TOSOH

TOSOH
THE CUSTOMER
MAGAZINE

NO. #01 2015

HIC / HILIC /HPLC

TOSOH BIOSCIENCE

02
EDITORIAL
DEAR READER

Dear reader, welcome to the first issue of the Tosoh Bioscience customer magazine in 2015. The motto of this issue is HIC/HILIC/HPLC. It is featuring our big conference event, the HIC/RPC Bioseparation Conference, which was held this spring in Malta. New products and a co-operation project are related to the HILIC mode. We visited the lab of the Department of Drug Sciences at the University of Pavia, one of the oldest universities in Europe. Our new addition to the TSKgel Amide-80 HILIC product line, small particle UHPLC columns, will be presented at the HPLC 2015 Conference in June in beautiful Geneva. We are looking forward to meeting some of you there to share impressions of the conference and discuss challenging UHPLC applications.

ENJOY READING AND STAY INFORMED.

REGINA ROEMLING I MARKETING MANAGER
TOSOH BIOSCIENCE GMBH

CONTENT

IMPRESSUM

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03 WHAT'S NEW COLUMNS

TSKgel AMIDE-80 HILIC COLUMNS FOR UHPLC USE

TSKgel AMIDE-80 COLUMNS PACKED WITH 2 µM SILICA BASED PARTICLES ARE THE LATEST ADDITION TO THE WELL-KNOWN TSKgel AMIDE-80 SERIES. THE AMIDE STATIONARY PHASE PROVIDES A UNIQUE SELECTIVITY UNDER REGULAR NORMAL PHASE CONDITIONS OR IN THE HYDROPHILIC INTERACTION (HILIC) MODE OF CHROMATOGRAPHY.

For years, TSKgel Amide-80 columns are used successfully for HILIC separations of polar compounds such as saccharides, glycans, oligosaccharides or peptides, documented in more than 250 scientific publications. In parallel to the growth of the biotherapeutics market the use of amide-phases for glycosylation analysis steadily increased.

Packed with spherical silica particles that are covalently bonded with non-ionic carbamoyl groups, TSKgel Amide-80 provides higher stability than conventional amino-phases and a unique selectivity. It shows higher retention of polar compounds than other Amide columns on the market. Figure 1 shows the characterization of the new 2 µm version of TSKgel Amide-80 (red line) compared to the renowned 3 µm Amide-80 (colored area) based on the system proposed by Y. Kawachi et al. (J. Chromatogr. A, 1218 (2011) 5903 ff).

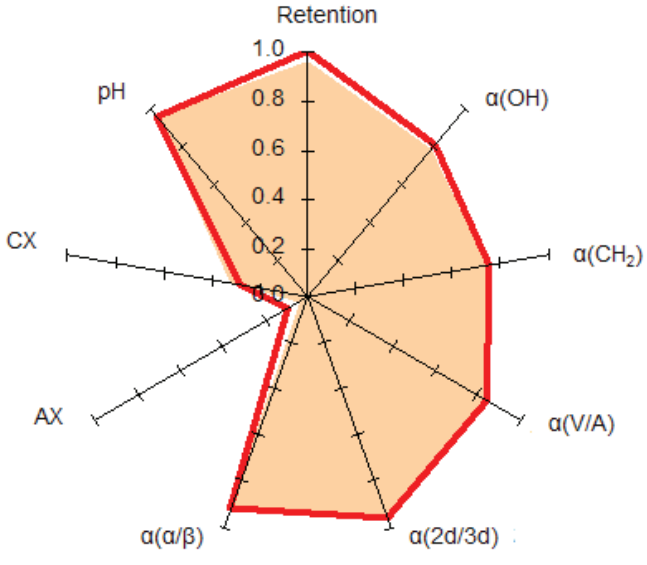


FIGURE 1: **SELECTIVITY PROFILE OF TSKgel AMIDE-80**

Figure 2 shows a typical analysis of labelled N-glycans on the new UHPLC column compared to the traditional 3 mm TSKgel Amide-80. The reduced particle size considerably increases theoretical plates and resolution. A 40 percent increase in resolution can be achieved when using the same method with the 2 µm material. The number of theoretical plates is increased by more than 60 percent.

The new 2 µm TSKgel Amide-80 material improves peak capacity and sensitivity for both, (U)HPLC and LC-MS analysis. When using short columns this can be exploited to considerably reduce analysis time. The columns are especially suited for use in UHPLC systems, as their reduced system volume and optimized detector specifications help to maintain the high resolution that can be achieved with 2 micron columns.

