TOSOH

TOSOH
THE CUSTOMER
MAGAZINE

NO. #02 2018

CA++PURE/CONGRATULATION/CONFERENCE

TOSOH BIOSCIENCE

02
EDITORIAL
DEAR READER

Welcome to the winter issue of the Tosoh Bioscience customer magazine. This magazine has a focus on downstream processing. It is featuring a new hydroxyapatite chromatography medium with exciting properties and presents a new approach for the purification of a bispecific antibody.

If you are working in downstream processing the HIC/DSP Bioseparation Conference will be an excellent opportunity to gather a thorough update on latest trends and discuss current challenges with specialists from all over the world. Check out page 8 and register for the Conference, which will take place in February 2019 in Interlaken, in the Swiss Alps.

In this issue, we continue our series of portraits of Tosoh partners and colleagues: We thank our distribution partner Sebio GmbH for 10 years of successful cooperation and introduce Manuela Sevilla to you, who recently joined our Technical Support Team.

ENJOY READING AND STAY INFORMED.

REGINA ROEMLING | SENIOR MARKETING MANAGER
TOSOH BIOSCIENCE GMBH

THE SUPER-T - COMIC #8

CONTENT

IMPRESSUM

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03
WHAT'S NEW CHROMATOGRAPHY MEDIA

CA++PURE-HA - THE NEW STANDARD FOR HYDROXYAPATITE

IN OCTOBER, TOSOH BIOSCIENCE LAUNCHED A NEW MEDIUM FOR THE PURIFICATION OF BIOPROTEINS. THE HYDROXYAPATITE CA++PURE-HA OFFERS SUPERIOR SEPARATION FOR MULTIPLE CLASSES OF BIOMOLECULES. IT IS EASY TO PACK AT PROCESS SCALE, AND ITS REDUCED COSTS AND IMPROVED CHEMICAL STABILITY INCREASE THE PROFITABILITY OF DSP PROCESSES. IT IS SPECIFICALLY DEVELOPED FOR THE PURIFICATION OF MONOCLONAL AND POLYCLONAL ANTIBODIES, ANTIBODY ISOFORMS, FUSION- AND PHOSPHOPROTEINS, AND THE ISOLATION OF SINGLE-STRANDED FROM DOUBLE-STRANDED DNA.

Ca++Pure-HA media is a spherical, macroporous form of the hexagonal crystalline structure of hydroxyapatite. It has been sintered at high temperatures for increased mechanical and chemical stability, allowing it to withstand the rigors of industrial-scale applications. The robust nature of Ca++Pure-HA allows for it to be used reproducibly for many cycles at high flow rates and in large columns. In large-scale processes, Ca++Pure-HA is easy to pack and unpack.

Ca++Pure-HA offers a dynamic binding capacity (DBC), at 5% breakthrough, of greater than 30 g/L human IgG at residence times as low as 2 minutes, and even DBC greater than 50 g/L at 5-minute residence time (Figure 1). It is effective at removing aggregates and degradation products from mAbs with an elution buffer such as potassium chloride.
This new hydroxyapatite medium is alkaline stable in 0.5 mol/L NaOH for greater than 65 CIP cycles with no appreciable loss of dynamic binding capacity. Its high mechanical stability allows packing of large process columns (Figure 2).

