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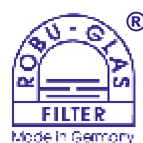

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SINTERED FILTERS

On the search

One of the most important newspaper of Germany recently published an article by Miriam Meckel, a scientist at the University of St. Gallen, Switzerland, about how the joy in searching and the joy in finding in journalism is always cause for surprises. You can also read this as – not only does it take some time to deal with a magazine - browsing and reading at least require some partial reward.

Therefore, to mediate knowledge, the factor of motivation is an absolute prerequisite – particularly since image-driven journalism in the online media is posing increasing new demands as well as expectations from the readers. The magazine we provide you with today therefore is a product clearly developed beyond what used to be called a specialist magazine. Text and image are inseparable. Therefore, we put great value on presentation of the message in the interest of our partners from industry as well as our authors.

This magazine lab&more, published in Germany, is produced and sold for the international markets around the world. You can find it at many congresses and trade fair events on all five continents. We are already looking forward to the interesting feedback that we always receive from the industry after such campaigns.

Presenting research and research results in an interesting and attractive manner is our strategy. Therefore, it was very interesting to find the following new quality approach to journalism in the article quoted above: "Man instead of machine". Colleague Meckel has also truly understood that people are first and foremost interested in people, and only then in what we in Germany sometimes refer to as "dry technology". As you can see and read, technologies really aren't all that dry.

Have fun reading and experiencing this issue.

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Price

Single issue 18 €
Annual subscription
Germany: 69 € zzgl. 7% MwSt.

Abroad: 81 €

Subscription

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Print

Frotscher Druck GmbH
Riedstraße 8 · 64293 Darmstadt
www.frotscher-druck.de

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Der CO_2-neutrale Versand mit der Deutschen Post

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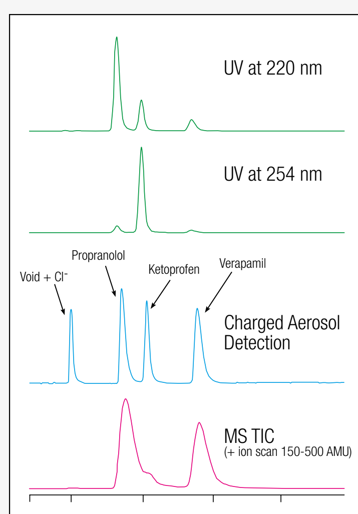

Comparison of Charged Aerosol Detection to UV and MS

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Merck Millipore: Grant from Massachusetts Life Sciences Center to collaborate with Promethera Biosciences

Merck Millipore, the Life Science division of Merck, received a $400,000 grant from the Massachusetts Life Sciences Center (MLSC) to fund a partnership with Promethera Biosciences, a Belgian pharmaceutical company whose mission is to discover, develop, and commercialize cell therapy products to treat liver diseases in an innovative way. This is one of four grants given to Life Science companies in Massachusetts to fund international collaboration as part of MLSC's International Collaborative Industrial Program. Through this collaboration, Merck Millipore's microfluidic technology will enable the researchers at Promethera to mimic the liver microenvironment long-term, allowing for increased consistency and scale-up potential for live cell models. Using liver stem cells provided by Promethera and Merck Millipore's CellASIC® microfluidic cell culture platform, both organizations strive towards improved preclinical liver toxicity testing methods.

→ **www.merckmillipore.de**

Rentschler: "Processes for Virus-based Biologics" is the focus of this year's 3. Laupheimer Zelltage

Rentschler invites under the heading Processes for Virus-based Biologics to the third Industrial Cell Culture Technology Conference. The conference will take place on June 2–3, 2014 at Laupheim's historical castle, Germany. Virus-based biologics play an increasing role in the development of innovative gene, immuno and virotherapeutics. Novel virus-based carrier technologies such as virus-like particles are breaking into new vaccine sectors. The first approval of a gene therapeutic (Glybera®) in Europe as well as various reports of success from gene therapy trials are bolstering confidence in the potential of gene therapy. Also the development of oncolytic viruses for cancer immunotherapy is attracting more and more attention.

The conference will bring together international academic and industrial experts in cell culture and process technology for the production of virus-based biologics and is the first conference of its type eagerly awaited by the scientific and industrial community. The keynote lecture will be held by Florian Wurm (Swiss Federal Institute of Technology). The full program and organization details can be found here: http://rentschler.de/en/information/laupheimer-zelltage/

→ **www. rentschler.de**

Fraunhofer IPA: Lab automation as a driver of progress for biotechnology and bioproduction

Lab automation is the key to scientific progress in biotechnology and bioproduction. Modern analysis automatons and economically efficient, reproducible processes are the decisive prerequisites for the step from basic research to practical use of modern procedures for therapy of diseases, sustainable raw material synthesis or in the field of energy generation.

With the cell production machine "Autranomics" and the Tissue factory for production of artificial skin models, Fraunhofer IPA decisively advanced automated cell and tissue culture. One future area is in automated production of personalised medication customised to the individual patient based on human cells.

Another biotechnology revolution is coming up in the area of biomolecule production. Conventionally, proteins and enzymes such as insulin for diabetics, vaccination components or detergents are produced industrially in cell cultures under high energy consumption. "Cell-free bioproduction" is much gentler on resources. It is being developed by Fraunhofer IPA and seven other Fraunhofer institutes in a project subsidised by the German Bundesministerium für Bildung und Forschung (BMBF).

Another focus is establishing standards in lab automation. The Fraunhofer IPA works intensely on the SiLA initiative (Standardization in Lab Automation).

→ **www.ipa.fraunhofer.de**

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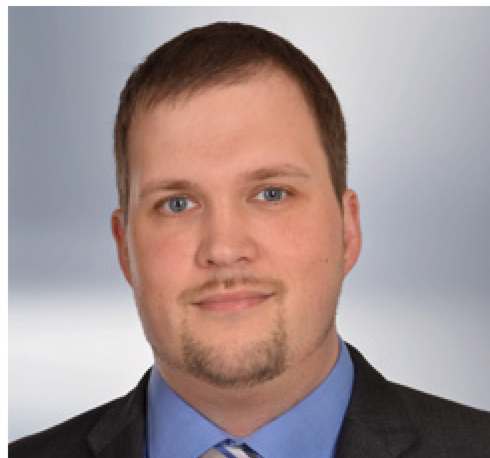
Eberhard-Gerstel-Preis 2014: For 2-dimensional liquid chromatography



Jakob Haun will receive this year's Eberhard-Gerstel-Preis at the analytical Conference in Munich on 2 April 2014. The prize, sponsored by GERSTEL GmbH & Co. KG, is awarded every two years by the work group Separation Science of the specialist group for analytic chemistry of the German chemist society (Gesellschaft Deutscher Chemiker; GDCh) for an outstanding publication in the area of analytic separation technologies. It comes with a prize money of 2,500 Euro. Eberhard Gerstel (1927–2004) founded the company named after him in Mülheim an der Ruhr in 1967. At the Institut für Energie- und Umwelttechnik e. V. in Duisburg, Haun was the first postgraduate student to deal with two-dimensional liquid chromatography based on miniaturised separating columns in connection with high-resolution mass spectrometry. Haun ventured into terra incogntia here, since this kind of coupling had not been described before. Samples from Life Sciences and environmental analysis can at times contain more than a hundred components in different concentrations. Therefore, procedures are required that permit recording many main and trace components at the same time. The new coupling could also be successfully applied, e.g. to analysis of fungal poison in house dust. This is considerable progress in the area of multidimensional separating technology in analytic chemistry.

→ **www.gdch.de**

Roche: FDA Panel recommends Roche HPV Test for first-line screening

Roche announced that the U.S. Food and Drug Administration (FDA) Microbiology Devices Panel of the Medical Devices Advisory Committee recommended unanimously that the benefits of the cobas HPV (Human Papillomavirus) Test as a first line, primary screening tool in women 25 years and older to assess their risk of cervical cancer based on the presence of clinically relevant high-risk HPV DNA outweigh the risks. The panel also voted unanimously that the cobas HPV Test is safe and effective for the proposed indication for use. If approved, the cobas HPV Test would become the first and only HPV test indicated as the first-line primary screen of cervical cancer in the United States.

→ **www.roche.com**

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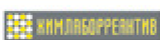

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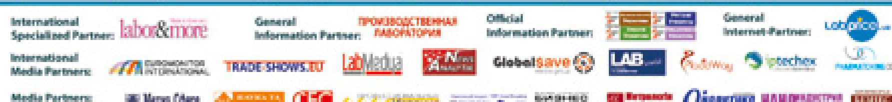

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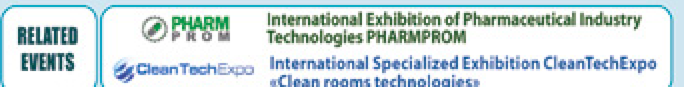

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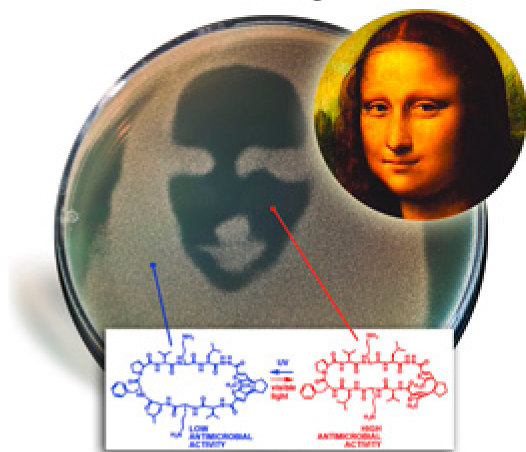
biochemistry

Antibiotic can be switched on and off with light



Scientists at the KIT and the university of Kiev produced an antibiotic the biological activity of which can be controlled by light. Thanks to the robust photo switch diarylethene, the antimicrobial effect of the peptidmimetic can be spatially used in a targeted manner regarding location and time. This could open up new treatment options in future in case of locally limited infection. Only this permits reducing side effects. In the magazine "Angewandte Chemie", the researchers present their photoactivatable antibiotic with the new photo module.

Fig.: © http://www.kit.edu/index.php
Figure (image): Babii et al., Angewandte Chem., 2014
Source: http://www.kit.edu
Original Publication: Oleg Babii, Sergii Afonin, Marina Berditsch, Sabine Reißer, Pavel K. Mykhailiuk, Vladimir S. Kubyshkin, Thomas Steinbrecher, Anne S. Ulrich, and Igor V. Komarov: Controlling Biological Activity with Light: Diarylethene-Containing Cyclic Peptidomimetics. Angewandte Chemie (2014). DOI: 10.1002/ange.201310019

materials and coastal research

Ship exhaust above the North Sea

Dr. Volker Matthias from the Helmholtz-Zentrum Geesthacht recorded the current pollutant emissions from commercially used ships in the North Sea in the scope of the EU project Clean North Sea Shipping. Assuming different scenarios, the coastal researcher also calculated possible future emissions. The models show: Without further statutory regulations and technical changes to the ships, the nitrous oxide exhaust from shipping may increase by 25 percent by 2030.

Source: http://www.hzg.de/public_relations/press_releases/051754/index_0051754.html.de

cardiology

Preventing sudden cardiac arrest

Scientists and doctors at the medical university clinic of Tübingen have published a paper on a newly identified phenomenon in the ECG in cooperation with the TU Muenchen and the university of Helsinki, permitting conclusions on the risk of sudden cardiac arrest.

The cause is usually malignant arrhythmia that will lead to irreversible brain damage if not treated. The timely recognition of high-risk patients is deemed an unsolved problem in medicine. Impairment of the nervous system, specifically the N. sympathicus, is essential in the development of malignant arrhythmia of the heart. The team of researchers presents a new electrocardiographic phenomenon that reflects the effects of the N. sympathicus on the heart.

Source: http://www.medizin.uni-tuebingen.de/
Original Publication: J Clin Invest. 2014; doi:10.1172/JCI70085

immunology

Brake for the immune system

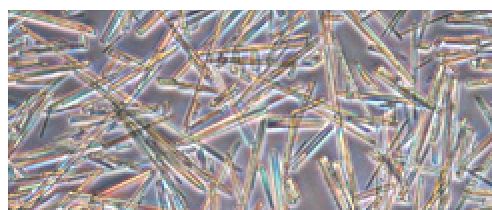


Fig. Recordings of urea crystals: Urea crystals form needle-shaped crystals that activate the immune system
Source: www.tum.de (Figure: K. Neumann/TUM)

For the first time, scientists have identified a receptor on human cells that recognises specific crystals. The new receptor appears on immune cells and binds urea crystals: They are considered the cause of gout, but also control immune reactions. The team managed by researchers of the Klinikum rechts der Isar at the Technische Universitaed Muenchen (TUM) presents its results in the specialist magazine Immunity.

Fig.: www.tum.de (Figure: K. Neumann/TUM)
Original publication: Konstantin Neumann, Mercedes Castiñeiras-Vilariño, Ulrike Höckendorf, Nicole Hannesschläger, Simone Lemeer, Danny Kupka, Svenia Meyermann, Maciej Lech, Hans-Joachim Anders, Bernhard Kuster, Dirk H. Busch, Andreas Gewies, Ronald Naumann, Olaf Groß, Jürgen Ruland; Clec12a Is an Inhibitory Receptor for Uric Acid Crystals that Regulates Inflammation in Response to Cell Death, Immunity (2014), http://dx.doi.org/10.1016/j.immuni.2013.12.015

materials research

How long do composites last?

Many materials that can only be glued together are used in airplanes, buildings and medical technology today. How do the glues resist environmental influences, though? How do they react to moisture, heat and constant mechanical strain? Materials researcher Wulff Possart from Saarbrücken examines chemical changes to composites, laminations and glued components. He wants to slow down these changes and predict ageing of glues precisely. Such forecasts are decisive for the industry regarding the lifetime warranty of components and product liability.

Source: http://www.uni-saarland.de/fak8/wwthd/

neurobiology

How a little worm can help fight Alzheimer's disease

Scientists from the Max-Planck-Institute (MPI) for the biology of ageing in Cologne have discovered that a molecule in the body could strengthen the defensive mechanisms against neurodegenerative diseases. If the small roundworm C. elegans is fed with this metabolic product additionally, the degradation of harmful protein aggregates in the body is supported and the worm's life is extended.

Source: http://www.age.mpg.de/
Photo: © MPI for the biology of ageing

physical chemistry

Recognising infection with the naked eye

In a new project, researchers of the university of Siegen are working on sensors that make bacterial infection in wounds visible by colour change. These sensors could be integrated into band-aids for treating burns in children. At the same time, the preventive administration of antibiotics can be avoided.

The system sounds as simple as that of traffic lights. Red light means you have to break, green light means that you can drive on. The colour change is visible to the unaided eye. This system can help recognise infection in wounds simply by looking at them.

