in the area of Process Analytical Technology (PAT), applied to both chemistry and bioprocessing.[13] PAT, an initiative proposed by the FDA in 2004, is a systematic analytical approach to designing, analyzing and controlling a process through timely measurements of critical quality and performance attributes, with the goal of increasing process understanding and ensuring final product quality.

The PAT strategy is very useful, for instance, in the field of continuous flow chemistry. Micro- and meso-reactor technology offers many advantages, such as rapid heat and mass transfer, and it has been studied actively at ChemTech over the past several years. In a recent Master project, carried out in collaboration with the fine chemicals industry, the integration of an online process

supervision platform in a mini-CSTR reaction screening system was investigated.[27] The system was designed such that different process monitoring probes (IR, Raman, *etc.*) could be installed interchangeably, depending on the need, providing a live analytical window into the process and enabling process development, control and optimization.

Several projects have also been conducted on the application of PAT in the field of bioprocess engineering. In one study, online biomass monitoring sensors were used to control the specific growth rate of microorganisms and to prevent the production of undesired overflow metabolites.[28] From the control point of view, this task is difficult because of the strong noise present in the online biomass concentration signal and due to the non-linear dynamics of the process. In the latest study, a new controller logic was proposed to address these problems.[29] The application of calorimetry as a PAT tool in bioprocessing is currently being investigated.

HES-SO Sion

The Institute of Life Technologies (ITV) is part of the School of Engineering of the HES-SO Valais-Wallis. Analytical Chemistry, Biotechnology and Food Technology are the three educational pillars of the Bachelor of Science degree program while Biotechnology & Sustainable Chemistry, Diagnostic Systems, Food & Natural Products as well as Peptide & Protein Technologies constitute the four research groups of the Institute. Various platforms, such as Analytical Chemistry & Biochemistry, strategically support the research groups with a portfolio of specialized high-end equipment, analytical methods, and expertise. A strong experience in analytical chemistry, cell cultures and purification methods, especially in the area of downstream processing (DSP), is at hand. In addition, one of the internationally leading laboratories in modern separation sciences and its applications is present at ITV. The highly qualified staff of ITV is organized in interdisciplinary, matrix-like research teams and works with high confidentiality, if requested. ITV has 2,200 m^2 of modern laboratories and pilot plants including a large set of wave bags and bioreactors up to 300 L. The platforms offer their services and know-how to industry partners such as companies from the biopharmaceutical sector. All platforms have a long and successful record of accomplishment in commissioned work for companies and scientific/applied science projects.

The comprehensive and modern analytical instrument park at ITV is organized as an analytical platform. It provides efficient support in scientific but also industrial projects and is accessible for contract work of industrial partners of ITV. The Analytical Platform works – if needed – under the quality management system ISO 17025, the microbiology section under GMP. Based on a long-term convention between the Federal Institute of Technology in Lausanne (EPFL), their pole 'EPFL Valais-Wallis' in Sion and the HES-SO Valais-Wallis, Sion, ITV has internal access to the high-end analytical instruments of the EPFL, which also leads to close scientific cooperation. Furthermore, the analytical laboratory of ITV functions as Agilent Technologies CH demonstration laboratory for analytical equipment and applications.

At the beginning of 2021, ITV including the Analytical Platform is moving to its new building at the new and modern HES-SO Valais-Wallis campus next to the EPFL common pole 'Energypolis', directly located at the train station of Sion. Hereinafter we present R&D project examples where the analytical chemistry & biochemistry platform was crucial in attaining project objectives.