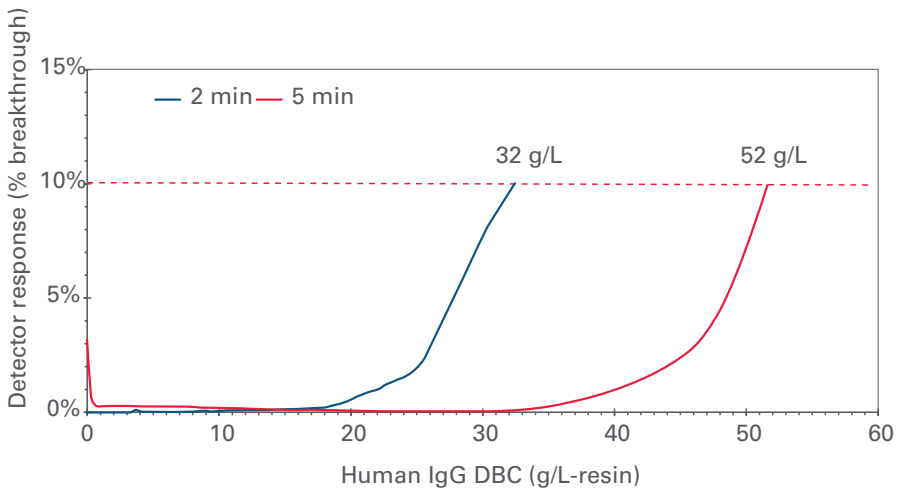
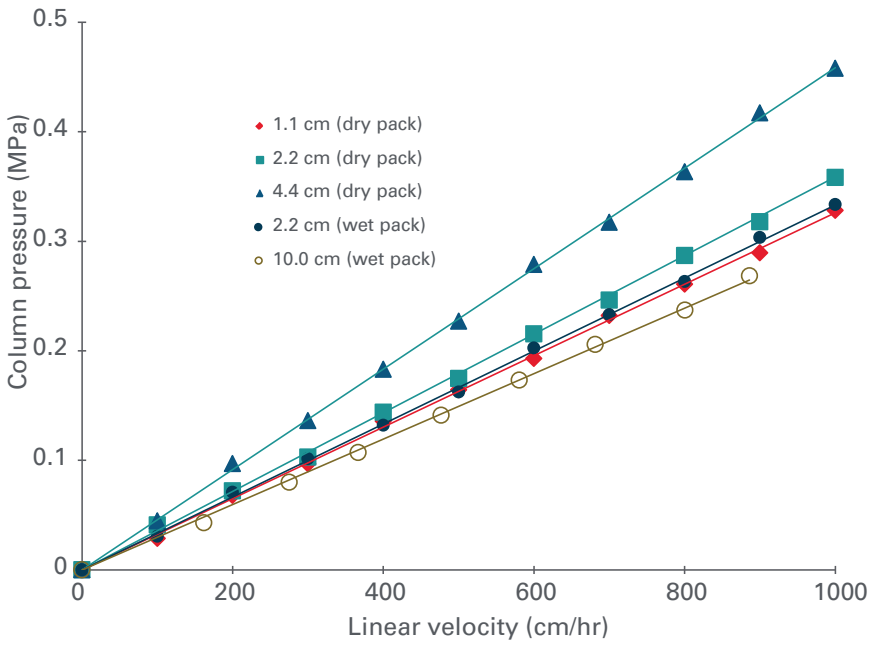


FIGURE 1: **CA++PURE-HA DYNAMIC BINDING CAPACITY**

Ca++Pure-HA media offer chromatographers the combination of exceptional separation properties and unequalled selectivity and resolution for multiple classes of biomolecules. The highly selective and robust nature of this medium provides the flexibility to use it at any stage in a process from capture to final polishing.



Resin:	Ca++Pure-HA
Column size:	1.1 cm ID × 19.3 cm (dry pack) 2.2 cm ID × 20.3 cm (dry pack) 4.4 cm ID × 19.3 cm (dry pack) 2.2 cm ID × 20.6 cm (wet pack) 10.0 cm ID × 21.3 cm (wet pack)
Mobile phase:	5 mmol/L sodium phosphate, pH 7.2
Linear velocity:	as noted
Detection:	pressure (MPa)