Source: http://idw-online.de/de/news575784
Image source: https://www.uni-siegen.de/start/news/forschung/571989.html (image source: G. Schulte, H. Schönberr, N. Voelcker, et al.).
Image text: Green turns red: A photonic sensor of nanoporose silicon indicates the replacement of air in the nanopores with alcohol by changing colour. Infection is to be indicated according to the same principle in future (image source: G. Schulte, H. Schönberr, N. Voelcker, et al.).

molecular biology

How brain tumours are formed

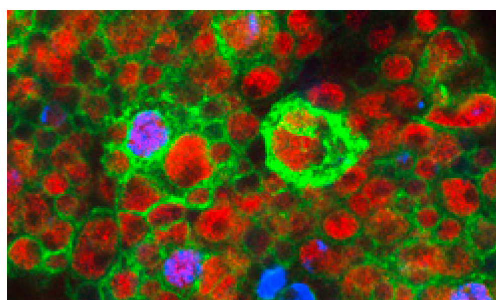


Fig. Brain tumours in drosophila, developed from neuronal stem cells. The tumour (green) contains mostly stem-cell-like cells (red) that reproduce strongly (blue).
Photo: University Basel

How does cancer form? This is still one of the most urgent questions of our time. Cancer stem cells are more and more moving into the focus of research. Researchers from the biocentre of the University of Basel and the Austrian Academy of Sciences now have identified a protein complex in neuronal stem cells of the fruit fly Drosophila melanogaster that ensures the correct development of stem cells and thus prevents the development of cancer. In drosophila, normal neuronal stem cells develop into immature predecessor cells first that become nerve cells through various maturing steps. The researchers around Prof. Heinrich Reichert and Prof. Jürgen Knoblich now have found important components – parts of the SWI/SNF protein complex and their target protein named Hamlet – which start a rigorous control programme in predecessor cells. It ensures that the cells will only divide within limitations and prevent return to a stem cell. It also warrants that the cell cycle ends in time and maintains the temporal identity of a predecessor cell.

Source: www.unibas.ch
Original publication: Cell, Volume 156, Issue 6, 1259-1273, 13 March 2014 | doi: 10.1016/j.cell.2014.01.053

Breakthrough

Tracera merges the GC-2010 Plus GC with the brand-new BID-2010 Plus detector

The brand-new universal BID-2010 Plus detector applies the patented, breakthrough Barrier Discharge Ionization (BID) technology. It targets organic and inorganic compounds at the ppb level and enables their high-sensitivity analysis in a single detector only. Coupled with the GC-2010 Plus capillary gas chromatograph, Tracera reveals trace components which are difficult to see for other GC detectors.

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SuperNova in the test tube

Nano Crystals for mega Fluorescence Amplification

Prof. Dr Reinhard Renneberg [1], Jan Engels[1],
Dr Hans-Georg Eisenwiener[2]
[1] Hong Kong University of Science and Technology (HKUST), Hong Kong, China
[2] Diagnostic Healthcare Consulting, Weil am Rhein, Germany

We tell the exciting story of how mega fluorescence amplification was invented. First, there was the question: How can previous biochemical evidence reactions be amplified million-fold to detect the biochemical "needles in a haystack" (substances at very low concentrations) in a multi-component system like a blood sample?

A mega idea supplied a possible solution based on the following idea: If a nano crystal, comprising of millions of potentially fluorescing molecules, is used as label ("marker") in immune-chemical antigen-antibody tests or hybridisation reactions (DNA tests) instead of one or a few singular fluorescing molecules, calculations suggest a potential million-fold stronger fluorescence and thus amplification. If this kind of nano crystal is dissolved quickly and then triggered to fluorescence by a chemical reaction – hydrolysis –, fluorescence of millions of potentially fluorescing molecules will suddenly occur. This can be compared to a "SuperNova explosion in space". The fluorescing molecule found and chosen was fluoresceindiacetate (FDA), a non-fluorescing organic molecule that fluoresces after hydrolysis.

A lack of detectability and quantifiability of a substance in a complex sample matrix is a recurring problem.

Where are highly sensitive detection reagents needed?

The proof of an analyte in the blood, serum/plasma, urine, CSF or saliva is "tedious and stressful" or not possible at all yet - particularly when DNA, antigens, antibodies or other substances are to be documented that are only present in very low concentrations (e.g. in the nano, pico or femto gram/mL range) or if it is difficult or painful to acquire a larger amount of sample material.

With our current determination methods – using colloidal gold or classic fluorescence markers – cardiac markers such as Troponin I/T, FABP or BNP/NT-proBNP can be determined in quality and quantity using 50 – 100 µL capillary blood. How to get 50 – 100 µL of capillary blood from the fingertip, though? This is not without pain. The super label FDA as a nano crystal does not require any more than 5 – 10 µL of blood. This amount is easy to acquire.

Heureca in the night lab

The FDA story

In 1999/2000, Dieter Trau, at that time professor at the National University Singapore (NUS), was working on his doctoral thesis at the Hong Kong University of Science and Technology (HKUST) in the lab of Prof. Dr Renneberg. His research area was layer-by-layer encapsulation of different enzymes using poly-electrolytes. Among others, he also used the organic substance fluoresceindiacetat (FDA), which is commercially provided in crystalline form.

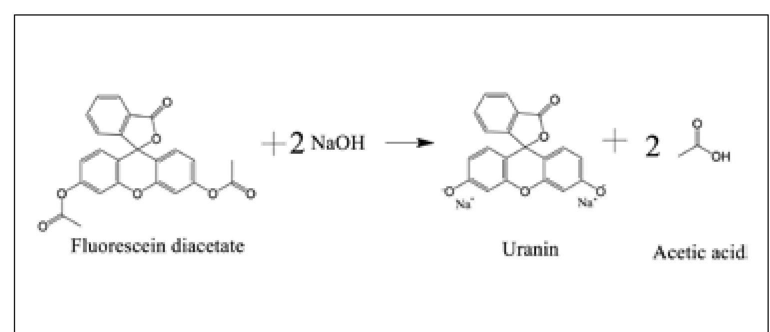

Fig. 1 Hydrolysis reaction of fluoresceindiacetate (FDA) with caustic soda into the greenly fluorescing uranin and acetic acid as a side product.

Reinhard Renneberg, born 1951, studied chemistry at the Lomonossov university in Moscow. After acquiring his diploma, he worked at the Zentralinstitut für Molekularbiologie (ZIM) in Berlin-Buch, where he acquired his doctorate in 1978 and habilitation in the area of bio sensors in 1991. From 1991 to 1995, he managed the immune sensor department of the Fraunhofer-Institut für Chemo- und Biosensorik (ICB), Münster. In 1994, he followed the call of the Hong Kong University of Science and Technology (HKUST) as Full Professor of Analytical Biotechnology. Renneberg is also active as a company founder. He is the author of "Bioanalytik für Einsteiger" and "Biotechnologie für Einsteiger", for which he received the literature award of the Fonds der Chemischen Industrie in 2008.

Photograph: © Viola Berkling, Oschersleben

Jan Engels, born 1989, studied bio engineering at the University of Applied Sciences Aachen. After acquiring his Bachelor's degree, Prof. Reinhard Renneberg suggested him for the fast-track programme of the Hong Kong University of Science and Technology (HKUST) for a directly subsequent PhD in chemistry. He was accepted. His interest is in nano particles for evidencing various reagents and their direct application. At the moment, his research is targeted at FDA nano crystals for various immune assays. Furthermore, his scientific interest has led to his activity in founding a start-up company spun off from the research group of Prof. Renneberg.

Hans-Georg Eisenwiener, born in 1939, studied physical chemistry at the Johannes Gutenberg-Universität in Mainz. After his doctorate, he increasingly focused on medical diagnosis and took over management of the neurology lab of the university clinic in Göttingen, before entering the diagnosis section of F. Hoffmann-La Roche in Basel, which was then under development. He was responsible for the R&D-department of the diagnosis section with the wide product range of clinical chemistry, immunochemistry, microbiology, blood clotting and analysis automation there. After a reorganisation, he looked for new diagnostic parameters and determination methods outside of the department. Since his retirement, he has become a consultant for introduction and commercialisation of new diagnosis parameters and point-of-care technologies.

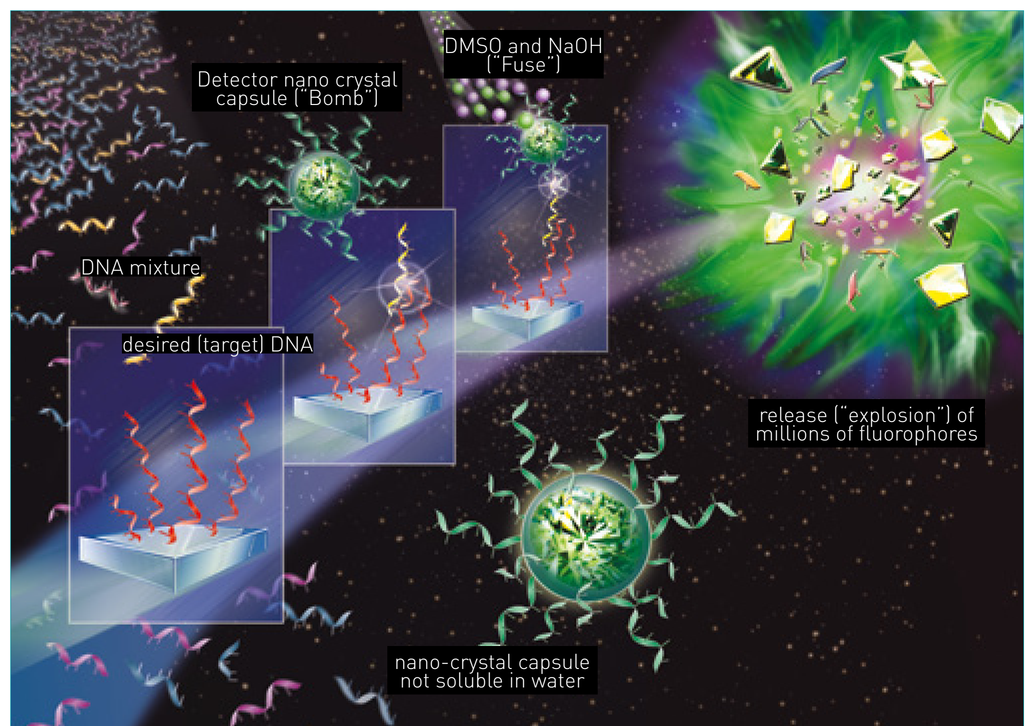

Fig. 2 Figure illustrating the SuperNova principle, here to document DNA: First, catcher DNA strands are bound to a specific surface. In the next step, the complementary DNA that is the target binds to the catcher. The non-fluorescing nano crystals were marked with supplementary detection DNA and then bind to the caught DNA strand as well. Last, the required mix of organic solvent (DMSO) and reaction reagent (NaOH) is added. The crystal is dissolved suddenly and the currently non-fluorescing molecules start emitting a green light after a subsequent reaction.

Illustration: Biotechnologie für Einsteiger, 4th edition

When Dieter Trau achieved polyelectrolyte labelling of large FDA crystals, he had an idea for how to continue using his previous experiments directly: “Why not use a fluorescing crystal for fluorescence testing instead of the usual evidence methods where only a few fluorescing molecules will adhere to every antibody?”

Dieter Trau labelled finely ground FDA crystals first with polyelectrolyte layers of PAH (polyallylamine hydrochloride) and PSS (polystyrene sulfonate). Then he attached antibodies to them to try out an immune test. After various tests and experiments, he was able to successfully label an FDA crystal with antibodies and then bind antigens to it. However, a reaction of non-fluorescing FDA into strongly fluorescing fluorescein was missing to produce the signal. For this, Trau first used enzymes (esterases) that are also used in cell cultivation. However, they react relatively slowly, which weakened the strong fluorescence effect again. Further tests led to hydrolysis of the acetate groups by highly concentrated sodium hydroxide (NaOH) into acetic acid and uranin (Fig. 1).

FDA is, however, an organic compound. They do not dissolve well in water, but very well in organic solvents, such as DMSO or IPA (isopropanol alcohol). With the addition of a “releasing reagent” comprising of, e.g., DMSO and sodium hydroxide, the crystals “exploded” like a super nova and the entire liquid suddenly turned into a strongly greenly fluorescing solution that can be seen by the unaided eye (Fig. 2). The basis for the “SuperNova fluorescence” is the immediate dissolution of the crystal, which then permits the quick chemical reaction between the caustic soda and the FDA. Prof. Renneberg enthusiastically called out “Super!” and Dieter Trau added “And new is nova”. This was how the name – “SuperNova” – was coined. It was patented not long after [1].

“Wonder substance” FDA

The prerequisites: It should be a compound that is present in crystalline form, well-specified and not fluorescent as such. It must be possible to grind or recrystallise the crystal down to nanometre size. Why crystals? They contain the tightest packing of molecules in nature. After the crystal had been dissolved by a “reaction mixture”, the dissolved molecules instantly continued to react and all dissolved molecules fluoresced. Dieter Trau thus discovered in Hong Kong that fluoresceindiacetate (FDA) was a mega fluorescence amplifier [2].

Fluoresceindiacetat (FDA) is a derivative of the known fluorescing molecule fluorescein, which is often used in immunoassays. The two substances differ molecularly by two acetate groups attached to the FDA. FDA as such has nearly no fluorescence at all and is mainly used for bacteria and cell cultivation. FDA reacts with hydrolysis catalysing enzymes (specifically esterases) to fluorescein because the two acetate groups are split off. This is reflected in the intense green colour and fluorescence that can be seen well in the living cells using a fluorescence microscope. This reaction permits differentiation between living and dead cells, since dead cells no longer produce any enzymes.

Enormous amplification potential

A single FDA crystal ball 100 nm is size holds an unbelievable 1,200,000 (1.2 M) FDA molecules. Comparing the roughly 1.2 M

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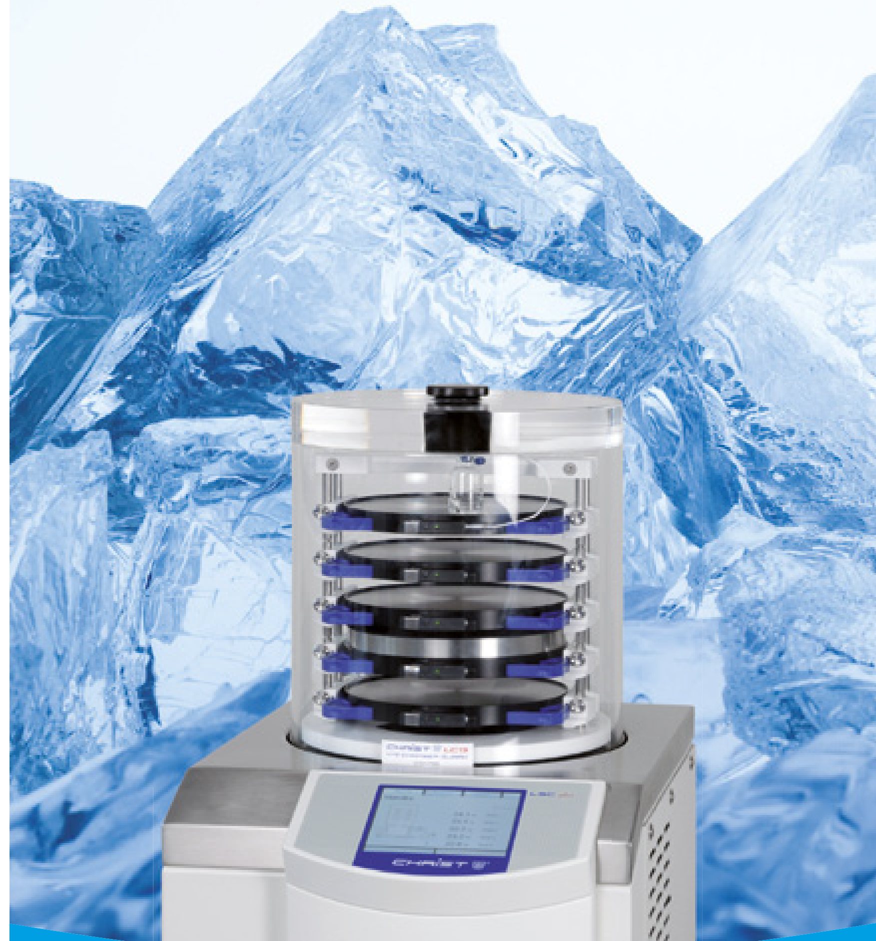

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molecules in the crystal used to roughly ten molecules directly bound to antibodies, there is a ratio of 1:120,000. This is, of course, reflected in the fluorescence strength as well.