The Analytical Platform at the Institute of Life Technologies: Examples of Established State-of-the-art Bioanalytical Applications

Proteins, particularly monoclonal antibodies (mAbs) are an important class of biotherapeutics. They have a strong presence and sustained growth in the pharmaceutical industry nowadays. About 70 mAbs have received first approval for human treatment in Europe, two in 2020.[30] They are used for a variety of indications including several forms of cancer, autoimmune and infectious diseases. Research on mAbs, but also on antibody-like compounds as for example, bispecific antibodies (bsAbs) or single chain variable fragments (scFv) and particularly antibody drug conjugates (ADC) is a growing field. Due to the upcoming patent expiry of top-selling originator products, more and more biosimilars are under development. Especially for the biosimilars comprehensive analytical characterization is needed in order to detect modifications in comparison to the originator, *e.g.* in the amino acid sequence or post translational modifications (PTM) as the glycosylation pattern, which may impact therapeutic efficacy, bioavailability and biosafety.[31]

To be able to characterize complex biomolecules like mABs the Institute of Life Technologies has established many state-of-the-art bioanalytical tools and techniques in the last years. They allow the development as well as the quality control of *e.g.* complex proteins to be monitored, including posttranslational modifications. Among many others, capillary gel electrophoresis, enzymatic sample preparation techniques, conventional as well as special LC techniques and mass spectrometry are present.

Capillary Gel Electrophoresis (CGE-SDS)

For many years, conventional sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) has been widely used to monitor identity and purity of therapeutic proteins.[32,33] At the Analytical Platform at the Institute of Life Technologies in addition to the traditional SDS-PAGE, also CGE-SDS was implemented for the analysis of proteins, especially mAbs. Advantages of CGE-SDS in comparison to SDS-PAGE include short analysis time, high reproducibility, and much higher resolution. It is less laborious, does not use toxic chemicals and has the possibility of full automation using an autosampler. Proteins are detected on-capillary, usually with UV light at 220 nm. The sensitivity is comparable to the Coomassie blue staining method. In high sensitivity impurity assays, proteins can be detected down to about 10 ng/mL after labelling with a fluorescent dye and using a laser-induced fluorescence detector (LIF). This sensitivity is similar to that achieved by using silver staining in SDS-PAGE. In Fig. 12 the analysis of an intact, non-reduced immunoglobulin (IgG) sample by CGE-SDS-UV is shown. 100 µg of protein were buffer exchanged to SDS-MW Sample Buffer (Sciex, SDS-MW Analysis Kit) and alkylated with iodoacetamide at 70 °C for 5 minutes. Separation was performed with SDS-MW Gel Buffer in a 30 cm bare fused capillary at 15 kV on a ProteomeLab PA800 capillary electrophoresis system from Sciex.

This analysis is used to determine, for example, the mAb content (titer) as well as its impurities such as lower molecular

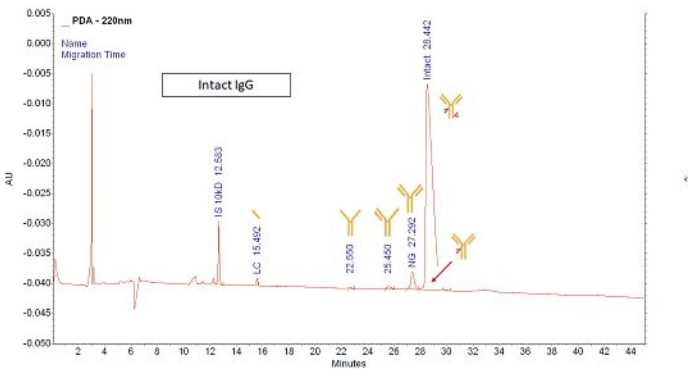

Fig. 12. Electropherogram of an IgG sample produced at Institute of Life Technologies and analyzed in the Analytical Platform laboratory; tentative peak assignment.

weight fragments in a formulation. In addition, the percentage of high molecular weight species, *e.g.* stable aggregates and the percentage loss of one N-glycan chain or two N-glycan chains from an intact humanized mAb, are assessed and monitored during stability studies.