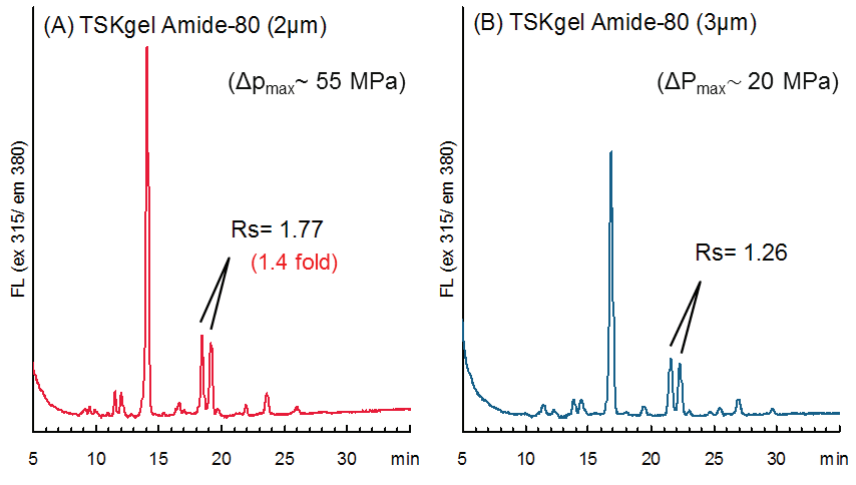
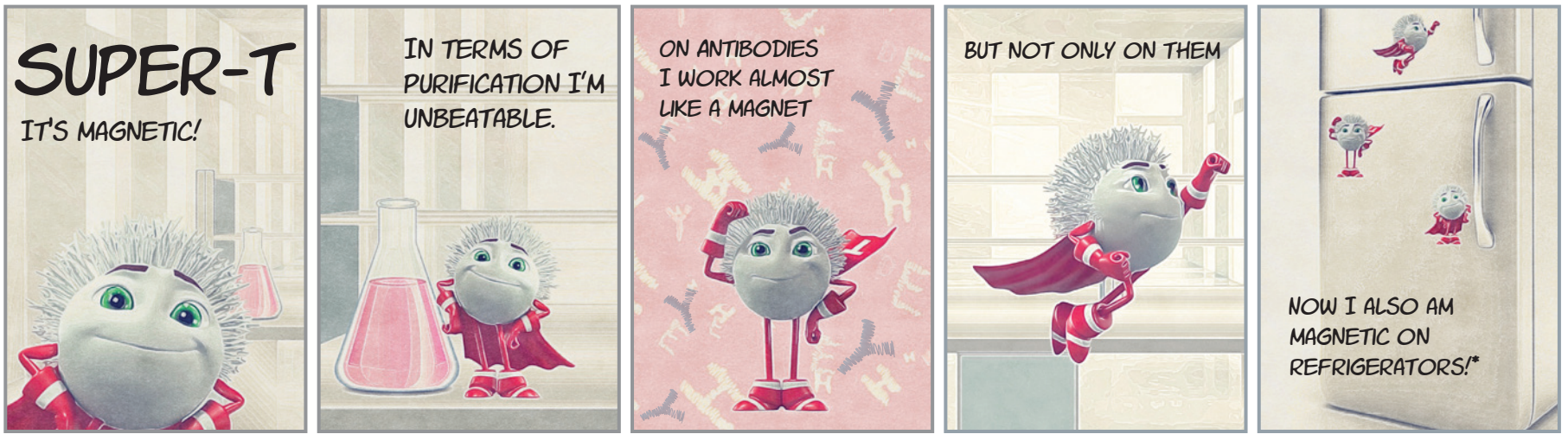


FIGURE 2 : **COMPARISON OF GLYCOSYLATION ANALYSIS**

THE SUPER-T - COMIC #1



* PASS BY OUR BOOTH #20 AT HPLC 2015 IN GENEVA TO PICK UP YOUR SUPER T - MAGNET!