Core question: How can a better or stronger signal or - more precisely - a higher signal/noise ratio be produced? Wouldn't it be better to use a nano crystal with millions of FDA molecules instead of a few fluorescing molecules of, e.g., FITC, to achieve the analysis sensitivity, characterised by the 3 NCCLS-parameters – limit of detection, limit of determination and limit of quantification – in the ng/pg- to fg-area for small available samples?

In contrast to today's "state-of-the-art" procedures, this is possible by not only binding molecules of fluorescence labels to antigens, antibodies or DNA, but by nano crystals of fluorescing molecules being bound instead. This means: Instead of the roughly ten fluorescing molecules that are covalently bound to antibodies, the reverse binding order uses a complete crystal comprising of a derivative of a fluorescing molecule to which the antibodies are bound directly. This bond between the crystal and the antigen or antibody can be adsorptive or covalent in origin. The strength of the bond can be increased by preliminary coating of the crystal with charged polyelectrolyte layers, the layer-by-layer encapsulation [3].

The great challenge: Competition for the PCR?

In addition to optimisation of particle size, immune assays for different antigens and antibodies with a lower limit of determination than conventional assets are being developed as well [4]. Direct evidence for just a few DNA molecules would be even more attractive. The current standard is – next to several other amplification methods - the polymerase chain reaction that leads to about 1 M DNA copies per hour. It is the unchallenged state of the art.

How close would the AmpCrystal (SuperNova)-method come to the range of DNA-PCR amplification? How many DNA molecules would be required for detection? What would be possible with SuperNova without cyclers and expensive enzymes?

The method: A nano crystal is not coated with antibodies but with single-stranded DNA. They bind to complementary DNA from a sample that is bound to a catcher DNA in a first step. It can be detected after addition of the releasing reagent and connected mega fluorescence generation [5]. It would be possible to combine the AmpCrystal method with PCR and thus save cycles - or to replace the PCR in a few tests (e.g. in microbiology).

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Tracers

Tracers use molecules marked by radioactivity or fluorescence to permit observation of chemical or biological processes. Tracers are used in many areas, such as hydrology, meteorology, nuclear medicine or air technology. Tracers are used in hydrology to determine the flow direction and speed of bodies of water (surface water and ground water). There are two different types of tracers: Environmental tracers, natural substances that are already present and can be detected in the water, as well as artificial tracers that must be added to the water.
Fluorescence tracers such as uranin have become particularly important. In addition to determination of water conduct, they can also be used to find leaks in roofs or water lines. Due to its extremely strong green colour, uranin is also used to colour the sea in case of airplane and ship accidents to enable helpers to find the location more easily.

Fluorescence spectacle on St. Patrick's Day: Every year on 17 March, the holiday of the Irish saint, the colour green becomes important around the world. For example, the Chicago River is dyed green on this day. Until 2003, this was done using uranin, which was then replaced by another dye due to intervention of the EPA.

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Detective work with molecular accuracy

Non-target screening, suspected-target screening and target screening – of technologies and philosophies, databases and crafts

PD Dr Thomas Letzel
Analytical Research Group at the Chair of Urban Water Systems Engineering of the Technische Universitaet Muenchen

The three terms in the title, as well as "Known Unknowns" and "Unknown Unknowns" are new keywords that are currently confusing the analytical water scene. The procedure in use of just these technologies, however, often is not consistent yet. This article will now try to put the many different terms and approaches into some semblance of order. At the same time, it will report on pragmatic applications.

Non-target screening, suspected-target screening and target screening as well as "Known Unknowns" and "Unknown Unknowns" are new keywords that are currently increasing in interest in water analysis. The search for unknown or expected molecules in the matrix water brought about new instrumental technologies and analytical strategies. A great share is based on liquid-chromatography separation (LC) with atmospheric pressure ionisation (API)-coupled mass spectrometric detection (MS) and is technologically very mature.

Analytical screening (by LC-API-MS): What is this?

Let us first have an entirely un-scientific (but properly scientifically quoted [1]) look at what the public platform Wikipedia contributes to the term of "sieving". The word screening is mainly used in the medical context here, and the results of screening studies are medical in origin as well. However, there also is a more general definition: "Screening (screening, grid application, selection, raying) is a systematic test method that is used for identifying certain properties of the test objects within a defined test area – usually comprising of a large number of samples or persons. Screening therefore is an orienting screen test aligned with certain criteria." [1]

Referring this definition to the currently highly popular screening area of analytical chemistry in water research, it may mean: "A LC-API-MS screening is a systematic analytical test method that is used to recognise the organic molecules within the matrix water, characterising them physico-chemically and determining their quantity. This screen test is thus aligned with the criterion of organic water contents determination."

The fact that screening is a clearly aligned but also limits application is important here. This "screening for organic water contents" says nothing about any other components such as micro-organisms or inorganic substances, nor are there any indications of toxicology or biological function. For this, further analytical screening technologies are necessary. In the best case, they can be coupled with this one [2].

Now, for the first time we stumble across the term of "non-target screening", which must be a "targeted analytic" in spite of its name.

Therefore, there cannot be any "non-target screening" in the actual sense.

Has the article arrived at its end now? As the following text shows: It has not!

Let us first have a look at figure 1, which tries to illustrate classification of the terms. Even if entirely untargeted analytic is not possible, these tests still can be performed without a prior objective. The results are two types of untargetedly detected target molecules, the "Unknown Unknowns" and the "Known Unknowns".

The class of "Unknown Unknowns" cannot be uniquely identified by any currently available evaluation method and also cannot be assigned to any known molecules. There remains only the option to determine structure by similarity comparisons with known molecules, such as hydrophobicity, molecular mass or mass-mass spectrometric fragmentation patterns.

The class of "Known Unknowns" has recently been described very nicely by J.L. Little et al. as "Known Unknowns – that is, species known in the chemical literature or MS reference databases, but unknown to the investigator." [3]. This definition conceals another dilemma of non-target screening. "not targeted" does not always mean "unknown". Non-target screening thus often is quick to use the same path as "suspected-target screening", which implements identification of expected molecules using analytical and chemical databases. The suspected-target screening, however, will start out with a list of expected substances and can be specifically aligned with this.

This approach and the "Known Unknowns" share the fact that there initially are no real reference substances but that substances can be identified via substance databases, chemical databases, analytical or mass spectrometric databases and/or reconciliation with in-silico predictions.

If this search or interpretation leads to success, the corresponding real reference substance can be synthesised and used. This clear identification and the further use are consistently also referred to as "Target Screening", which contains the quantitative analysis using isotopic labelled reference materials.

Non-target screening: Possibilities of an analytical technology

Now using the non-target screening approach in the text consistently as a technology approach and less strictly according to the definition, we can continue in good spirits:

This approach typically is a water analytic with a) sampling, b) sample preparation, c) liquid-chromatographic separation, d) ionisation and ion transfer of the contained analytes and e) mass spectrometric detection of the same (poss. incl. structure determination by tandem-MS).

Now, clearly targeted and analyte-focused methods are used (even though these are preferably kept as universal as possible to do more justice to the name):

a) Sampling

Sampling is typically performed using different sampling devices (e.g. trowel or hose); the sample is then transferred to sampling vessels and stabilised with chemicals or by storage in the frozen condition. All steps may falsify our sample, e.g. by loss, enrichment and modification of the organic molecules. Practical sampling that is correct as well as impeccable under statistics aspects must be ensured here. In the latter case, the sampling area as well as the sampling duration (e.g. short sample intervals [4] or 24 h mixed sample [5]) plays a very important role.

b) Sample preparation

The best sample preparation is, of course, no sample preparation at all!

Since this is frequently not possible, an attempt is made to separate the desired analytes (by enrichment) from the undesired

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matrix molecules (by reduction) by many enrichment and reduction methods. The most common ones are solid phase extractions (SPE); e.g. by online-SPE [5,6] and high polarity value SPE [6]), where – as in sample separation - mainly chromatography material is used.

c) Sample separation

Classically, the reversed phase – high performance liquid chromatography (also RP-HPLC) with C18-phases or other unpolar to medium-polar phases is used for separation of organic analytes in water. This has increasingly become established in the recent past, since this separation can be very well connected with mass spectrometric detection. We also refer to the following sections for this. A currently completed round robin test on standardisation of the retention times achieved in turn facilitates transferability of the results interlaboratory results [7], with analytical data becoming easier to reconcile.

For some time, the numbers of users of the hydrophilic interaction liquid chromatography (HILIC) have been increasing. This is due to the also-excellent possibilities of coupling to the MS as well as due to its ability of separating polar molecules. A very current application with RPLC-HILIC-MS-coupling [8] even promises increased use of chromatography in the expanded polarity range (e.g. logD -5 to logD +5). This value reflects, among others, the hydrophobicity that was characterised highly variably over the last decades [9].

Thus, the separation properties of organic molecules can be used as specific physicochemical indices for characterisation of the individual molecules.

d) Ionisation and ion transfer

Molecules must ionically be transferred to the gas phase after separation so that they can enter the mass spectrometer. Particularly observe the nature of molecules (e.g. their functional groups) as such, but also the characteristics of the mobile phase (e.g. signal-influencing matrix effects or pH-values). Finally, ion sources with different properties (e.g. API-techniques, such as ESI, APCI and APPI) in positive and/or negative ionisation are mainly used [9,10].

The technology of ion mobility increases in importance. It permits another independent molecule separation (after entry into the mass spectrometer) and is driven by the geometry of the molecules [11].

e) Mass spectrometric detection (incl. structural analytic by tandem-MS)

The mass spectrometric detection can be used highly variably, both in the type of application and, specifically, in the type of instruments. We refer to the present literature for detailed treatment of the mass spectrometric possibilities (e.g. [9,10]).

Some questions are of special importance here, however: Which detection mode, which detection range, accurate, internal standard, neutralisation effects, isotopic shift [12], adduct formation, which sensitivity, in-source fragmentation, tandem-MS, significant fragments, which dissociation technologies, which analytical evaluation software, etc.?

By combining these analysis methods, more or less "non-targeted" measurement is now possible. However, the data gained can often be processed further well – as shown below.

Suspected-target screening: Possibilities of different databases

The suspected target screening typically starts with a list of analytes to be measured (since expected). The results of these measurements are subsequently subjected to the "molecule search" (see fig. 1). As with the "Known Unknowns", most molecules are stored in the databases [13] (but not specifically present in the lab as reference substances). The "suspected targets" and the "Known Unknowns" can be further characterised using substance databases (such as STOFF-IDENT), chemical databases (such as Chemspider or Chemicalize), analysis or mass-spectrometry databases (such as DAIOS, MassBank, local databases [5,6,14] or commercial MS spectrum databases [15]) as well as in reconciliation with in-silico predictions (such as the UM-BDD, EPI SuiteTM, filter for MS-similarity trees [16] or MetFrag). If these databases lead to clear results, the user can procure (or synthesize) the corresponding substances and then use them in target screening (see * in fig. 1).

Target screening: Possibilities of the LC-API-MS-"craft"

Identification from the non-target and suspected target screening lead to use of reference substances (see above and * in fig. 1) and their use in Target Screening. By use of isotopic labelled reference substances, concurrent quantity analytic of many molecules is possible as well (examples include 72 analytes by "MRM" [5] and 88 analytes by "SRM" [6]).

Application of different screening strategies in water

a) Use of conventionally gained non-target screening data

A pragmatic path of using results from the non-target screening is direct comparison of analytical RT-MW-plots (retention time (RT) against molecular weight MW) at different times (also see fig. 2a –upper left), received using LC-API-MS.[4] This strives not solely for the identification of individual analytes but also compares the analytical results by "imaging" and takes their differences into consideration.

b) Use of the suspected-target screening path for "Known Unknowns" (with data of accurately measuring MS)

Use of LC-QqToF [14] and Orbitrap-MS [17] (this applies to FTICR-MS and IT-ToF-MS accordingly) in the non-target screening leads to the above RT-MW-plots with accurate masses (i.e. elemental formula) for the respective molecules. Thus, they can be subjected to the molecule search and characterised as "Known Unknowns" via databases (see fig. 2a).

Molecules that cannot be identified or assigned via this path thus correspond to the current "Unknown Unknowns" and thus must be supplied to the "Similarity search" (see fig. 1 –left and [11]).

c) Use of the suspected-target screening path for "Known Unknowns" (with data of inaccurately measuring MS)

Using the non-target strategy with inaccurately measuring mass spectrometric as in the LC-QqQ (this also applies to IT and linearIT), the databases can be used as well – with limitations (fig. 2b). Due to the missing mass spectrometric accuracy, no elemental formulas can be generated in this approach and equipment sensitivity will be very low at the large mass spectrometric detection range of the quadrupole devices. Considering these disadvantages, these devices are still justified in these screening approaches. This is particularly important because these mass spectrometers are much easier and cheaper to operate and thus also are very common in analytical labs. Thus, it is possible that labs, which typically operate target screening, use their devices for non-target examinations as well.

d) Use of target screening

The known analytes are sensitively detected and quantified with e.g. LC-QqQ-MS and the respective MRM-methods [5,6].