Mass Spectrometry (MS) Peptide Mapping

Bottom-up proteomics by proteolytic digestion of proteins prior to MS analysis is a common approach to identify proteins based on their amino acid sequence and to detect post-translational modifications. The general procedure of protein identification involves digestion of the intact protein using trypsin or other proteolytic enzymes followed by mass analysis of the resulting peptides. In general, tryptic digestion in solution is a time-consuming process, which is easily tainted with operator's mistakes resulting in poor repeatability. In order to overcome those drawbacks, a workflow including an easy-to-use and quick digestion kit (Smart Digest™, Thermo Fisher Scientific) without tedious sample preparation prior to accurate MS analysis (HPLC-qTOF 6530, Agilent) was established at the Institute of Life Technologies. As a result, highly reproducible digestions (RSD < 5 %, n = 3, 5 target peptides) were obtained with a sequence coverage ranging from 71 to 99% for five model proteins, including two different mAbs. Sample preparation time was decreased substantially. Conventional in-solution tryptic digestion takes about 16 h. Using the smart Digest™ approach, digestion time takes 20 to 60 min, depending on the protein complexity. For example, in Fig. 13 bovine serum albumin (BSA) was digested during 20 min leading to 96% of sequence coverage. The protein was separated on a HPLC 1260 from Agilent equipped with a BioZen 2.6 µm Peptide XB-C18 column from Phenomenex.

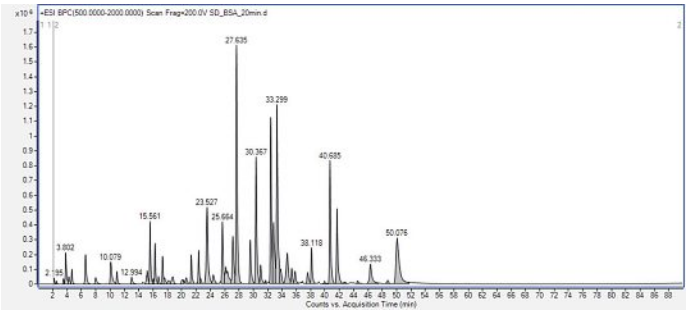

Fig. 13. Base peak chromatogram (BPC) of a tryptic digest of BSA analyzed in the laboratory of the Analytical Platform 'HES-SO Sion' with a qTOF 6530 MS from Agilent Technologies. Elution was achieved with a linear gradient of 5–70% solvent B in 90 min with a flowrate of 0.2 mL/min, where solvent A was MS grade water with 0.1% formic acid and solvent B was MS grade acetonitrile with 0.1% formic acid. An AJS ionization source was used and MS data were acquired in the positive ion mode in the range of 100–3200 m/z at a rate of 1 Hz in extended dynamic range (2 GHz) mode with a qTOF 6530 from Agilent Technologies. Data were analyzed with BioConfirm software.

Intact Mass and Drug to Antibody Ratio (DAR)

Antibody drug conjugates (ADCs) are designed for targeted treatment of a wide variety of cancers. These complex molecules, composed of a monoclonal antibody linked to a small organic biologically active cytotoxic (anticancer) payload or drug, bind to a surface antigen of the cancer cell. After phagocytosis by the cell the highly cytotoxic payload is release by *e.g.* lysosomal cleavage.[15] The number of payloads per antibody is one of many key parameters that requires determination to ensure clinical efficiency and safety for patients. To support industrial research projects on bio-conjugation development and optimization at the Institute of Life Technologies, a general workflow was established using mass spectrometric techniques. It comprises the determination of the intact mass of the native protein and the labelled one, the comparison of both and finally the assessment of the number of labels as well as the determination of the mean drug to antibody ratio (DAR). An example is shown in Fig. 14. Based on the masses detected from the unmodified and modified mAb, it was found that in total five payloads were attached to the mAb, three on the heavy chain and two to the light chain, leading to a mean DAR of 3.3. Results could be confirmed by comparing them to capillary isoelectric focusing (cIEF-UV) analysis of the same samples (data not shown). The same pattern was obtained, since cIEF separation is based on charge differences and with each payload the mAb loses one positive charge.