04 CONFERENCE REVIEW

9TH HIC/RPC BIOSEPARATION CONFERENCE IN MALTA

IN THE PREVIOUS ISSUE OF THIS MAGAZINE WE ANNOUNCED THE 9TH HIC/RPC BIOSEPARATION CONFERENCE HELD FROM MARCH 16-19, 2015 IN SLIEMA MALTA. THE HIC/RPC CONFERENCE SERIES, WHICH ALTERNATES BETWEEN EUROPE AND THE USA, PROVIDES A UNIQUE FORUM FOR IN-DEPTH DISCUSSION OF DOWNSTREAM PROCESSING. FOLLOWING ITS TAGLINE 'ADVANCEMENTS, APPLICATIONS, AND THEORY IN DOWNSTREAM PROCESSING' THE CONFERENCE TRADITIONALLY FOCUSES ON BIOPROCESS APPLICATIONS AND ON FUNDAMENTAL RESEARCH ON HYDROPHOBICALLY INFLUENCED MODES OF CHROMATOGRAPHY. IN THE COURSE OF TIME THE EMPHASIS OF THE CONFERENCE SHIFTED TOWARDS HYDROPHOBIC INTERACTION (HIC) AND IN RECENT YEARS ESPECIALLY TOWARDS MIXED-MODE CHROMATOGRAPHY.

The island of Malta, the venue of the Conference concentrates on 316 square kilometers splendid witnesses to an eventful history that goes back to the Bronze Age and saw periods of Arabian, Italian, French, and British dominance. The Conference offered various opportunities to experience Maltese hospitality and encouraged the exchange of ideas and thoughts among the attendees and to establish and renew friendships with colleagues from around the world.

The Scientific committee of the conference, headed by Alois Jungbauer (BOKU, University of Natural Resources and Life Sciences Vienna, Austria) composed a striking program, balanced between fundamentals and industrial applications. The program started with a keynote lecture of Arieh Ben-Naim, who reflected on "Hydrophobic hydrophilic phenomena in aqueous solutions". The lively and engaged presentation was a remarkable opening of the conference that will certainly be remembered.

The evening event of the first day was marked by honouring one of the long time fellows of this conference, Milton Hearn. Alois Jungbauer proposed a toast to Milton who has been awarded the 2015 American Chemical Society (ACS) Alan S. Michaels Award and the prestigious 2015 ACS Award in Chromatography. In addition, he was recognized as Member of the Order of Australia for his significant service to science through major contributions to advances in chemical manufacturing. The delicious dinner was the icing on the cake to the first day of the conference.

Back to the conference talks: The fundamentals session offered a range of approaches on fundamental understanding of hydrophobic interaction and mixed-mode chromatography. While Steven M. Cramer (Rensselaer Polytechnic Institute, Troy, US) focused on ligand design for multimodal resins, Christian Frech (University of Applied Science, Mannheim, DE) presented modelling of dual salt gradient elution in multimodal chromatography.

The industrial case studies and the practical applications of HIC or mixed-mode chromatography presented this year covered a broad range of target molecules: monoclonal antibodies, therapeutic enzymes, virus like particles (VLP), and new antibody formats such as antibody drug conjugates and bispecific antibodies. Guy de Roo from Synthon, Nijmegen, NL, gave an excellent keynote lecture on antibody-drug-conjugates (ADC). New antibody formats were also covered by the joint presentation of Jean-Francois Depoisier from NovImmune and our colleague Judith Vajda on the development of a DSP platform process for a bispecific antibody.

A round table discussion of all members of the scientific committee on Wednesday covered various aspects of HIC/RPC/MMC related downstream processing, ranging from the acceptance and the future of mixed-mode chromatography in the industry over the development regarding disposable columns and the options for continuous processing for HIC and mixed-mode. The last day of the conference was dominated by presentations on novel stationary phases for HIC and mixed-mode chromatography.

In keeping with tradition, the conference offered not only a high class scientific program but also various opportunities to network with colleagues. The rich Maltese history, the nice conference venue, tasty meals, and an excursion to the famous old town of Valetta - a UNESCO world heritage preparing for being European culture capital in 2018 - combined with a dinner in the historic surrounding of M'dina further contributed to making this conference an unforgettable event for all participants.

Tosoh Bioscience is the sole sponsor of this conference series and provides support for logistics and organization for the scientific committee. The 10th HIC/RPC Bioseparation Conference will be organized by Tosoh Bioscience LLC and will take place in Arizona, USA in late winter/early spring 2017.