FIGURE 2: **MECHANICAL STABILITY OF CA++PURE-HA**

YOU CAN ORDER A DIGITAL INFORMATION PACKAGE AND A FREE SAMPLE HERE: HTTPS://WWW.SURVEYMONKEY.DE/R/CAPUREHA_CM

04
APPLICATION
DOWNSTREAM PROCESSING

INTERMEDIATE PURIFICATION OF BISPECIFIC ANTIBODIES WITH A NON-AFFINITY PLATFORM

THE THERAPEUTIC BENEFITS OF MONOCLONAL ANTIBODIES (MABS) HAVE BEEN DEMONSTRATED IN RECENT DECADES AS TREATMENTS FOR VARIOUS HUMAN DISEASES. DESPITE MABS' KEY FEATURES SUCH AS SPECIFICITY, SELECTIVITY, AND SAFETY, THE FORMAT HAS LIMITATIONS. BISPECIFIC ANTIBODIES MAY OVERCOME A NUMBER OF DIFFICULTIES. A NEW PLATFORM FOR INTERMEDIATE PURIFICATION OF κλ-BODY BISPECIFIC ANTIBODIES USES HYDROPHOBIC CATION EXCHANGE (CEX) CHROMATOGRAPHY AND HYDROPHOBIC INTERACTION CHROMATOGRAPHY (HIC) TO REPLACE TWO AFFINITY-CHROMATOGRAPHY STEPS AND A POLISHING STEP.

Multiple formats of bispecific antibodies have been developed, although only the κλ-body is fully human and devoid of linkers or mutations. It does not require any genetic modifications of heavy and light chains and results in bispecific antibodies with natural sequences.
Different affinity chromatography steps have been developed for purification of bispecific mAbs. However, development of a nonaffinity-based platform leads to more cost-effective production processes. The advent of hydrophobic cation-exchange resins, often referred to as mixed mode, provides opportunities to reduce the number of affinity steps in a platform process.

SCOUTING OF INTERMEDIATE DOWNSTREAM PROCESSING

A one-step purification of other bispecific mAbs using HIC has been described previously. Therefore, HIC was chosen as the first chromatographic mode to be evaluated for separation of the κλ-body from the monospecific mAbs after an initial protein A capture step. Linear gradient scouting experiments were performed on TOYOPEARL Phenyl-600M and TOYOPEARL Butyl-600M for separation of κκ-monospecific mAb, λλ-monospecific mAb, and the κλ-body present in the protein A eluate pool. Resolution using Butyl-600M was greater than using Phenyl-600M, with the λλ-monospecific mAb and the κλ-body resolved to baseline. Thus, Butyl-600M was selected for subsequent optimization experiments.

In contrast to analytical cation exchange (CEX) chromatography, baseline resolution could not be achieved at process scale using CEX chromatography resins. A combination of hydrophobic and ionic interactions may provide sufficient selectivity to accomplish separation of both monospecific mAbs and the κλ-body. A hydrophobic CEX (mixed mode) resin was therefore evaluated.

Chromatographic scouting runs of the separation of the κκ-monospecific mAb, the λλ-monospecific mAb, and the κλ-body on the mixed mode resin TOYOPEARL MX-Trp-650M have been performed in a linear sodium chloride gradient at several pH. At pH 6.0, the flow through fraction contained λλ-monospecific mAb, whereas the κλ-body and the λλ-monospecific mAb were adsorbed to the resin.

STEP-GRADIENT ELUTION MIXED-MODE AND HYDROPHOBIC INTERACTION CHROMATOGRAPHY

Mixed-mode chromatography and HIC discriminated the κλ-body and the monospecific mAbs with orthogonal retention criteria. The selective flow-through of the λλ-monospecific mAb observed at pH 6.0 in the scouting experiments with TOYOPEARL MX-Trp-650M provided an opportunity to develop an efficient step elution protocol for purification of the κλ-body. Figure 1 shows a chromatogram of a step-elution separation at pH 6.0. The purity of κλ-body was approximately 65%. The majority of the remaining contamination was κκ-monospecific mAb. Hence, a subsequent purification step was required.