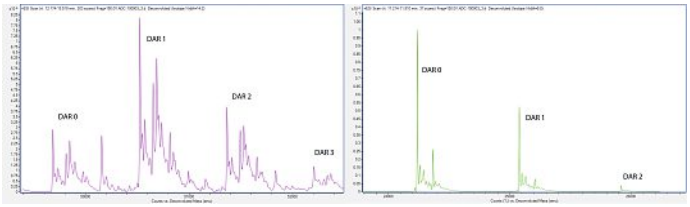

Fig. 14. Deconvoluted mass spectra of a mAbs heavy chain with different numbers of the small payload (left) and light chains with different numbers of the small payload (right), analyzed with a qTOF 6530 from Agilent Technologies. The ADC was reduced with 5 mM DTT at 56 °C for 20 min to obtain the light and heavy chains. After dilution, the sample was separated on a HPLC 1260 from Agilent equipped with a BioZen 3.6 µm Intact XB-C8 column from Phenomenex. Elution was achieved with a gradient of 5–95 % solvent B in 20 min and a flowrate of 0.3 mL/min, where solvent A was MS grade water with 0.1% formic acid and solvent B was MS grade acetonitrile with 0.1% formic acid. An AJS source was used and MS data were acquired in the positive ion mode in the range of 100–3200 m/z at a rate of 1 Hz in extended dynamic range (2 GHz) mode with a qTOF 6530 from Agilent Technologies. Data were analyzed with BioConfirm software.

To conclude, the Analytical Platform of the Institute of Life Technologies possesses state-of-the-art and well-established generic workflows and a wide catalogue of methods and techniques that enable the support of research projects in the area of characterization and quality control of complex biomolecules.

The Analytical Platform Supports the Development and Performance Evaluation of Next-generation Point-of-care Diagnostic Systems

The Diagnostic Systems Research Group, for more than a decade now, has worked on innovative solutions to address unmet needs in the field of *in vitro* diagnostics (IVD) and more specifically point-of-care (POC) diagnostics.

The IVD domain is required to meet stringent quality standards, therefore, a specialized and powerful analytical platform is necessary to ensure a detailed characterization of developed assay reagents such as derivatized proteins, labeled antibodies and modified nucleic acids (*cf.* Table 1) and to support validation of diagnostic test performance characteristics. One of the gold standard reference methods in clinical laboratories is mass spectrometry (MS).[34,35] Hence, we have established several liquid chromatography (LC) coupled to MS methods ranging from quantitative therapeutic drug monitoring (TDM) to qualitative protein sequencing approaches for benchmarking purposes during the development of POC diagnostic tests.

As an example in the context of the Nano-Tera project ISYPEM II, a demonstrator was designed and developed for TDM of the drugs Tacrolimus, Everolimus and Tobramycin by the Diagnostic Systems research group in collaboration with the Systems Engineering Institute in order to enable personalized, continuous

Table 1. Project examples requiring a reference analytical method for head-to-head comparison and performance verification.

Biomarkers	Envisioned POC diagnostic test	Reference method
Immunosuppressive drugs *e.g. Tacrolimus*	Development of a compact POC diagnostic demonstrator to perform reliable therapeutic drug monitoring (TDM) based on FPIA.	LC-MS^2 quantitative assay in whole blood as reference method. LC-qTOF for characterization of synthesized ligands.
Antibiotics *e.g. Tobramycin*	Development of a compact POC diagnostic demonstrator based on FPIA to conduct reliable TDM for newborns.	LC-MS^2 quantitative assay in whole blood as reference method.
Cholesterol	Development of a one-step colorimetric and electrochemical IVD quantitative assay at the POC.	LC-MS^2 quantitative assay in whole blood as benchmark method.
Cortisol	Development of a quantitative lateral flow assay.	LC-MS^2 quantitative assay in whole blood or saliva as benchmark method.
Thyroxine (T4) (endocrine analytics)	Proof of concept study of a POC device for the diagnostic testing of hypothyroidism in newborns.	LC-MS^2 quantitative assay in whole blood as reference method.
***Chlamydia Trachomatis* (Infectious disease)**	Feasibility of a POC diagnostic device for detection of *Chlamydia* infections based on isothermal amplification and an LFA readout.	Quantitative Realtime PCR as reference method.
Mild Traumatic Brain Injury (mTBI)	Development of an electrochemical POC diagnostic device to diagnose mTBI on-site	ELISA IVD validated method
IgG/IgM **COVID-19**	Evaluation of the requirements to use POC diagnostic tests to support public health actions during the deconfinement phase of COVID-19 outbreak.	IVD laboratory-based validated methods and collaboration with clinical central laboratories.

and accurate monitoring of treatments. Measurements using the POC diagnostic demonstrator device were performed within a low-volume chamber equipped with a compact optical reader, enabling drug quantification using a fluorescence polarization immunoassay (FPIA). Processing of blood samples obtained from a finger prick were performed using a microfluidic cartridge. To verify that the developed demonstrator met the challenging analytical and clinical specifications,[33] results of tested samples were compared to data obtained with LC-MS methods.[36,37] Fig. 15 depicts the current stage of integration of the POC diagnostic device for TDM at the HES-SO Valais-Wallis.