THE CONFERENCE WEBSITE WWW.HIC-RPC.ORG WILL KEEP YOU UPDATED

HERE'S WHAT THE ATTENDEES SAID ABOUT THE CONFERENCE:

"Good mix of academia and industry"

"Overall very good and well organized. HIC/RPC never disappoints"

"Good mixture of fundamentals, applied science, process development and networking opportunities"

**"This was my first time at the conference.
I truly enjoyed it. The topics were as diverse as the audience.
I definitely learned a lot."**

06 FEATURED LAB

PHARMACEUTICAL ANALYSIS LABORATORY - DEPARTMENT OF DRUG SCIENCES, UNIVERSITY OF PAVIA

THE UNIVERSITY OF PAVIA, ITALY, IS ONE OF THE OLDEST UNIVERSITIES IN ITALY. ALESSANDRO VOLTA (1745-1827) AND CAMILLO GOLGI (1843-1926, NOBEL PRIZE IN 1906) ARE TWO OF MANY OF FAMOUS PROFESSORS WHO TAUGHT IN PAVIA. THE DEPARTMENT OF DRUG SCIENCES IS RENOWNED FOR THE HIGH QUALITY OF THE RESEARCH AND APPLICATION WORK AND CONTINUOUSLY PUBLISHES A BROAD RANGE OF SCIENTIFIC PAPERS UNDER THE GUIDANCE OF PROFESSOR GABRIELLA MASSOLINI, THE DIRECTOR OF THE DEPARTMENT. GABRIELLA MASSOLINI IS RESPONSIBLE FOR THE LAB DEVOTED TO PHARMACEUTICAL ANALYSIS. WE MET HER AND HER CO-WORKERS IN PAVIA TO DISCUSS ABOUT THE DEVELOPMENT OF THE DEPARTMENT AND CURRENT TOPICS OF THEIR RESEARCH ACTIVITIES.

TB: Professor Massolini, could you first of all give us a short summary of the research activities of your lab?
GM: The research activities of my lab are essentially directed towards the development and application of novel methods in pharmaceutical and biomedical analysis by high-performance liquid chromatography coupled with diode array and mass spectrometric detectionWe have been working for a long time in the development of stationary phases based on immobilized proteins/enzymes. These HPLC stationary phases have been used in our lab as chiral selectors and as biochemical probes for the determination of stereoselective ligand-protein binding interactions, ligand-ligand interactions and for the determination of binding constants. We carried out innovative researches on the exploitation of immobilized enzymes as bioreactors in liquid chromatography and we have been involved in the development of frontal affinity chromatography-MS methods for drug discovery.

TB: Which areas besides the development of protein-based stationary phases are you working on?
GM: The experience in the field of immobilized macromolecules allowed us to direct the scientific interest to the development of bioreactors for the on-line digestion of proteins. We have developed new on-line HPLC-MS systems for the digestion/identification of proteins with particular regards to the characterization of post-translational modifications (glyco- and phospho-proteins) and for the analysis of biopharmaceutical such as new glyco-vaccines against tuberculosis.

Further research activities are devoted to the development and validation of HPLC methods for food products quality control and authenticity testing, cosmetic products quality control and support to technological development, and pharmaceutical analysis for the assay of active compounds as well as for impurities profiling and characterization.

TB:Your group is very open to co-operations with industrial partners. Where do you see the values in this type of projects?
GM: We enjoy close collaborations with thought leaders in both academia and industry. These collaborations provided excellent input for the development of our research ideas. Discussion with experts from companies producing columns with innovative stationary phases, such as Tosoh, enabled us to improve the chromatographic methods that we design to tackle pharmaceutical and biomedical problems. HPLC columns provided by industrial partners are also used for the training of students in pharmaceutical analysis and help to overcome insufficient financial resources.

TB: What do you think are the key challenges for the analytics of pharmaceuticals today?
GM: The advent of protein-based bio-pharmaceuticals and antibody drug conjugates (ADCs) has resulted in increased reliance on the chromatographic and spectrometric techniques used to determine the key quantitative and qualitative attributes of such complex therapeutic entities. HILIC, in conjunction with tandem mass spectrometry (MS/MS), has steadily gaining acceptance in the analysis of polar compounds from complex biological matrices.

Another important issue is the characterization of genotoxic impurities and their impact on the pharmaceutical industry. There is a demand of appropriate strategies used to select and develop analytical methods relevant for the particular impurities identified. Also in this context HILIC as an HPLC mode orthogonal to reversed-phase, can be used by itself or in multidimensional approaches, for the separation of a variety of pharmaceuticals and their impurities in both R&D and Drug Discovery laboratories.