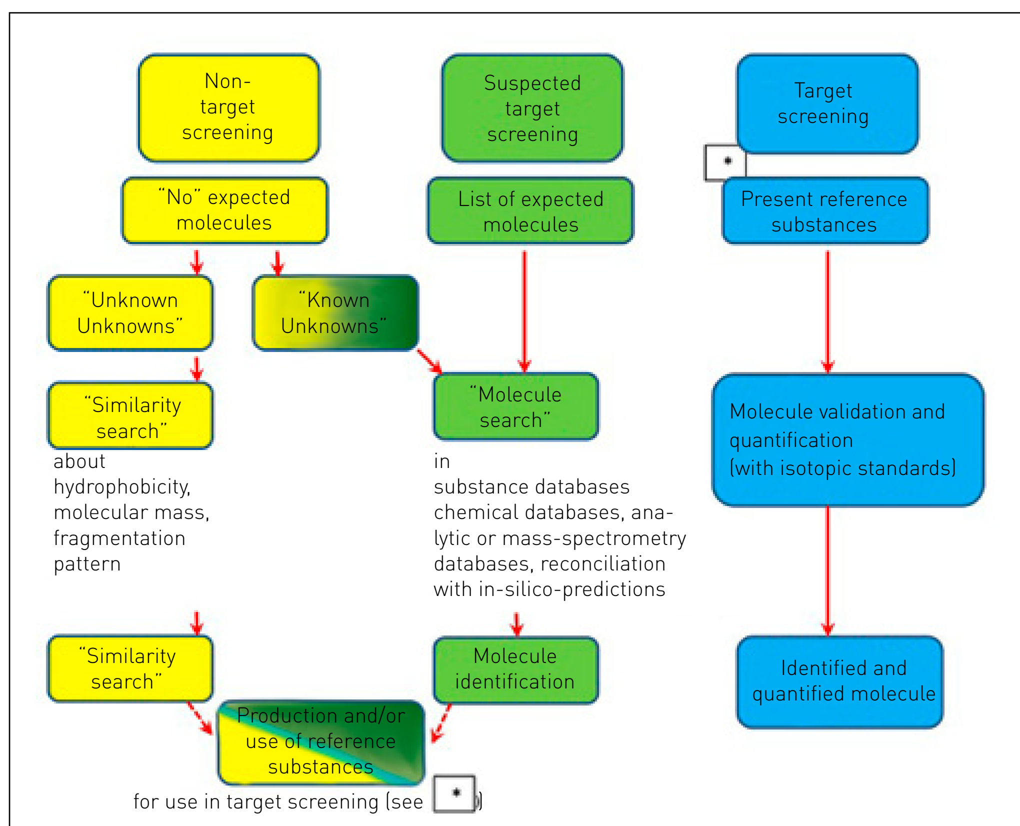

Fig. 1 Overview and details for use of analytical strategies in water analytic

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Fig. 2 a) "Known Unknowns" (via suspected-target screening pathway)

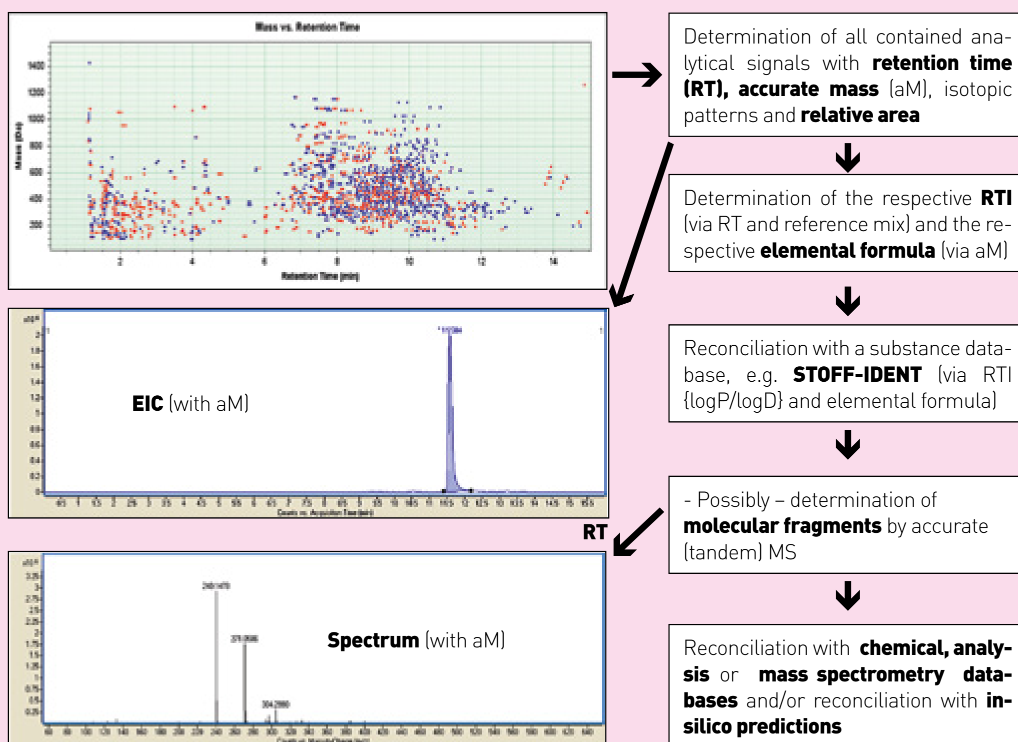
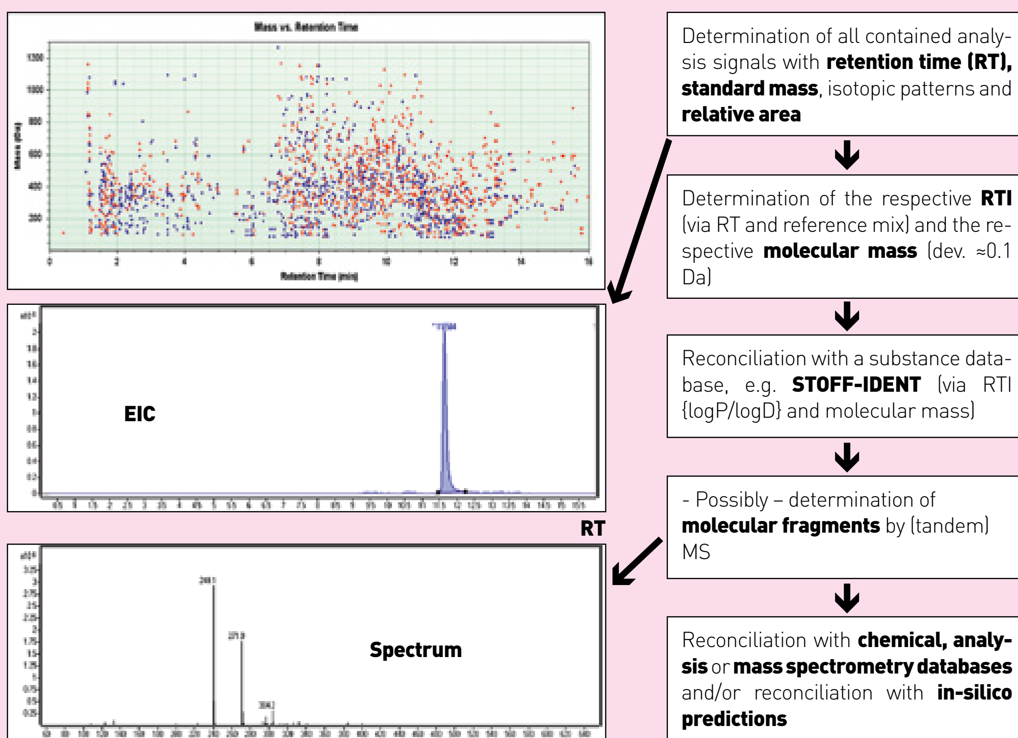


Fig. 2 b) "Known Unknowns" (via suspected-target screening pathway) with inaccurately measuring (tandem) mass spectrometers



Summary

The time in which analytical chemistry was considered an auxiliary science is now actually past. Due to the current diverse use of the described LC-API-MS screening techniques in many different disciplines (e.g. in proteomics [9], metabolomics [16], human samples [15], in foods [18] as well as in wine [8]) analytic even enters the foreground. Now, however, great care must be taken to ensure that the earlier problem is not turned around, pushing the applicative disciplines into the background. Consider: Only a strongly linked analysis is a good analysis system. Therefore, "the analysts" are only excellent when they also look over the rims of their teacups, get comfortable in the other disciplines and closely cooperate with their representatives.

The screening techniques as such are currently subject to great interest. This up-to-datedness is also the reason for most quotes from this publication from 2012/2013. It is certain that the subject will remain in the focus and that further papers will deal with screening in water [10]. Now we are ready for this and for the screening terms.

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Thank you

The research project "RISK-IDENT" is a network project in the research focus "Risk management of new hazardous substances and pathogens in the water cycle" (RiSKWa) of the German Bundesministerium für Bildung und Forschung (BMBF) with the promotional ID: 02WRS127 3. We cordially thank all partners of the RISK-IDENT project for their cross-institution commitment to harmonisation and standardisation of water analytic and development of STOFF-IDENT. Special thanks go to Dr. Giorgia Greco, who contributed to the article's structure with figure 2. By the way, the original analysis information from this figure was gained with other systems and red wine. This is clear proof that the devices can be used (or illustrated) universally, that the data sources cannot always be determined directly and that we are honest ;-).

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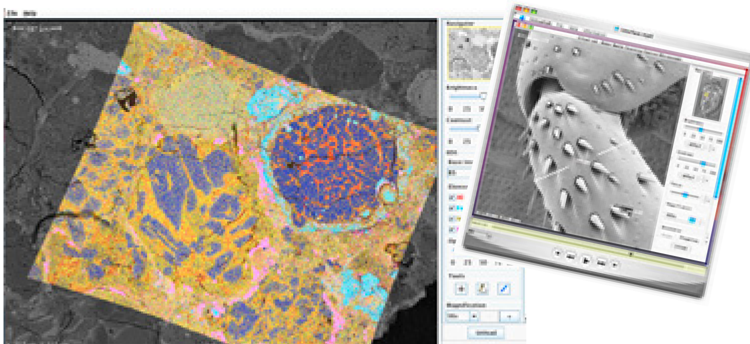

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The materials: You can choose objects from a total of 90 samples with extraordinary detail and load them into the microscope by a click of the mouse. The authors do not conceal that sample preparation and handling in real life means a lot more delicate work. The section "training" shows some truly well-done videos on preparation (cutting, embedding, dying) in English. The short clips and animations on the theory and practice are so great that they alone are worth visiting the page; we recommend the clips to any student as preparation for an EM internship.

The images currently available on the website for studies together makes up more than 60 giga pixels. Most of it comprises of biological samples, but there are also meteorites, moon dust and technical samples in the collection. With Virtual Microscope, the user can microscope the image data recorded first as in real time. Functions include loading/unloading of samples, navigation in the object, changing the amplification factor, setting image parameters (contrast and brightness), selecting, analysing and measuring the focus. There are tools for labelling of image areas. The operating elements of the software are nearly self-explanatory (but of course the page also contains detailed tutorials and teaching clips for this).
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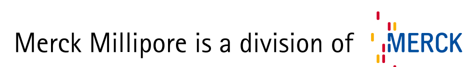


Meat as a doping trap

Clenbuterol testing in doping control samples: drug abuse or food contamination?

Prof. Dr Mario Thevis & Prof. Dr Wilhelm Schänzer
Centre for Preventive Doping Research and European
Monitoring Centre for Emerging Doping Agents,
German Sport University Cologne

Clenbuterol, a β_2-sympathomimetic drug, has been on the list of substances prohibited in sport for over 2 decades. Due to its putative performance-enhancing properties, urine samples are routinely tested for its presence during doping control: by using modern liquid chromatography-mass spectrometry instruments, detection limits at the level of just a few picograms per millilitre are possible. This sensitivity not only makes detection possible long after use of the illegal substance has been discontinued but, for a number of regions outside Europe, has also uncovered a food contamination problem that could make life very difficult for professional athletes.

Clenbuterol is a racemic mixture of two enantiomers (Fig. 1). Banned in sport since 1992, its presence is usually tested for in doping control with the aid of liquid chromatography/(tandem)-mass spectrometry. On account of its anabolic properties, clenbuterol is not listed in sport as a β_2 agonist, but in the category S1.2 ("Other Anabolic Agents") and is thus prohibited at all times, i.e. both during the sporting event itself and at any other time [1]. There is no threshold limit for clenbuterol: the detection of the analyte in doping control samples immediately results in an "adverse analytical finding" (AAF). To maximise the potential time frame for detection following discontinuation of a (presumably illegally) consumed substance, instrumental analysis has been pursuing significant improvements over the last few years. This has resulted in very low detection limits and, as a consequence, athletes' consumption of clenbuterol has been proven in a great many cases. Figure 2 gives a typical example of analytical clenbuterol testing, on a urine sample containing approx. 3 pg/ml clenbuterol. The method used here consists of a liquid-liquid extraction from urine into methyl tert-butyl ether with subsequent re-extraction into aqueous hydrochloric acid (0.06 M) and LC-MS/MS analysis.

Clenbuterol in foodstuffs

The fact that clenbuterol exhibits anabolic effects – especially at high doses of the drug – has also led to abuses of the substance in the area of meat production. As a result, this industry has banned its use in food production at an international level [2]. Yet in 2010 and 2011, athletes tested positive for clenbuterol after participating in overseas sporting events outside the European Union (in China and Mexico). The results are traceable back to contaminated foodstuffs. In 2010, routine doping control of a German team returning from a tournament in China found low – yet clearly detectable – concentrations of clenbuterol in the urine of every single member of the squad [3]. A follow-up study, conducted with people residential in China and with tourists staying in China for various lengths of time and in various locations, further illustrated the problem of illegal use of clenbuterol in animal feed: based on current anti-doping regulations, no fewer than 22 of the 28 volunteers tested would have returned "positive" test results. As a number of anti-doping organisations were aware of this problem in advance of the Olympic Games in 2008, advisory notices warning against eating meat in China were circulated to athletes, so as to guard against inadvertent consumption of clenbuterol and falling into this "doping trap". After all: differentiating between a drug abuse incident sometime in the past – and thus relevant for doping control – and a low dose as consumed in contaminated food is, in analytical terms, a highly sophisticated task.