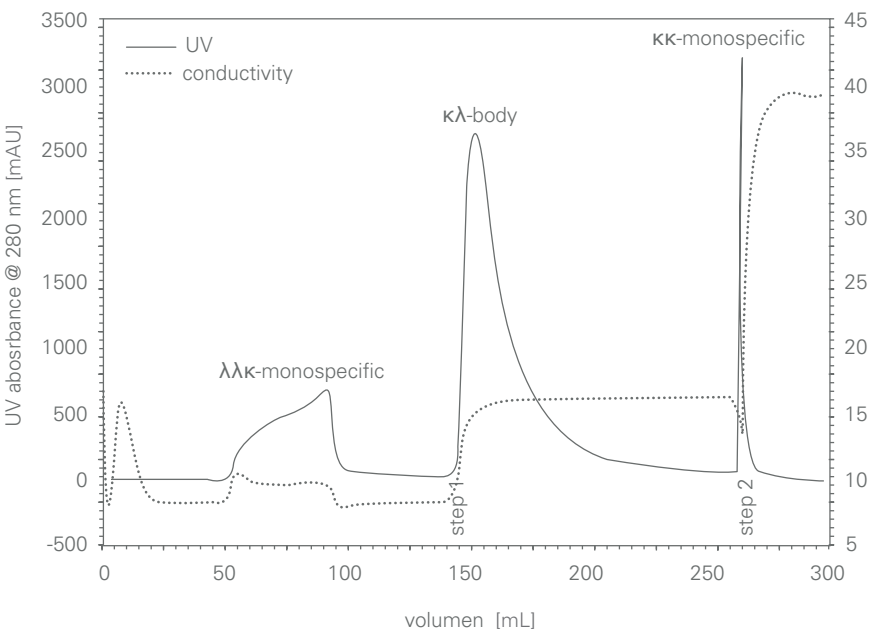


FIGURE 1: **SEPARATION OF THE PROTEIN A ELUATE POOL ON THE MIXED MODE RESIN TOYOPEARL MX-TRP-650M IN A STEP-GRADIENT ELUTION**

05 APPLICATION DOWNSTREAM PROCESSING

The selectivity of TOYOPEARL Butyl-600M is less susceptible to variations in conductivity and pH. Furthermore, the use of HIC adds another orthogonal separation criterion to the process. This is advantageous with regard to other process-related impurities, such as viruses and DNA. The TOYOPEARL MX-Trp-650M step 1 eluate pool containing κλ-body was loaded onto a TOYOPEARL Butyl-600M column. The κλ-body was recovered at a purity of 99.5% (Figure 2).

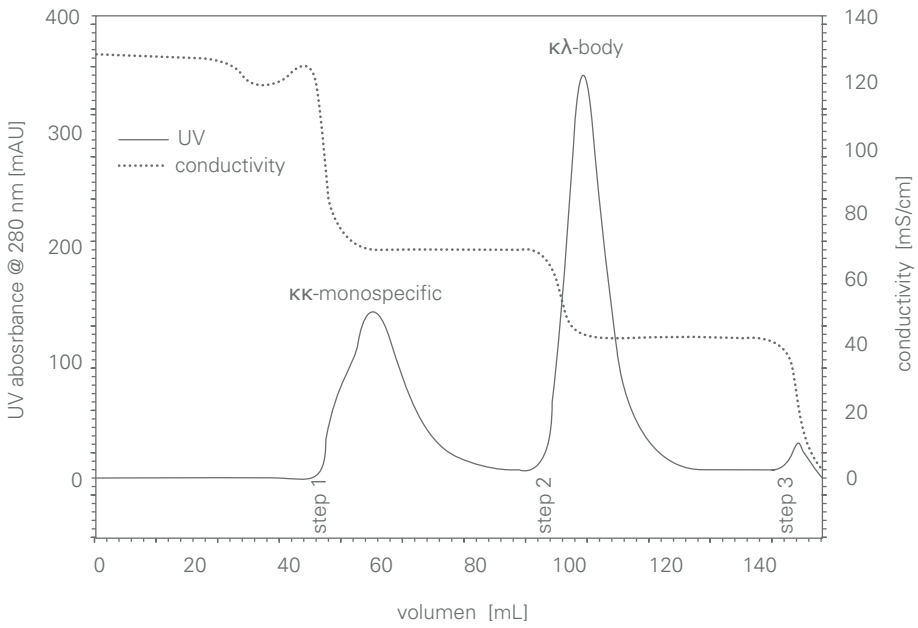


FIGURE 2: **PURIFICATION OF THE κλ-BODY FROM THE MIXED MODE STEP 1 ELUATE POOL WITH TOYOPEARL BUTYL-600M IN A STEP-GRADIENT APPROACH; 99.5 % PURE κλ-BODY IS RECOVERED DURING STEP 2.**