TB: Professor Massolini, thank you for the fruitful discussions here in Pavia. We are looking forward to future results of our ongoing co-operation.

IF YOU WANT TO KNOW MORE ABOUT THE WORK OF THIS GROUP, ATTEND THE LECTURE OF PROF. MASSOLINI AT THE PBA 2015 CONFERENCE IN TIBLISI, GEORGIA.

CONTACT FOR SCIENTIFIC COOPERATIONS AT TOSOH BIOSCIENCE GMBH: P.D. DR. EGBERT MÜLLER [TECHNICAL DIRECTOR]

07 HILIC IN THE LITERATURE

HILIC APPLICATIONS IN PHARMACEUTICAL RESEARCH

HYDROPHILIC INTERACTION LIQUID CHROMATOGRAPHY (HILIC) HAS GAINED AN IMPORTANT ROLE IN PHARMACEUTICAL AND BIOPHARMACEUTICAL ANALYSIS. HEREIN WE SUMMARIZE ONE OF THE MOST RECENT PAPERS FROM OUR FEATURED LAB, THE DEPARTMENT OF DRUG SCIENCES, PAVIA. TARGET MOLECULES OF THEIR RESEARCH RANGE FROM COSMETICS COMPONENT OVER PURINE AND PYRIMIDINE BASES AND NUCLEOSIDES TO INTACT GLYCOPROTEINS. THEIR WORK ON THE ANALYSIS OF INTACT NEO-GLYCOPROTEINS BY HILIC WAS PUBLISHED IN 2014 [1].

Oligosaccharides conjugated to carrier proteins (neo-glycoproteins) have been successfully developed as semi-synthetic vaccines. The concept of using synthetic glycoproteins has also been discussed for the prevention and therapy of several non-infectious diseases. The complexity of glycosylated proteins necessitates the development of new analytical strategies to characterize these biopharmaceuticals as their structure is important for their stability, folding, efficacy, and safety.

Characterization of glycoproteins in terms of identity, heterogeneity and impurity can be accomplished by a variety of analytical methods: NMR, MS, CE, HPLC, and spectrophotometric methods. However, liquid chromatography (LC) coupled to electrospray ionization mass spectrometry (ESI-MS) is the most commonly used approach and has been applied for the analysis of intact glycoproteins, characterization of glycan structures and glycopeptides. The analysis of the intact proteins is an elegant approach that simplifies sample preparation, but protein heterogeneity can limit resolution.

The paper focusses on the development of a simple HILIC-UV method for the analysis of intact glycoproteins to be used for the monitoring of synthetic glycosylation processes. As proof of concept ribonuclease A (RNase A) and RNase B which exists in five isoforms varying in the number of mannose residues were separated to optimize the method. Figure 1 shows the chromatographic profiles for RNase A (red traces), RNase B (green traces) and their equimolar mixture (black traces) on TSKgel Amide-80 (2 x 150 mm, 3µm), eluted at a flow rate of 0.2 mL/min and a temperature of 50 °C.

The method was applied to the separation of neo-glycoproteins prepared starting from the RNase A by chemical conjugation of different glycans. The presence of RNase A and its glycosylated reaction products were further confirmed by ESI-MS analysis. Applying the developed HILIC-UV method it was possible to monitor the glycosylation reaction of RNaSe A with Ara (1-6)Man-IME and assess the distribution of neo-glycoprotein isoforms without laborious sample workup prior to analysis (Figure 2).