In Mexico, a similar situation can be found: here, too, a series of results from food testing have uncovered the unlawful use of clenbuterol in animal feed over the last few years [4, 5]. The potential repercussions for professional athletes were highlighted first in May 2011, when five

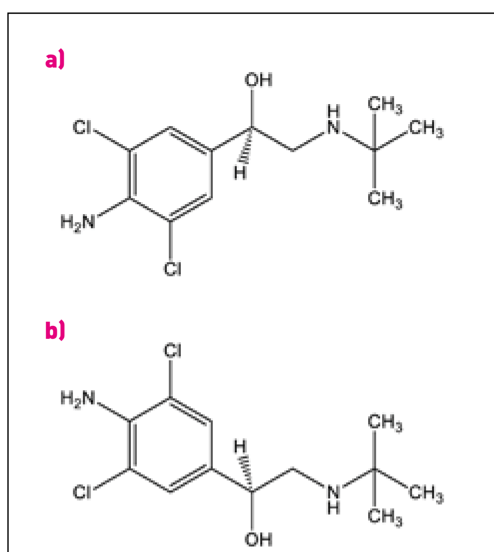


Fig. 1 Clenbuterol enantiomer structures: a) (-) clenbuterol (active isomer) and b) (+) clenbuterol (inactive isomer)

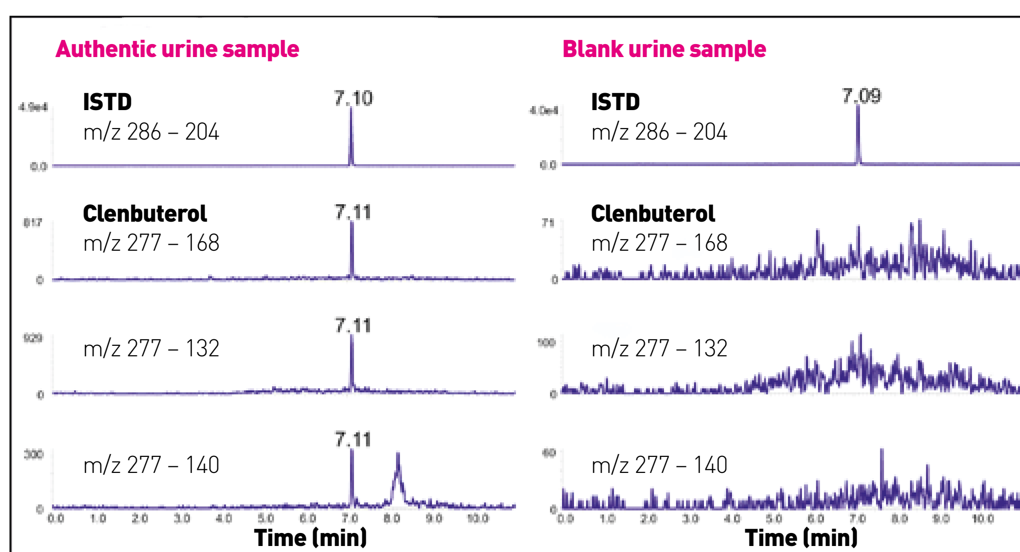
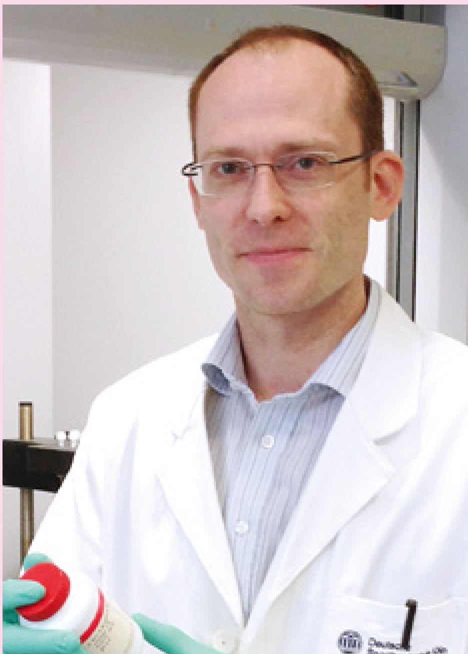


Fig. 2 Extracted-ion chromatograms of a urine sample with approx. 3 pg/ml clenbuterol (left), compared to a blank urine sample. The enantiomers remain unseparated by conventional liquid chromatography.



Mario Thevis studied chemistry at RWTH Aachen and sports science at German Sport University Cologne. After receiving his doctorate in biochemistry (2001), he completed a post-doc at UCLA (University of California, Los Angeles). Following his habilitation in biochemistry in 2004, he was appointed Professor of Preventive Doping Research in 2006. He is Chair of the Centre for Preventive Doping Research at German Sport University Cologne and Head of the European Monitoring Centre for Emerging Doping Agents. His key areas of research focus on the development of new detection procedures for analytical anti-doping tests (especially new agents and peptide hormones currently undergoing clinical trials).

Wilhelm Schänzer followed a first degree in sports and chemistry with a doctorate from the Institute of Biochemistry at German Sport University Cologne, completing his habilitation there in 1995. He was appointed Professor of Biochemistry and Head of the Institute of Biochemistry and its WADA-accredited doping control lab at German Sport University Cologne in 1997. His key areas of research focus involve the clarification of steroid metabolism and the identification of new long-term metabolites; improving analytical techniques for autologous doping-relevant substances such as erythropoietin (EPO), testosterone and related prohormones; and clarifying the structural properties of new designer substances.

players of the Mexican national squad (men's football) tested positive for the β_2 agonist [6]. This finding proved to be especially problematic, since the U-17 World Cup for junior football teams was scheduled to take place the following month. As a result, soccer governing body FIFA organised a comprehensive programme of food quality testing alongside routine doping controls during the tournament. All in all, athletes submitted 208 urine samples for doping control testing. The analytical findings revealed the presence of clenbuterol in 109 cases, with concentrations of up to 1500 pg/ml urine [7]. The food samples taken in parallel were tested in a suitably-equipped laboratory in the Netherlands, with 14 of the 47 samples containing – in some cases considerable – quantities of clenbuterol. Viewed as sufficient to account for the drug's presence in the doping control samples, no player was sanctioned for breaching the anti-doping rules. A breakdown of the samples by tournament location revealed no clear trend: the matches had been played in Guadalajara, Mexico City, Monterrey, Morelia, Pachuca, Querétaro and Torréon, with 8 to 36 players being tested at each location. Since some teams played matches at several locations, no conclusions can be drawn from urine samples about the place where contaminated food was eaten. One interesting aspect may be noted, however: of the 24 teams taking part, five teams returned consistently negative sets of doping control results. Of these, at least one had heeded the advisory notice and refrained from consuming meat for the entire competition.

Challenges for analytical testing

These examples clarify the analytical difficulties surrounding clenbuterol in the context of doping control testing. Accordingly, a number of approaches have been taken to generating analytical methods capable of differentiating between foodstuff contamination and previous (non-recent) deliberate consumption of the drug. One of the more recent strategies adopted relies on the fact – as mentioned above – that clenbuterol is a racemate (see Fig. 1) in its drug formulation. After administering clenbuterol, a pig study has shown that enrichment in edible tissue (e.g. muscle) is con-

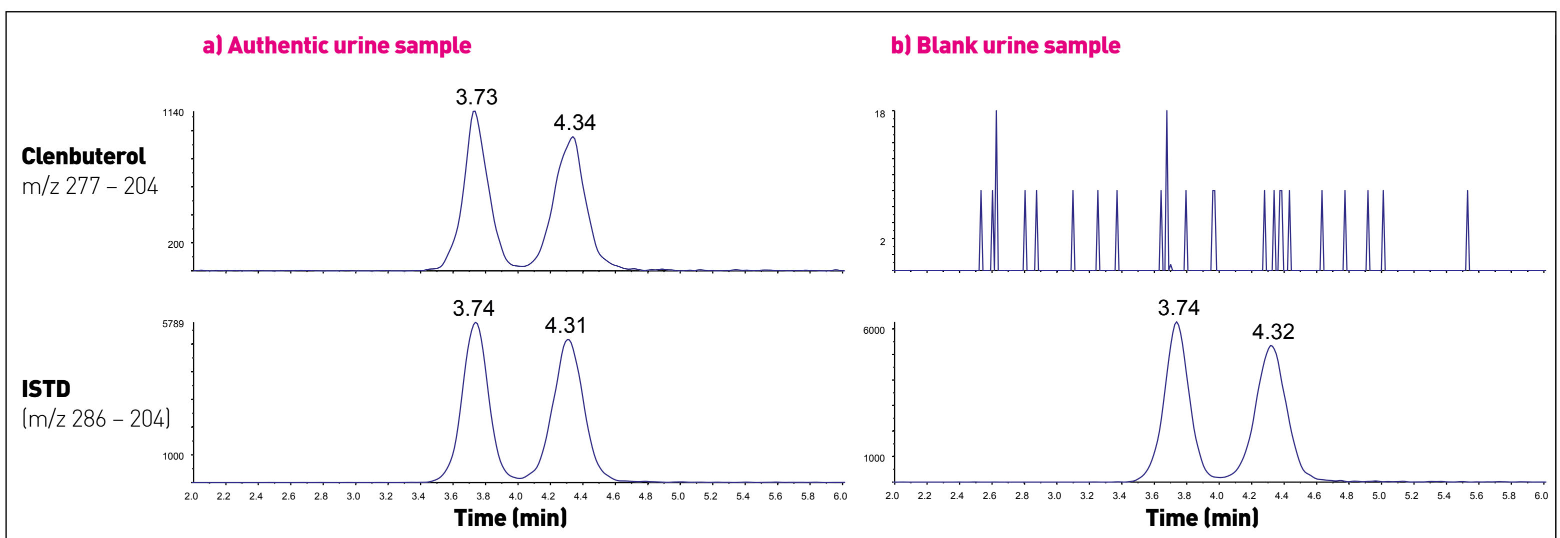


Fig. 3 Extracted-ion chromatogram of a urine sample with approx. 200 pg/ml clenbuterol (A) with separation of the (-) and (+) enantiomers at 3.7 and 4.3 min. The upper patterns represent clenbuterol, while the lower patterns depict the 9x(2)H-labelled internal standard, also present as a racemate. As a comparison, (B) shows a blank urine sample.

siderably higher for the therapeutically inactive (+) stereoisomer than for the (-) stereoisomer. Accordingly, once clenbuterol treatment is discontinued, a significant disparity in concentration between the two components will develop over time [8].

Enantiomer analysis offers new potential

This would mean that there is a functional difference between a drug product dose and food contamination: a difference that is detectable insofar as the (-) stereoisomer complement of clenbuterol has undergone sufficient depletion. To test this hypothesis, a method for enantiomer separation with subsequent isotope dilution analysis using LC-MS/MS was developed, with the aim of determining the enantiomeric ratio and thus potentially identifying either a case of therapeutic drug dosage or a case of contamination. Figure 3 presents a chromatogram of a urine sample with clenbuterol following enantiomer separation: baseline separation is clearly visible here. Two excretory studies with simple therapeutic doses of clenbuterol were conducted. Results were as expected: the (-)/(+) enantiomer ratio in urine never fell below 1 at any point during the 160 hours of testing. This is due to the fact that the (+) stereoisomer also exhibits elevated tissue retention in such cases, meaning that the (-) stereoisomer is excreted to a significantly greater degree [9]. Following the oral intake of a clenbuterol enantiomer mixture with the (-) stereoisomer component already depleted (as might occur in cases where contaminated meat is consumed), the ratio may show a decremented value that cannot be successfully reconciled with therapeutic administration of the substance. While the authors are not aware of clearance studies involving enantiomer-depleted clenbuterol mixtures, results from athletes' urine samples have nonetheless shown that (-)/(+) ratios significantly lower than 1 have been determined in a number of cases. In consideration of the fact that a value greater than 1 cannot prove doping took place, yet a value less than 1 is, on the basis of current scientific knowledge, inconsistent with the therapeutic administration of clenbuterol as a drug in humans, the determination of the enantiomer ratio for clenbuterol findings is certain to contribute some useful data. While other – potentially wide-ranging – studies are required to evaluate the potency of enantiomer analysis, one possible approach seems nonetheless practicable.

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An extra portion of zinc

Biofortification of vegetables with micronutrients

Prof. Dr Stephan Clemens
Department for Plant Physiology, University of Bayreuth

Powerful forearms, a pipe in the mouth, a sailor's hat: It takes him only seconds to open and empty the can of spinach. He faces his next brawl with superhuman strength. This is how we all know Popeye, the sailor. The secret of his strength is in the high iron content of spinach. This idea got innumerable parents to trying to get their children to "like" the rather unpopular vegetable. Unfortunately, some things are quite wrong here: While spinach is tasty and healthy, it is not a wonder drug. As in most plant tissues, its iron content is around 30 µg/g – and not 10 times as much, as it was often assumed because the dry and fresh weights were once swapped. However, it is perfectly accurate that iron gives strength. It is an essential micro nutrient.

However, the World Health Organisation WHO estimates that about half the global population is suffering from an iron undersupply [1]. A deficit like this is referred to as "hidden hunger" today, because the consequences are often not evident at once. Insufficient intake of essential minerals and vitamins can lead to severe health impairment and millions of years of life lost by sickness and premature death (DALYs = disability-adjusted life years in the language of epidemiology). Many people are suffering not just from iron deficits. The "Big Four" also include zinc, iodine and vitamin A. Estimates suggest that zinc deficiency threatens up to 2 Bn people. Figures for iodine are similar. Approx. 20% of the children in the world suffer from Vitamin A deficiency. Globally, iron and zinc deficits are on place 9 and 11 of the most frequent causes of death respectively [1].

Why are iron and zinc essential?

Nothing can live without these metals. Iron can be present in two oxidation states under physiological conditions and therefore has been recruited for innumerable redox reactions during evolution. Cellular respiration, for example, would be impossible without iron-sulphur proteins. Metals are indispensable for the interaction of proteins with small molecules as well. Most of the iron in our body is localised in the haemoglobin of the red blood cells, where it binds oxygen. An easy to diagnose consequence of iron deficit therefore is anaemia. The consequences if iron deficit include reduced mental and physical performance.

Zinc is part of approx. 9% of the proteins in eukaryotes [2]. Zinc is found in enzymes of all six classes as a catalytic co-factor, particularly frequently in hydrolases. Zinc is an important structure element particularly in DNA-binding regulatory proteins (zinc-finger motif). In spite of the diversity of biological functions of zinc, a deficit has been difficult to determine, in contrast to the situation with iron. The evidence for a deficit is mainly based on the positive effects of zinc addition to the food (= zinc supplementation). The results here are clear. Meta analyses document a significant reduction of diarrhoea (about 20%), pneumonia (about 15%) and mortality (about 18%) in children supplied with additional zinc [3]. A case study in the latest World Health Report of the WHO cites comparable figures for children under two in Bangladesh to whom 70 mg zinc per week were administrated [4]. The positive effect on the immune defence that can be derived from these findings has also been evidenced for older people. Both zinc and iron deficits in early childhood also result in stunted growth that cannot be balanced out even by sufficient supply in later development stages.

How can supply with iron and zinc be improved?

In the light of the severe consequences of "hidden hunger", improved supply with essential minerals has become an important goal around the world. A body of laureates of the Nobel Price for economy, evaluating possible solutions for the most urgent global welfare problems in the "Copenhagen Consensus", created a ranking with three measures for fighting "hidden hunger" among the first five positions in 2008. In 2012, this body assigned bundled measures to improve micro nutrient supply the highest priority (www.copenhagenconsensus.com). The food industry is also paying increasing attention to the "Big Four".