Another ongoing project is dedicated to the development of an innovative POC diagnostic system in order to deploy in the future a cost-effective, yet quantitative and accurate procedure

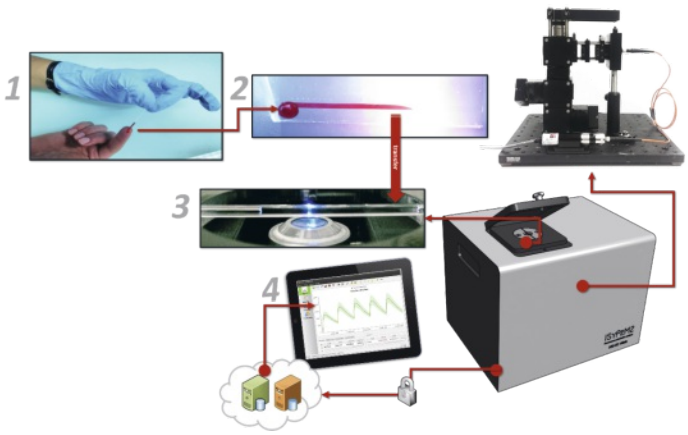

Fig. 15. Sample-to-result workflow of the POC diagnostic system that includes capillary blood collection (1), therapeutic drug extraction (2), quantification of the drug content in a microchamber with a compact fluorescence-polarization (FP) reader (3) and data processing on a secured dedicated platform (4).

to support diagnosis of hypothyroidism with a quick TAT. The time factor is critical in the context of neurocognitive and physical development disorder[38] that can occur when the disease is not treated in the first hours of human life.[39,40] In developed countries, it is usually possible to provide such diagnostic testing within a well establish laboratory network. However, in remote locations of large countries or in resource limited areas of the world, the time between collection, transport, measurement and finally diagnosis prior treatment can take at least three days to weeks, which is too long to prevent irreversible brain damage and growth deceleration to infants.[41] The HES-SO Valais-Wallis activities have focused on providing the bioassay based on a low-cost system and, at the same time, evaluating the performance of existing solutions by direct comparison with a quantitative LC-MS^2 analytical method. Based on the support given by the Analytical Chemistry & Biochemistry Platform it was possible to determine the limited quantitative performance of commercially available rapid tests and pinpoint challenges towards a successful implementation of a next-generation POC diagnostic system.

To promote the development of new and innovative POC diagnostic solutions, the Diagnostic Systems Research Group is organizing jointly with the Swiss Center for Electronics and Microtechnology (CSEM) the 3rd Swiss Symposium in Point-of-Care Diagnostic to take place in Visp, Valais on 29 October, 2020. This event brings together stakeholders from academia, research, medicine and industry to foster innovation in diagnostics (more information on our website: *www.pocdx.ch*).

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[1] P. Brodard, M. Dabros, R. Marti, E. Vanoli, M. Zinn, U. Frey, C. Adlhart, L. Kind, F. Koch, F. Burgio, J. Stenqvist, S. Saxer, U. Pieles, P. Shahgaldian, S. Wendeborn, *Chimia* **2019**, *73*, 645; doi: 10.2533/chimia.2019.645.

[2] S. Kemmerling, S. A. Arnold, B. A. Bircher, N. Sauter, C. Escobedo, G. Dernick, A. Hierlemann, H. Stahlberg, T. Braun, *J. Struct. Biol.* **2013**, *183*, 467; doi: 10.1016/j.jsb.2013.06.012.