[1] ALICE PEDRALI ET AL., MOLECULES 2014, 19, 9070-9088

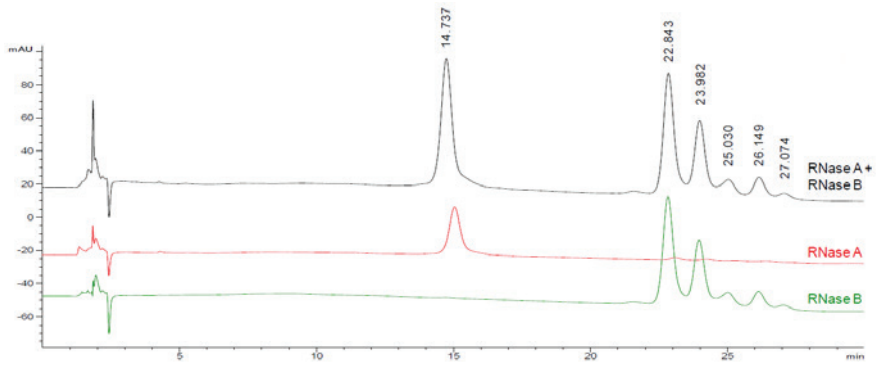


FIGURE 1: **HILIC SEPARATION OF RNASE A AND RNASE B ISOFORMS ON TSKgel AMIDE-80**

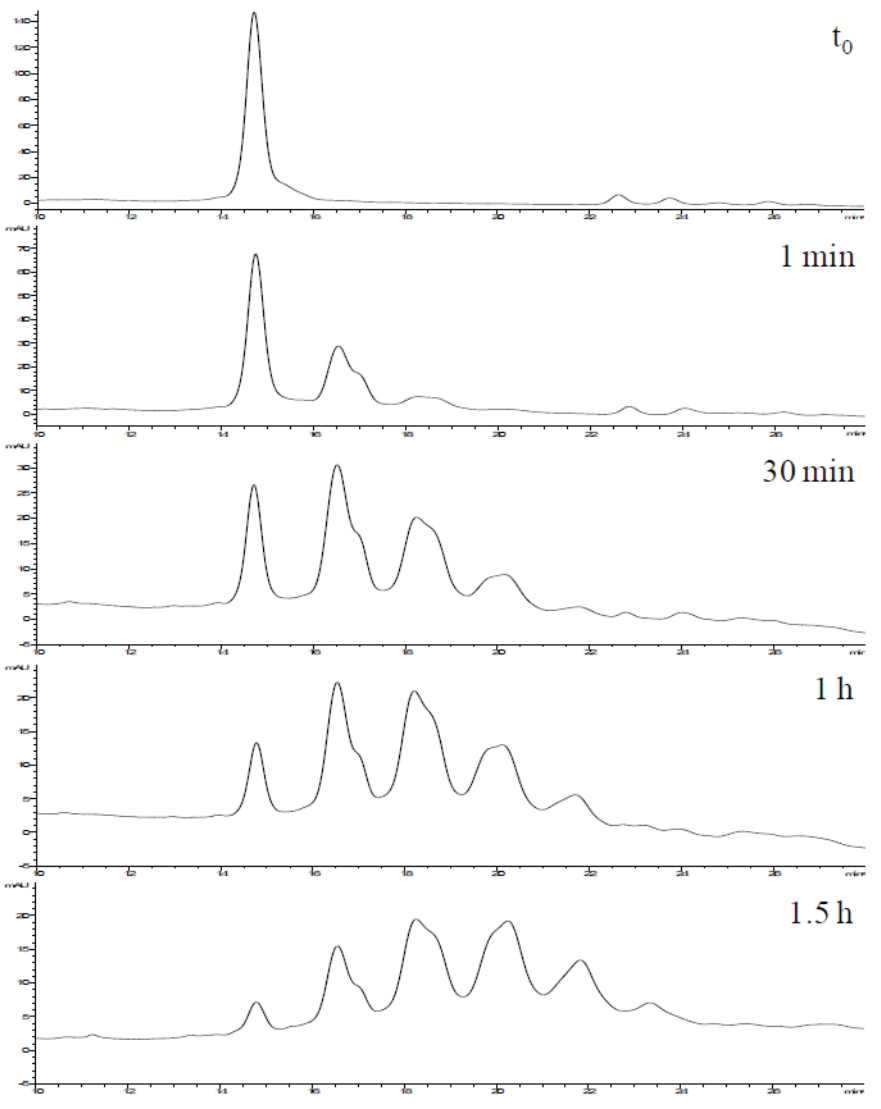


FIGURE 2: **MONITORING OF THE SYNTHESIS OF NEO-GLYCOCONJUGATES**

08
WHAT'S HAPPENING
ARRIVAL AT RHINE-MAIN AREA

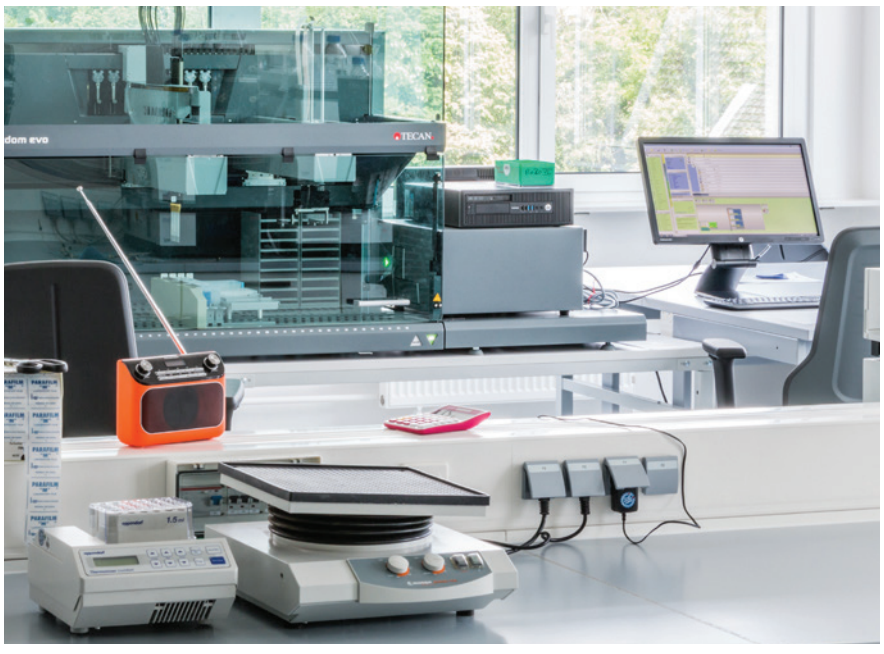
MISSION ACCOMPLISHED – OFFICE AND LAB MOVED TO THE NEW FACILITIES IN GRIESHEIM

AS ANNOUNCED IN THE LAST ISSUE OF THIS MAGAZINE TOSOH BIOSCIENCE CENTRALIZED OFFICES, PRESENTATION AND LABORATORY FACILITIES OF BOTH SUBUNITS, THE SEPARATION BUSINESS UNIT AND THE DIAGNOSTICS BUSINESS UNIT, IN THE RHINE-MAIN AREA. AFTER MOVING THE OFFICES JUST BEFORE CHRISTMAS 2014 THE MOVE OF THE TECHNICAL SUPPORT GROUP COULD BE TACKLED IN APRIL 2015 AFTER THE COMPLETION OF OVER 230 SQUARE METERS OF NEW LAB FACILITIES.

The new site at the Leuschnerpark in Griesheim is close to Frankfurt Airport and very close to the highways A5 and A67, two of the central highways linking the northern and southern parts of Germany. Thus, the new location is easy to reach for customers and cooperation partners, but also for colleagues from other Tosoh subsidiaries in Europe and overseas.