Supplementation, e.g., with zinc tablets or fortification of foods by addition of io-

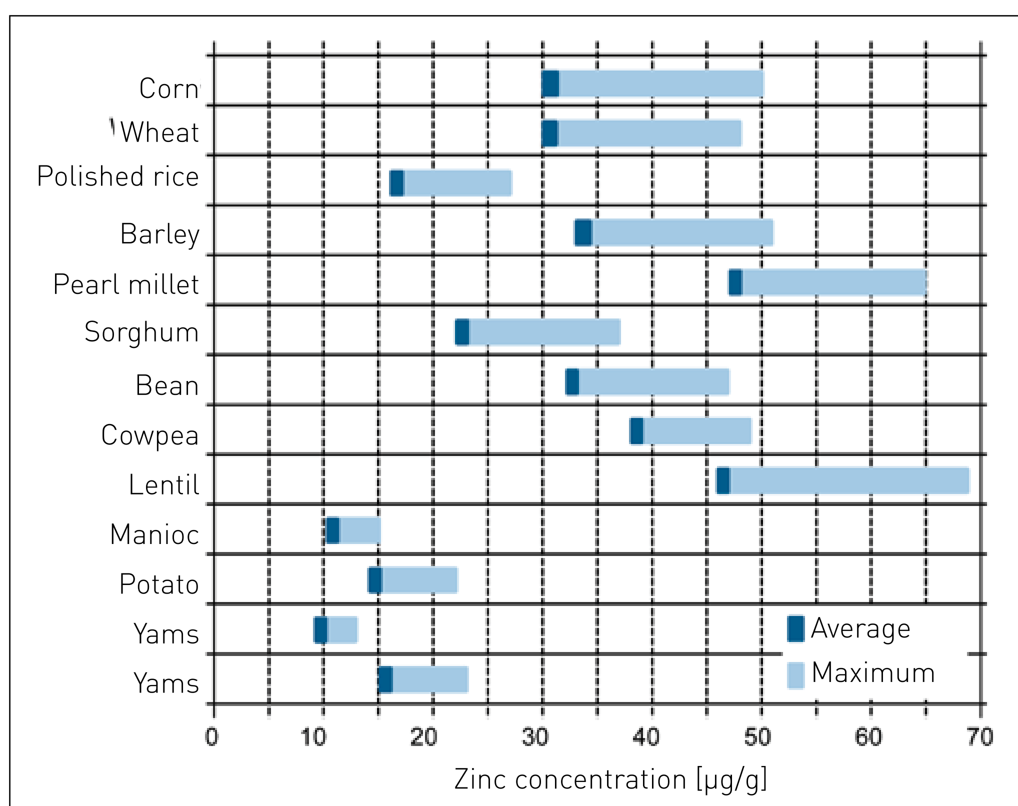

Fig. 1 Typical natural variation of the zinc contents of different plant foods. The averages and maximums from the HarvestPlus database according to Pfeiffer & McClafferty [2007] Crop Sci. are shown. 47, p. 88 – p. 105.

Fig. 2 The hyper-metal-accumulating plant *Arabidopsis halleri*. It grows in metal-polluted habitats as shown in this figure (strong zinc and lead contamination in the area of a zinc foundry) as well as uncontaminated soils. *A. halleri* is able to accumulate more than 10,000 and up to 50,000 µg of zinc/g dry weight anywhere. (I would like to thank Ricardo Stein, Romário Melo; University Bochum and Stephan Höreth; University Bayreuth, for the photograph).

dine and iron, can counteract deficiencies but requires an infrastructure such as working distribution paths. Additionally, specifically the locally produced agricultural products are the main food source in many regions of the world. Therefore, diversification of food and increase of micro nutrient content of the plant staple foods (= biofortification) would promise much more success in implementation and range. Increasing the diversity of their diet, e.g. by adding more meat and vegetables, however, often fails due to poverty.

Strategies of biofortification

For about ten years, the basics and implementation of biofortification have been subject to increasingly intense research. Some important activities are bundled in the HarvestPlus programme, which is essentially funded by the Gates Foundation (www.harvestplus.org). The goal is to increase the micro nutrient contents in the most important food plants to about two to three times the current values. "Hidden hunger" is expected to reduce significantly by this increase.

Generally, there are three strategies: Changing the growing practices, classic plant breeding and bioengineering The zinc content of grain seeds specifically can be increased by corresponding fertilisation. Like supplementation and fortification, this method requires the corresponding infrastructure and capital. Particularly in African countries, however, even fertilisation with the nutrients of nitrogen and phosphate, which are most important for quantity, is not common. Therefore, the most sustainable progress is expected by permitting development of micro-nutrient-rich varieties of the most important food plants and establishing them.

Natural Variation of the Micro Nutrient Content

Breeding of agricultural crops with a higher micro nutrient density requires the corresponding genetic potential, i.e. a natural variation of these properties. In fact, the zinc and iron contents in the consumed organs of some agricultural crops can differ by a factor of 2–4 (fig. 1). The first success is based on this range. A few weeks ago, for example, a rice type developed in the HarvestPlus programme with its zinc content raised by about 50% on average was introduced in Bangladesh (www.harvestplus.org/content/media).

Bioengineering approaches

Introduction of additional genes could achieve higher increases, is applicable even with species where classic breeding is virtually impossible (e.g. banana) or could even add new properties. The likely most prominent example for biofortification is the "Golden Rice" that was developed by scientists in Zürich and Freiburg. This rice synthesises provitamin A not only in its leaves but also in the starch-rich endosperm of the grain. Eating about 100 g of "Golden Rice" per day would cover 60% of the Vitamin A-demand of an 8-year old child. This could help millions of children. Nevertheless, the "Golden Rice" is not cultivated yet due to strong political resistance.

Zinc and iron biofortification is an even more complex biological problem than vitamin biosynthesis. Metal ions cross a long distance from the ground to the seeds. Their reactivity - the reason why they were recruited during evolution - requires a precisely regulated net-

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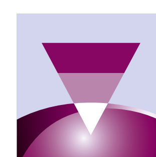
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Stephan Clemens, Jg. 1963, studied biology in Münster and Brighton, then acquired his doctorate in Münster. Since his postdoc-stay at the University of California San Diego, his scientific interest has been mainly targeted at metal homoeostasis in plants. He uses the models *Arabidopsis thaliana*, barley and *A. halleri*, a metal-hyper-accumulating plant, to examine the molecular mechanisms of metal transport and accumulation. As a group manager at the Leibniz-Institut für Pflanzenbiochemie, he qualified as a professor at the University of Halle-Wittenberg in 2003 . Since 2006, he has held the chair for plant physiology of the University of Bayreuth; since 2012, he has been managing the research office for food quality in Kulmbach as well.

work of transport and storage processes. Potentially harmful interactions with proteins and other cellular components must be suppressed by complex formation with designated ligands. At the same time, the metal ions in and between cells must be mobile. We do not have comprehensive understanding of how this metal homoeostasis works in detail in any organism. Some of the processes involved have been explained molecularly in the meantime. The examination of metal-hyper-accumulating plants that may acquire zinc concentrations in their leaves up to 1000 times as high as those of virtually any other organism is one successful research approach (fig. 2). The molecular analysis of this extreme property has, e.g., disclosed the key role of zinc-pumping P-type-ATPases and the metal-ligand nicotianamine for the long-distance transport of zinc from the roots to the leaves (fig. 3). Genetic engineering of the synthesis of nicotianamine in rice permitted the development of plants containing three times as much iron and zinc in their grains [7,8]. The changed expression of three genes for metal homeostasis in a rice species grown in Myanmar led to an increase of the iron content in the grain by 3.4 times. In Myanmar, 75% of the children and 71% of the pregnant women suffer from anaemia caused by iron deficiency [9].

Bioavailability of iron and zinc

In the end, the micro nutrient content in the plant products is not solely decisive for biofortification. Their bioavailability, i.e. the share that can actually be absorbed by the human digestive tract, is essential as well. The reactivity of iron and zinc ions mentioned permits highly stable complexes to be formed with food components such as phytate (Myo-Inositol-hexakisphosphate), which prevent absorption in intestinal epithelial cells. Only about 15–35% of the zinc and 5–15 % of the iron taken up in the food are absorbed by the body. The bandwidth is due to the influence of other food components and one reason for the benefits of larger variety in one's diet. For example, ascorbic acid supports iron intake, because it reduces the less-available Fe(III) to better-available Fe(II). Again, breeding and bioengineering can help. Low-phytate-species of some cultural crops have been developed. However, reduction of the phosphate storer phytate has often coincided with yield reductions. The above genetically engineered rice with increased nicotianamine synthesis can highly effectively balance out the iron deficit of mice suffering from anaemia and thus evidently provides bioavailable iron [7].

It can be expected that our molecular understanding of metal homoeostasis in plants will increase quickly over the next years, leading to growing potentials for biofortification by breeding and bioengineering. It remains to be hoped that we as a society will learn to assess crops according to their properties instead of according to how they are developed as quickly as possible. Then we should take the first step and at least admit those genetically engineered species that only use variations available in their natural gene pool. These are types where only genes from the same species or a closely related, sexually compatible species, have been transferred (="Cisgenesis").

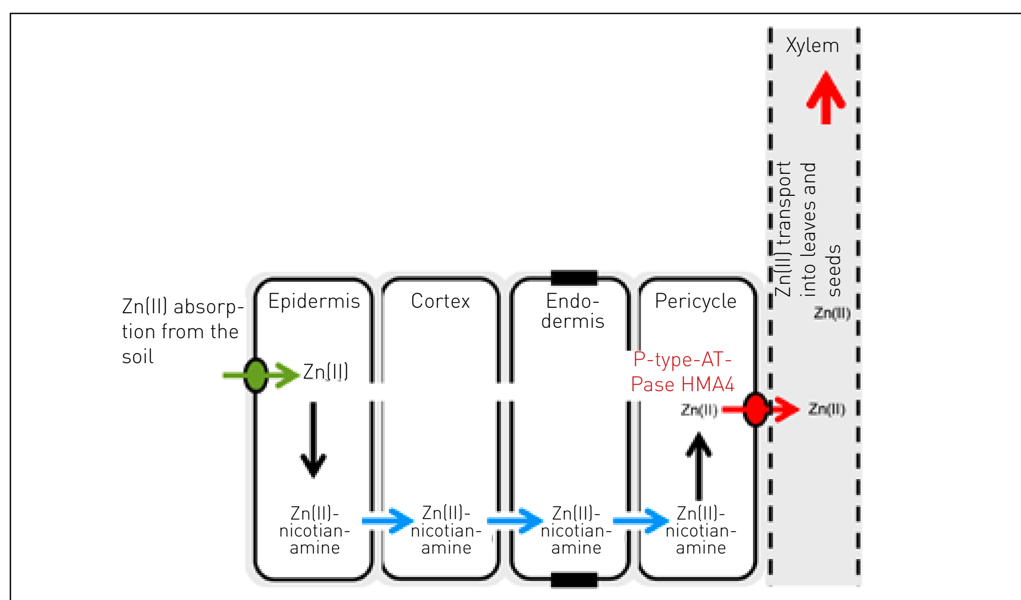

Fig. 3 Mechanisms of the long-distance transport of zinc from the roots to leaves and seeds This simplified chart shows cell types of the roots and the xylem as part of the transporting tissue. The absorption of zinc ions from the soil solution through the plant membrane is conveyed by specialist transporters. The formation of complexes of the metal ligand nicotianamine with Zn(II) ensures the mobility of Zn(II) in the cell-cell transport. Zn(II) is finally pumped to the xylem by P-type-ATPases such as HMA4 and can thus reach the above-ground organs.

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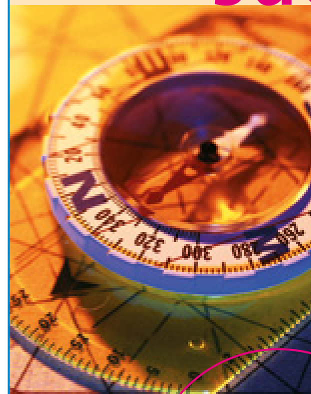

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Non-invasive cancer diagnosis

A new application for microscopy

Cancer has been a part of human life from our earliest days. In research conducted by Aachen-based oncologist Dr Leo Habets, a non-invasive, microscope-based diagnostic procedure has the potential to revolutionise research and progress in understanding the circumstances of the disease, so as to develop new therapeutic approaches. Microscope-based high-content screening systems in basic clinical research: can a non-invasive procedure replace the biopsy?

A 2,000-year quest

Hippocrates, the founding father of modern medicine, described growths whose blood vessels spread in all directions and were arranged like the legs of a crab. Once translated to the Latin word for crab, "cancer", the disease now had its modern name. Yet progress was slow for both diagnosis and treatment. For two long millennia, cancer was believed to result from a surfeit of "black bile" (from the Greek "melancholia"). In the absence of any empirical data, metaphysics long reigned supreme. Today, while "black bile" won't be found in any diagnosis, many of the mechanisms by which cancer cells develop and propagate still remain shrouded in mystery. As one example, researchers have seen how cancer patients will initially respond well to modern monoclonal antibody treatments, yet some patients then suffer recurrences, relapses with new metastases, even though the disease appeared to be already beaten. Why – and how – do these new metastases occur? Are there warning signs or early indicators that hint at the danger?

Empirical basic clinical research

Aachen-based oncologist Dr Leo Habets has been studying these questions, examining samples from cancer patients on a day-to-day basis in his lab in Aachen, supported by funding from METARES – the "Society for the Promotion of Cancer Diagnosis and Treatment at the Micrometastasis Stage". His work focuses on monitoring the success of treatment regimens for breast cancer patients. The approach he follows is empirical basic clinical research, which identifies indicators for metastases, as well as their propagation routes and mechanisms. "Our lab has studied samples from around 16,000 cancer patients," explains Dr Habets. "And we add 16 new datasets every day, on average." Even to the untrained eye, Habets' workplace would seem rather unusual, since refrigerators full of tissue samples – the predominant

feature of this kind of lab – are entirely absent. Apart from blood analyser units, only a few optical microscopes meet the eye. "What you see here," he says, pointing to a microwell plate on a microscope's specimen stage, "is the modern approach to tissue sampling: 20 millilitres of patient blood."

Biopsy as standard – "liquid biopsy" as the alternative

Previously, cancer diagnosticians were reliant on the biopsy, i.e. the surgical removal and analysis of tissue from a patient. Nor is it merely the case that the operation is complex, expensive and fairly unpleasant for the patient concerned. The biopsy itself is now suspected of propagating tumour cells in the body. Blood tests certainly seem to indicate that the blood tumour cell count rises after tissue piercing for biopsies. A less drastic method therefore seems to be called for. "With our procedure, we can largely eliminate patient stress," states Dr Habets. "Instead of surgery, patients merely need to have blood drawn – and we need just 20 millilitres."

"Liquid biopsy" is what researchers are calling this new diagnostic method, which has the potential to revolutionise cancer diagnosis. "The procedure offers advantages at all stages of cancer diagnosis and treatment monitoring," says Dr Habets. "We only have to look in the microscope to see whether or not a treatment is working. Non-invasive diagnosis even makes it possible to test drugs against patient blood before administering them. This enables the identification of potentially effective substances without inconveniencing the patient."

The dangers of circulating epithelial tumour cells

"The method we use was first developed by Professor Katarina Pachmann at the University of Jena," explains Dr Habets. "Her Maintrac procedure is extremely effective at identifying circulating epithelial tumour cells (CETCs) in blood. Their proliferation in blood is a clear warning sign. The underlying hardware is a Scan^R screening station from Olympus, running Scan^R software for data acquisition and analysis, which was specially developed by Olympus for this procedure. We use fluorescent dyes to label the cell types and cells of interest to us with antibodies."