[3] C. Schmidli, L. Rima, S. A. Arnold, T. Stohler, A. Syntychaki, A. Bieri, S. Albiez, K. N. Goldie, M. Chami, H. Stahlberg, T. Braun, *J. Visual. Exp.: JoVE* **2018**; doi: 10.3791/57310.
[4] a) J. V. Leonard, C. Dezateux, *Paediat. Child Health* **2011**, *21*, 56; doi: 10.1016/j.paed.2010.10.011; b) S. Sahoo, L. Franzson, J. J. Jonsson, I. Thiele, *Mol. bioSyst.* **2012**, *8*, 2545; doi: 10.1039/C2MB25075F.
[5] R. Fingerhut, M. L. Silva Polanco, G. D. J. Silva Arevalo, M. A. Swiderska, *Rap. Commun. Mass Spectrom. RCM* **2014**, *28*, 965; doi: 10.1002/rcm.6856.
[6] S. Gaugler, J. Rykl, I. Wegner, T. von Däniken, R. Fingerhut, G. Schlotterbeck, *Int. J. Neonatal Screen.* **2018**, *4*, 2; doi: 10.3390/ijns4010002.
[7] M. Luginbühl, S. Gaugler, *Clin. Biochem.* **2020**; doi: 10.1016/j.clinbiochem.2020.02.007.
[8] D. Goulson, E. Nicholls, C. Botías, E. L. Rotheray, *Science* **2015**, *347*, 1255957; doi: 10.1126/science.1255957.
[9] J.-P. Faucon, L. Mathieu, M. Ribiere, A.-C. Martel, P. Drajnudel, S. Zeggane, C. Aurieres, M. F. A. Aubert, *Bee World* **2002**, *83*, 14; doi: 10.1080/0005772X.2002.11099532.
[10] N. Desneux, A. Decourtye, J.-M. Delpuech, *Ann. Rev. Entomol.* **2007**, *52*, 81; doi: 10.1146/annurev.ento.52.110405.091440.
[11] V. Christen, M. Schirrmann, J. E. Frey, K. Fent, *Environ. Science Technol.* **2018**, *52*, 7534; doi: 10.1021/acs.est.8b01801.
[12] K. Fent, T. Haltiner, P. Kunz, V. Christen, *Journal?* **2020**, submitted.
[13] M. Dabros, O. Vorlet, R. Marti, W. Riedl, G. Grundler, A. Vaccari, M. Zinn, A. Ecker, C. Hinderling, *Chimia* **2015**, *69*, 482; doi: 10.2533/chimia.2015.482.
[14] F. Biasioli, F. Gasperi, C. Yeretzian, T. D. Märk, *TrAC Trends Anal. Chem*. **2011**, *30*, 968; doi: 10.1016/j.trac.2011.03.009.
[15] F. Biasioli, C. Yeretzian, T. D. Märk, J. Dewulf, H. van Langenhove, *TrAC Trends Anal. Chem.* **2011**, *30*, 1003; doi: 10.1016/j.trac.2011.04.005.
[16] A. N. Gloess, C. Yeretzian, R. Knochenmuss, M. Groessl, *Int. J. Mass Spectrom.* **2018**, *424*, 49; doi: 10.1016/j.ijms.2017.11.017.
[17] J. A. Sánchez-López, A. Ziere, S. I. F. S. Martins, R. Zimmermann, C. Yeretzian, *J. Breath Res.* **2016**, *10*, 36005; doi: 10.1088/1752-7155/10/3/036005.
[18] D. Mayr, T. Märk, W. Lindinger, H. Brevard, C. Yeretzian, *Int. J. Mass Spectrom*. 2003, *223-224*, 743; doi: 10.1016/S1387-3806(02)00967-3.
[19] D. D. Roberts, P. Pollien, N. Antille, C. Lindinger, C. Yeretzian, *J. Agricult. Food Chem*. **2003**, *51*, 3636; doi: 10.1021/jf026230+.
[20] M. Wellinger, S. Biollaz, J. Wochele, C. Ludwig, *Energy & Fuels* **2011**, *25*, 4163; doi: 10.1021/ef200811q.