The space for offices and laboratories remarkably increased and the new office also offers facilities for instrument demonstrations of both, separation and diagnostic systems, as well as training facilities. Besides new working benches, hoods and latest technical infrastructure, one of the highlights of the new lab is the new TECAN robotic system that enables the colleagues to perform resin screening and method development for our resins using the RoboColumn format.

We already had several internal meetings and visits form suppliers and customers in the new facilities and are looking forward to welcoming you there one day.



DON'T FORGET TO UPDATE YOUR RECORDS WITH OUR NEW CONTACT DETAILS: TOSOH BIOSCIENCE GMBH, IM LEUSCHNERPARK 4, 64347 GRIESHEIM, GERMANY, TEL: +49 6155 7043700, FAX: +49 6155 8357900

NEWS & EVENTS | MEET TOSOH BIOSCIENCE

MEET TOSOH AT TRADESHOWS AND CONFERENCES OR JOIN ONE OF OUR RENOWNED WORKSHOPS

UPCOMING EVENTS

- JUNE 21 - 25 | 2015 — HPLC 2015 | GENEVA [SWITZERLAND]
- OCT. 06 - 08 | 2015 — BIOTECHNICA | HANOVER [GERMANY]
- OCT. 14 | 2015 — BIOSEPARATION FORUM | SYMBION SCIENCE PARK / COPENHAGEN [DENMARK]

TRAININGS/WORKSHOPS

- SEP. 22 - 24 | 2015 — CHROMATOGRAPHY IN PROCESS DEVELOPMENT & PRODUCTION / BASIC COURSE IN GERMAN LANGUAGE | STUTTGART [GERMANY]
- SEP. 29 - OCT. 1 | 2015 — CHROMATOGRAPHY IN PROCESS DEVELOPMENT & PRODUCTION / BASIC COURSE IN GERMAN LANGUAGE | STUTTGART [GERMANY]

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