Dr Habets, oncologist, Aachen, Germany.

The differentiated display of the fluorescent dyes is a major advantage offered by the system. Cells that display all dyes as spots of colour are of particular interest. As one example, Figure 1 shows CETCs that exhibit specific oncogene characteristics due to the HER2 and EpCAM markers. Habets explains: "As you can see, some cells take up the fluorescent dye and show saturation over a wide area, while some have only spots of colour. Others have diffuse colouring or lack colours. As our data repository grows, we constantly improve the precision of our statements about the correlation between the occurrence of certain cell types and antibodies with cancer treatments and disease progression. All of the data are analysed in software, stored as image files and accessible from the database for later comparative studies."

Scan^R: a modular high-content screening solution

The Scan^R system as deployed by Dr Habets combines the modular and flexible approach of a microscope-based system with ability to meet the requirements of screening applications in terms of automation, speed, throughput, reliability and reproducibility (Fig. 2). The system is equally capable of handling both fixed and living cells. This makes the system – developed by Olympus in close collaboration with the European Molecular Biology Laboratory (EMBL) – suitable for use in a wide range of high-content screening applications. Results achieved include multiparameters and functional data about the interaction of a substance with the target or other cell components – such as absorption, permeability, selectivity, specificity or substance metabolism. Dr Habets: "The entire system is managed via a user interface which is for the most part self-explanatory. Users have access to all of the image acquisition and image analysis parameters." The images are acquired with multiple dimensions (X, Y, Z, t, λ), which can be evaluated subsequently with the aid of the integrated analysis system. "The options available to users in

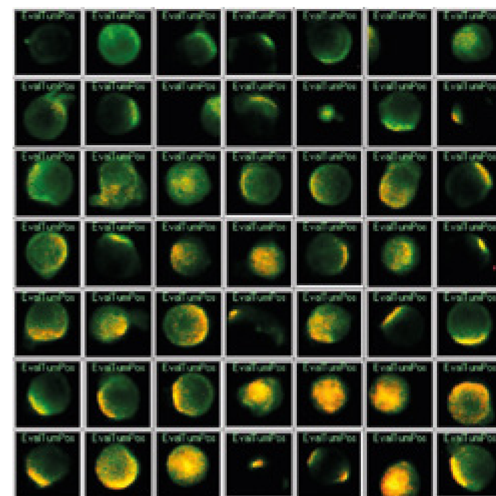

Fig. 1 Maintrac procedure for cancer cell profiling with the Olympus Scan^R system. Detection of oncogenic molecular labels HER2 (orange) on EpCAM-positive cells (green).

terms of image and data analysis are effectively unlimited," says Habets. The procedural approach taken by the Scan^R system's analysis module is oriented on cytometry, which enables the analysis of a large volume of multidimensional datasets. This sort of analysis is applied in infection biology, for example, to gain new insights in relation to infectious diseases. As two examples, the Scan^R system has previously been used in work studying Chlamydia bacteria and gastrointestinal viruses. While Chlamydia bacteria predominantly affect Africa – where they can cause blindness in many cases – gastrointestinal viruses are also widespread in Europe. Across Europe, 50% of its inhabitants are host to such viruses, which are suspected of causing cancer. Other fields of application for the system include diabetes research and the investigation of disorders such as coronary heart disease or atherosclerosis – each at the molecular level. Or – as in the case of Dr Habets' work – cancer.

Fig. 2 Olympus Scan^R high-content screening system. The modular all-in-one solution forms the basis for the Maintrac procedure deployed.

Extraction guaranteed

The Olympus system currently records up to five fluorescent tumour markers in parallel per scan. For each patient, this means some 10,000 blood cells are recorded at maximum resolution using specialised auto-focus routines. Such a precise quantitative analysis is ultimately dependent on the use of a highly stabilised fluorescent light source. The analysis software setup is based on the scatter plot approach used in flow cytometry, and enables the extraction of cell populations on the basis of parameters related to both intensity and morphological features. From those 10,000 blood cells, this enables the detection of a tumour-relevant population of as few as 1–50 cells, which can be displayed directly as an image gallery. Importantly, the Scan^R software offers bidirectional linking between the data point and the individual image of the cell in question, which enables interactive assessment and manipulation of the analysis.

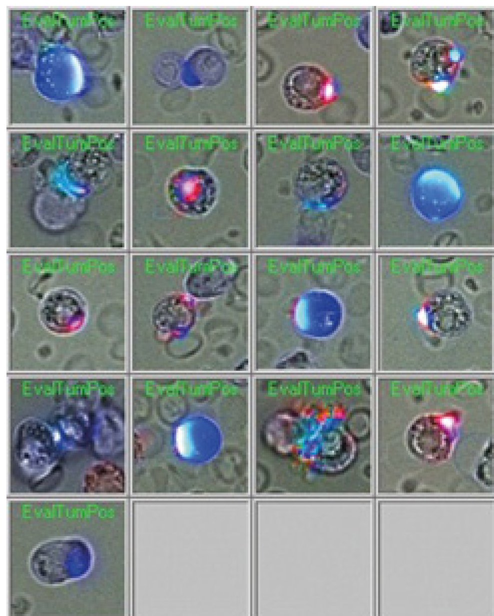

Fig. 3 Visualisation of metastasis risk indicators with the aid of the Maintrac procedure. Detection of CD44 antigen markets (blue) on CETC with the aid of fluorescently labelled antibodies.

Vast volumes of data

The datasets involved are tremendous: every month, the research lab fills a terabyte-sized hard drive with its data. "We'd be lost without software-driven data management," confesses Dr Habets. The fees charged for testing are moderate, however: "Our going rate for the identification of three antigens per patient is about 10 euros. That's incomparably cheaper than similar procedures." For the moment, analysis work with the Scan^R system is confined exclusively to basic research: in the future, it could help to develop or establish diagnostic procedures.

New insights, new questions

Within oncology, there is growing demand for affordable methods in basic clinical research. For every new insight, new questions arise. Tumour cells are usually epithelial cells, i.e. cells from the surface layers of tissue complexes. If cells taken from a breast cancer patient after "successful" treatment for cancer can be shown to be circulating epithelial tumour cells (CETCs), for example, this indicates the cells have survived the therapeutic bombardment unharmed in a kind of "hibernation". Alongside this survival, it's also a mystery why they are in the blood system in the first place. As epithelial cells, their path into this system should technically be blocked. "In a sense, they change their 'identity'," explains Dr Habets. "They undergo epithelial–mesenchymal transition (EMT), penetrate the blood vessels, roam about the body and can then metastasise. The Maintrac procedure gives us a reliable technique for identifying them in blood (fig. 3). Our next task is to explore the treatment options that are most effective in hampering EMT."

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Figs. 1 and 3 with kind permission of Dr Leo Habets.
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R-Biopharm offers different test systems for the analysis of antibiotic residues in food and feed. While Premi®Test allows a fast and easy qualitative screening for a broad spectrum of antibiotics; RIDASCREEN® ELISAs enable quantitative and specific screening of foodstuff for antibiotic residues. Additionally, EASI-EXTRACT® and RIDA® immunoaffinity columns allow sample clean-up of chloramphenicol from complex matrices such as honey, wine, enzymes and seafood prior to analysis with ELISA or LC/MS.

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Precise dosing for fully automatic CMC measurements At analytica 2014, KRÜSS GmbH will present the Micro Dispenser, a dosing unit for fully automatic measurements of the critical Micelle concentration (CMC). The Hamburg based manufacturer of measuring instruments has developed the Micro Dispenser especially for the Force Tensiometer K100, which has taken up an established position in the market for some time now. Two combined Micro Dispenser units create the whole concentration series for the surfactant to be analyzed directly inside the measuring vessel of the tensiometer. The K100 measures the surface tension between the respective dosing steps fully automatic by using the ring, plate or rod method. By software-controlled dosing and subsequent removing the added volume between measurements, a vast number of concentrations can be analyzed without being limited by the vessel size.

www.kruss.de

Eppendorf's new Mastercycler Nexus X2 Eppendorf has launched the latest offering in its range of molecular biology instruments. The new Mastercycler nexus X2 is ideal for researchers looking to carry out two runs of PCR simultaneously, without any compromise on the number of samples. The instrument comprises of two asymmetric blocks, consisting of 64 and 32 wells, which can be programmed and run completely independently, enabling two separate PCR protocols to be run in parallel. With reduced noise emission (< 40 dB), low power consumption and a small, well-designed footprint, the Mastercycler nexus X2 is perfectly suited for use in busy academic laboratories, or any institution with multiple users or large research groups. In comparison to other dual block cyclers, it provides an elegant solution for users wishing to run procedures using a large number of samples, without taking up a large amount of bench space. As the latest addition to the popular Mastercycler range, the Mastercycler nexus X2 continues Eppendorf's legacy of exceptional design and ease of use combined with efficiency and accuracy.

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Smallest spectrometer in the world Hamamatsu Photonics K.K. has developed the world's smallest "fingertip size" micro-spectrometer, an ultra-compact, lightweight and low-cost device which, according to Hamamatsu's research, provides nearly the same performance as Hamamatsu's MS series mini-spectrometer while being roughly half its size. The new C12666MA micro-spectrometer offers measurement in the visible wavelength range, and can be used for applications such as colour sensing in printing machines and LED lighting, point-of-care testing connected to smartphones, and other types of portable measurement. Samples of the C12666MA will be available to manufacturers from the 25th December 2013.

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House lights down, stage lights up

Pharmaceutical counterion determination

Dr Frank Steiner, Dr Carsten Paul, Dr Mark Tracy
Thermo Fisher Scientific, Germering and Sunnyvale/CA

Fig. 1 Schematic presentation of cation and anion functional groups in Thermo Scientific™ Acclaim™ Trinity P1 mixed-mode columns.

In contemporary practice, roughly half of all active pharmaceutical ingredients (APIs) are administered as salts. The use of the protonated or deprotonated form of the drug substance combined with the selection of counterions enables the targeted variation of key parameters, such as solubility and stability. Analysis of the corresponding counterions constitutes an essential part of the development process for new pharmaceuticals and is now an indispensable procedure within the quality control process for these products.

Increasingly sophisticated techniques – such as combinatorial synthesis – are now being applied in the search for new APIs. Apart from modern chromatographic approaches such as ion chromatography and HPLC, counterion analysis has been tended to be left in the shadows to date. Often, the methods and techniques deployed are not particularly powerful. By optimising separation conditions and detection technology, HPLC offers a modern, high-output alternative: the result is automated counterion analysis that is more efficient and more powerful – reflecting the state of current technology standards.

Contemporary methods of counterion determination

In the pharmaceutical lab, we principally find potentiometric titration or ion chromatography (IC) used for counterion determination. Titration is both time-consuming and labour-intensive. While ion-exchange chromatography can analyse multiple anions or cations within one measurement, IC is nonetheless unable to analyse anions and cations simultaneously on a single stationary phase. Examples of highly-specific methods for cation analysis include spectroscopic methods such as inductively coupled plasma optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS). Yet both of these methods are limited to a narrow range of applications while also being expensive to implement. All in all, none of the methods mentioned offers the option of analysing a series of ions with different charges during a single analysis run. Ion-exchange chromatography is certainly a powerful form of liquid chromatography that offers detection functions specific to anion or cation analysis and enjoys widespread deployment. Yet modern pharmaceutical formulations are now exhibiting increasing complexity. This complexity increases further if we then include the field of biopharmaceutics. If both cations and anions are present in a single sample, then at least two separation setups will be necessary, and analysis on the basis of IC is correspondingly more complicated. Pharmaceutical counterion analysis tends to involve the analysis of more complex samples, requiring a methodology that is both flexible and straightforward.

LC separation techniques for the simultaneous analysis of anions, cations and other compounds

The potential of LC methods to separate out substances depends on the selective interaction of analyte molecules with the stationary phase, i.e. it results from their "selectivity". As a separation mechanism, ion-exchange chromatography is unrivalled in the separation of small ionic substances. It depends on the selective electrostatic interaction of charged analytes with functional groups bearing an opposing charge on the surface of the stationary phase. Alongside primary separation mechanisms, secondary interactions also play a decisive role in selectivity for all LC separation techniques. In the

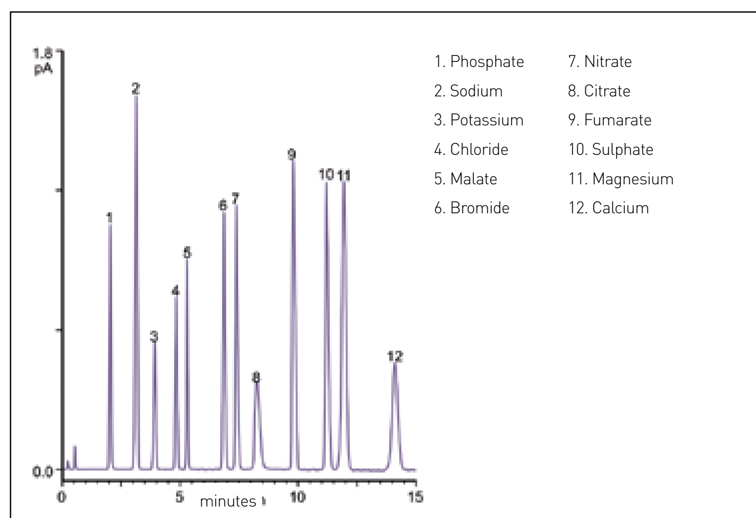

Fig. 2 Separation of anions and cations commonly used as pharmaceutical counterions using an Acclaim Trinity P2 column and detection with a Thermo Scientific™ Dionex™ Corona™ Veo™ RS charged aerosol detector.

development of novel phases for LC, increasing attention is now being paid to the targeted application of multiple and distinct – yet virtually equivalent – mechanisms. These are "mixed-mode" phases. These mechanisms are termed "mixed-mode" phases. The first columns of this type combined reversed-phase (RP) mechanisms with cation or anion exchange. Although these phases provided novel kinds of selectivity while also permitting the simultaneous separation of ionic and neutral substances, they did not allow the simultaneous retention of hydrophilic cations and anions. The inclusion of functional groups for both cations and anions on a single phase is effective only if physical separation of both groups prevents their reciprocal deactivation as a result of internal salt formation. Thanks to an ingenious system of synthesis, this challenge was first overcome by the use of "trimodal" phases: the structure of such phases is illustrated by the diagram given in Figure 1. These innovative mixed-mode phases combine anion and cation exchange with the reversed-phase mechanism of separation or with hydrophilic interaction liquid chromatography (HILIC), for example. Nor do these trimodal phases merely permit the simultaneous analysis of positively- and negatively-charged ions: for most formulations in the traditional world of small-molecule pharmaceuticals, these systems also enable simultaneous determination of the active substance.