[21] S. Crelier, O. Vorlet, P. Corvini, P. Lienemann, *Chimia* **2018**, *72*, 652; doi: 10.2533/chimia.2018.652.
[22] C. Demuth, I. Poggendorf, R. Lüthi, M. Frank, K. McNeel, *Gen. Engin. Biotechnol. News* **2016**, 36, 22; doi: 10.1089/gen.36.12.13.
[23] C. Demuth, T. Nacke, A. Barthel, I. Poggendorf, J. Varonier, M. Eggli, 18. Heiligenstädter Kolloquium 2016, **2016**.
[24] F. Bastkowski, P. Spitzer, R. Eberhardt, B. Adel, S. Wunderli, D. Berdat, H. Andres, O. Brunschwig, M. Máriássy, R. Fehér, C. Demuth, F. B. Gonzaga, P. P. Borges, W. B. da Silva Junior, A. Vospělová, M. Vičarová, S. Srithongtim, *Accred. Qual. Ass*. **2013**, *18*, 469; doi: 10.1007/s00769-013-1016-5.
[25] C. Rohrbasser, D. Rhême, S. Décastel, S. Roth, M. d. L. A. Montes, J.-L. Veuthey, S. Rudaz, *Chimia* **2009**, *63*, 890; doi: 10.2533/chimia.2009.890.
[26] J.-P. Bourgeois, O. Vorlet, *Chimia* **2018**, *72*, 905; doi: 10.2533/chimia.2018.905.
[27] L. Albergati, Master thesis HES-SO, **2020**.
[28] L. Habegger, K. Rodrigues Crespo, M. Dabros, *Fermentation* **2018**, *4*, 79; doi: 10.3390/fermentation4030079.
[29] Y. Brignoli, B. Freeland, D. Cunningham, M. Dabros, *Processes* **2020**, *8*, 679, doi: 10.3390/pr8060679.
[30] H. Kaplon, M. Muralidharan, Z. Schneider, J. M. Reichert, *mAbs* **2020**, 12; doi: 10.1080/19420862.2019.1703531.
[31] M. Lies, C. Lew, R. Lakshmanan, A. Guttman, *Drug Discov. Devel. ABSciex* **2018**, 1.
[32] L. Zhang, B. Yeung, J. Liu, *Am. Lab.* **2006**, *38*, 34.
[33] A. Taddeo, D. Prim, J.-M. Segura, M. E. Pfeifer, *J. Appl. Lab. Med.* **2020**, *5*, 738, doi: 10.1093/jalm/jfaa067.
[34] S. E. Conklin, C. E. Knezevic, *Clin. Biochem.* **2020**; doi: 10.1016/j.clinbiochem.2020.03.008.
[35] A. W. S. Fung, V. Sugumar, A. H. Ren, V. Kulasingam, *J. Clin. Pathol.* **2020**, *73*, 61; doi: 10.1136/jclinpath-2019-206269.
[36] F. Aucella, V. Lauriola, G. Vecchione, G. L. Tiscia, E. Grandone, *J. Pharma. Biomed. Anal.* **2013**, *86*, 123; doi: 10.1016/j.jpba.2013.08.001.
[37] J. Dinéia Perez, D. Sanches Aragão, F. Aparecida Ronchi, A. C. Febba, C. F. Rosso, H. Tedesco-Silva Junior, J. O. Medina de Abreu Pestana, D. E. Casarini, *Transplantation Proc.* **2020**; doi: 10.1016/j.transproceed.2020.01.077.
[38] M. D. Rappley, *Med. Update for Psychiatrists* **1996**, *1*, 64; doi: 10.1016/S1082-7579(96)80028-8.
[39] A. J. Bauer, A. J. Wassner, *Endocrine* **2019**, *66*, 51; doi: 10.1007/s12020-019-02024-6.
[40] F. Delange, Baillière's *Clin. Endocrinol. Metabol.* **1988**, *2*, 637; doi: 10.1016/S0950-351X(88)80057-0.
[41] S. R. Rose, R. S. Brown, T. Foley, P. B. Kaplowitz, C. I. Kaye, S. Sundararajan, S. K. Varma, *Pediatrics* **2006**, *117*, 2290; doi: 10.1542/peds.2006-0915.