Charged aerosol detection: it's universal

Conventional detection procedures are unsuitable for exploiting the benefits offered by the trimodal stationary phases described above, however; since the vast majority of typical drug substance counterions lack chromophores, this rules out the use of UV detection. While conductivity detection is in fact suitable for such counterions, neither the variant without suppression of background

Frank Steiner graduated in chemistry before obtaining his doctorate in 1995 from Saarland University in Saarbrücken. This was followed by a postdoc position at the Centre d'Études Nucléaires de Saclay in France, where he worked on elemental analysis and isotope analysis with IC and IC-ICP/MS. He then returned to Saarland University, where he completed his habilitation in 2003. After a two-year period of research and teaching, he accepted in 2005 a position as LC Systems Manager at Dionex Softron GmbH, a part of Thermo Fisher Scientific.

Carsten Paul studied chemistry (focus environmental chemistry) at Friedrich Schiller University (FSU) Jena from 2004 to 2008. After a short spell at the Scripps Institute for Oceanography (La Jolla/CA, USA) he completed his doctorate on a scholarship from the Jena School for Microbial Communication at the Dept. of Instrumental Analytics (also FSU Jena) from 2008 to 2012. Since 2012, he has been working as a solutions specialist for liquid chromatography applications at Dionex Softron GmbH, a part of Thermo Fisher Scientific.

Mark Tracy graduated in chemistry from the University of California, Davis, obtaining his Ph.D. there in 1984. He then worked as a chemist in the U.S. Air Force and at the California Animal Health and Food Safety (CAHFS) Laboratory System and Pickering Laboratories, Inc. In 2001, he began working for Dionex – since 2011 Thermo Fisher Scientific – in Sunnyvale, California, where he works in the Applications Development and New Column Development unit.

conductivity nor the use of a suppressor system enables the direct simultaneous detection of anions and cations. At this point, one detection system can fully realise its potential, a system generally held to be a truly universal detection procedure within LC: charged aerosol detection (CAD). CAD begins with the nebulisation of the mobile phase, followed by the generation of a dry aerosol and the subsequent adsorption of ionised nitrogen onto the dried particle surface. In a final step, this charge is then measured within an electrometer. With full coverage of aerosol particles, this charge is proportional to the surface and is thus – as a first approximation – to its mass. This principle of measurement supplies truly universal detection proportional to the mass flow, capable of detecting both organic and inorganic anions/cations as well as neutral molecules. Therefore, in principle it is possible to detect all non-volatile or semi-volatile components of a pharmaceutical formulation.

An end-to-end counterion determination solution in modern pharmaceutical analysis and its applications

It is self-evident that the combination of trimodal mixed-mode phases and charged aerosol detection provides the technical tools for a solution of this nature. The final touches to this technical solution are provided by the corresponding pre-programmed analysis setups (e-workflows) in the chromatography data system, which smooth the way to the result and analysis report with minimal training. The result is an all-in-one solution encompassing all of the key components and data, enabling even operators inexperienced in using LC to get off to a real "flying start". Figure 2 shows the deployment of this complete LC solution for the analysis of pharmaceutical counterions. During development of the stationary phase as utilised here, particular attention was paid to the simultaneous analysis of uni-/multivalent anions and cations.

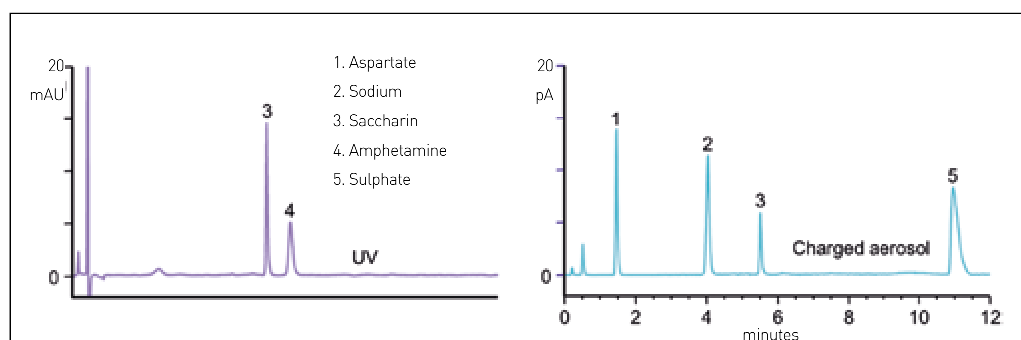

Fig. 3 Chromatogram of the primary components of the drug Adderall® (Shire Pharmaceuticals), a formulation for the treatment of attention deficit disorder; saccharin and amphetamine are detectable using UV detection, aspartate, sodium and sulphate via charged aerosol detection.

This apparatus makes it possible to separate and detect both inorganic and organic anions as well as univalent and bivalent cations within a single measurement. Overall, this makes it a simple matter to analyse twelve pharmaceutically relevant counterions within one gradient run in 15 minutes.

At this juncture, it should once again be emphasised that the proposed method not only facilitates the simultaneous screening of multiple counterions but also makes it possible to analyse the API. Depending on the third retention mechanism preferred, operators have a choice of trimodal reversed-phase columns or HILIC retention.

The proposed solution, consisting of a trimodal stationary phase and charged aerosol detection, is simple and straightforward to expand without forfeiting productivity. Figure 3 shows the separation of the primary components of a complete formulation. Note that this requires the analysis of volatile components, which exceeds the capabilities of the charged aerosol detector. Aspartate, sodium and sulphate are detected using the Corona Veo detector; for highly volatile amphetamine, a UV detector is used, connected in series. This approach ensures that an even greater spectrum of substances can be analysed with equal speed and simplicity.

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For an in-depth illustration of the principle of operation for a charged aerosol detector, please visit www.thermoscientific.com/veo.

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Saved on safety

Modern safety cabinets can recognise usage situations and react to them

The energy price increases and so do the operating costs for technical equipment. In the last five years alone, the costs for a kilowatt hour in the industrial area has increased by more than 30% [1]. "Reducing power" or "Switching off if there is no demand" could be some answers to this development. However, this is frequently not possible. Technical devices intended to protect persons and products must provide sufficient safety reserves in any situation.

Safety cabinets are part of this category of protection devices. As miniature containments, they permit handling of sensitive materials in a highly clean environment. At the same time, they prevent particular toxic substances from impairing the health of staff [2].

Protection through air

The protection performance of a safety cabinet essentially depends on its electrically generated air flows. Sufficiently high air flow velocities ensure that airborne particles are removed from hazardous areas and are separated in highly efficient HEPA filters. While contaminations from the environment are kept away from the materials by the downflow, the inflow prevents particles from escaping from the working space (fig. 1). On the other hand, a too high inflow may carry foreign substances into the working area, while a too-strong downflow will convey particles into the environment. A balanced ratio between both air flow velocities is, therefore, required for an effective separation between the clean, inner zone and the unclean environment [3]. Minimum values for the flow velocities are given by relevant standards [4, 5]. These standards are the basis for the assessment of conformity by which the manufacturer confirms that the prescribed minimum requirements are met (CE marking). In concrete terms, this means that the manufacturer specifies defined flow values to warrant sufficiently safe operation of the cabinet ("nominal set point").

Protection in handling

These are the framework conditions. What about the settings under actual lab conditions, though? Most safety cabinets are used according to the specifications at the nominal set point, but it is tempting to reduce the fan output with regard to energy consumption. As a consequence, not only the flow speeds drop, the flow ratio shifts as well. Whether and how such changes affect the protective performance of a safety cabinet can be best determined using a graphical representation in the form of a "performance envelope" [6]. This technical term designates an area of inflow-downflow combinations in which the standard tests for person and product protection were verifiably passed (fig.2). If a combination of inflow and downflow velocities is outside the envelope, the required protection cannot be ensured. Combinations near the limits offer only a limited safety reserve either. Therefore, the goal should be to operate the cabinet far within the safe range. The examinations for drawing up such a chart are, however, elaborate, as every value pair conceals comprehensive microbiological examinations. Inspections for generating a "performance envelope" chart are not yet part of the German and European standards but should be considered in future versions.

Protection with reason

A reduced safety reserve as a consequence of lowered air flows becomes evident when personnel activities are simulated in the environment of a safety cabinet. Tests with reproducible, static and dynamic disturbances that imitate the influence of a sitting, standing and moving person (fig. 3) clearly show

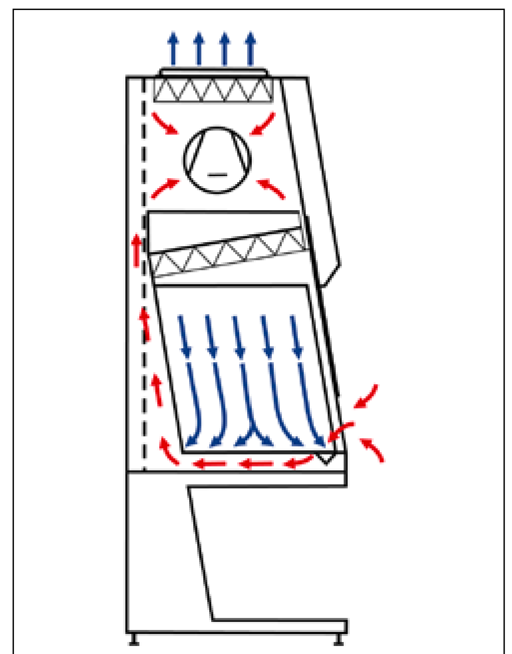

Fig. 1 Air flows in a safety workbench By now, high-resolution sensor systems permit recording what is happening before the workbench and assessing it. Most of the filtered, clean air (blue arrows) is returned to the working space with the displacement flow.

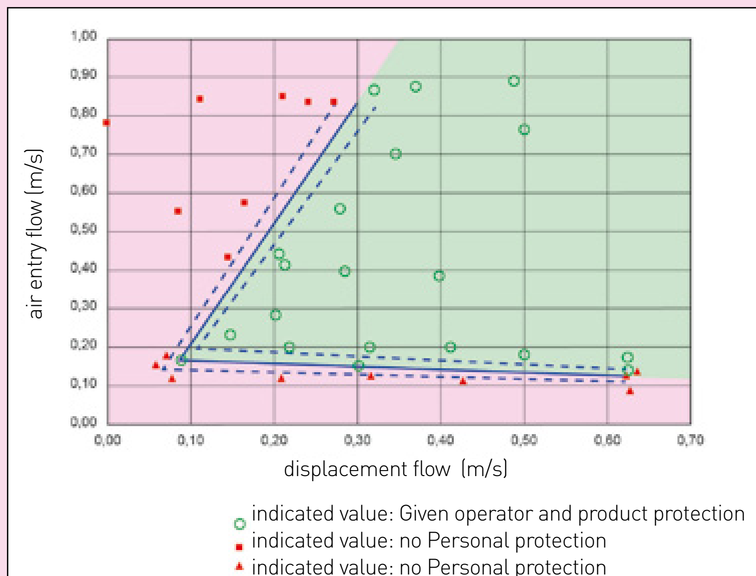

Fig. 2 Illustration of the interrelationship of the air entry and displacement flow in the form of a "Performance Envelope" chart The values within the "change" (green area) correspond to a safe workbench setting regarding protection of persons and products.

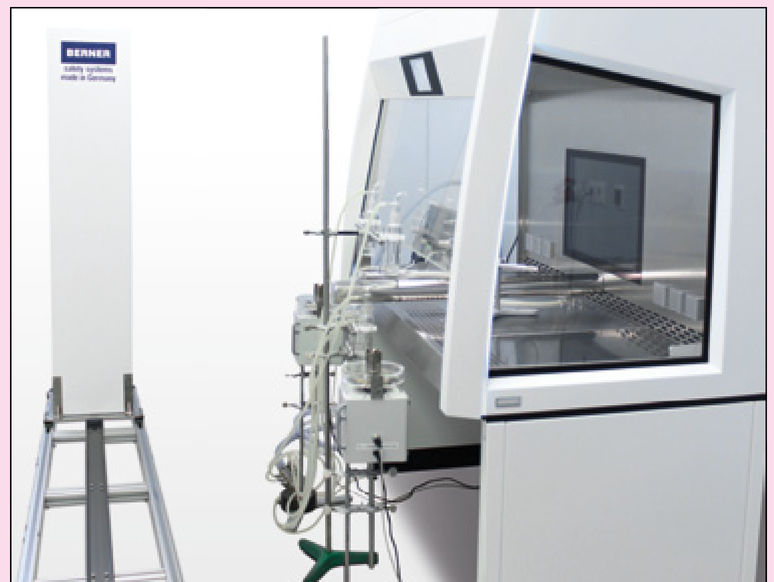

Fig. 3 Example for an artificial interference value for simulation of dynamic influences on the protection potential of a safety workbench Personal activities can be reproducibly simulated and evaluated with a moving plate.

that only sufficiently high and balanced air flow values permit successful compensation of outer interferences. Mainly fast personnel movements near the front aperture endanger the barrier function. Thus, the air flows can only be reduced by 24 % on average as compared to the nominal set point values before particles "break out" from the inside of the cabinet (in the unimpaired condition: 64 %) [7]. Flow impairment thus poses high demands to the safety system, but cannot be avoided in regular lab operation. Therefore, it is all the more important that the safety cabinet has enough safety reserves in form of a sufficiently high, balanced and standard-compliant ventilation setting.

In order to use scarce resources effectively, minimizing energy consumption is an important goal in safety cabinet development. However, in order to ensure safe working in any situation this objective should not be achieved by changing the operating parameters, but by using innovative components and smart control systems. By now, high-resolution sensor systems permit recording of what is happening in front of the cabinet and assessing it. In phases of low work activity, the power consumption can be reduced to a resource-protecting level. Power can be saved with safety workbenches – reasonably and not to the detriment of safety.

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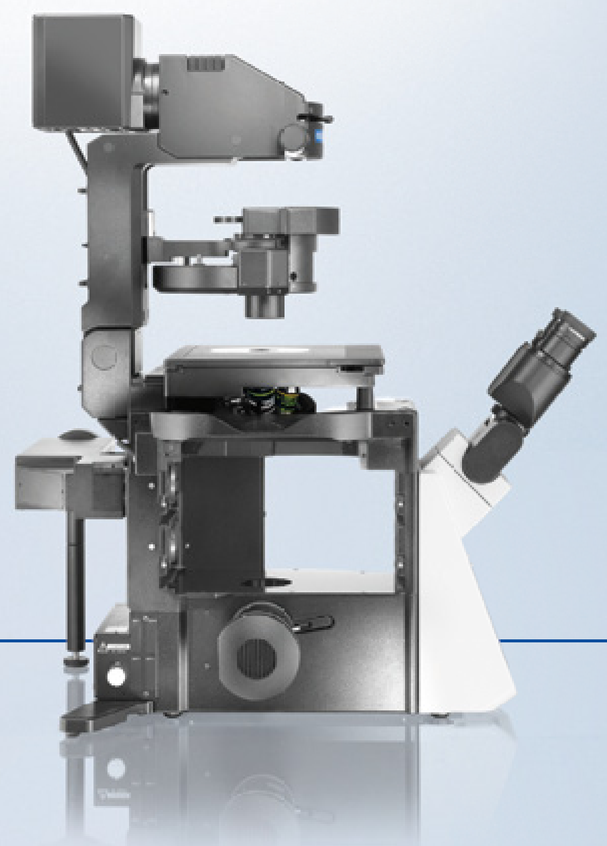

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