COMPARABLE PURITY, LOWER COSTS

Modern chromatography resins were evaluated for purification of a κλ-body. Hydrophobic CEX and HIC can replace two subsequent affinity-chromatography steps and a polishing step for purification of a κλ-body from monospecific mAb by-products (Figure 3). The three-step process using mixed mode and HIC chromatography showed comparable yields to a three-step affinity platform process currently used to purify a κλ-body.

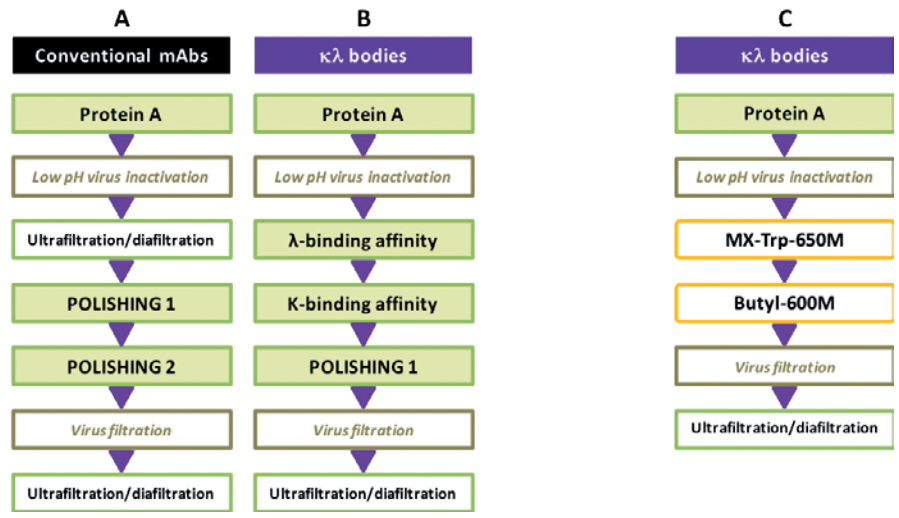


FIGURE 3: **STATE-OF-THE-ART DOWNSTREAM PROCESSING WORKFLOW FOR THE PURIFICATION OF MABS (A) AND κλ-BODIES (B), COMPARED WITH THE NEW PURIFICATION PROCESS FOR κλ-BODIES (C)**

Although the protein A elute pool did not contain significant aggregate levels, even after a low pH hold for virus inactivation, both TOYOPEARL MX-Trp-650M and TOYOPEARL Butyl-600M can be used for aggregate removal at conditions similar to those of the operating conditions applied here. Hence, it can be expected that the applied conditions would provide aggregate removal if a particular κλ-body candidate contained a higher level of aggregates. This is especially important with regard to platform applicability.

The excellent selectivity of TOYOPEARL MX-Trp-650M and TOYOPEARL Butyl-600M paves the way for future implementation at research, clinical, and commercial manufacturing scales. This approach combining reduced cost of goods and higher binding capacities offers an attractive new version of the purification process for the future manufacture of κλ-bodies.

More on this topic in the October issue of the BPI magazine

06
PARTNERS
10TH ANNIVERSARY SEBIO GMBH

SEBIO GMBH CELEBRATES 10 SUCCESSFUL YEARS IN BUSINESS IN SWITZERLAND

IN 2008, FELIX SENN DECIDED TO START-UP AN OWN COMPANY FOR THE DISTRIBUTION OF LABORATORY PRODUCTS FOR CHROMATOGRAPHY AND FILTRATION. AT THAT TIME HE COULD BUILD UPON 10 SUCCESSFUL YEARS AS A SALES PERSON FOR TOSOH BIOSCIENCE PRODUCTS. SINCE 2009, HIS COMPANY SEBIO GMBH IS AN OFFICIAL DISTRIBUTOR OF TOSOH BIOSCIENCE IN SWITZERLAND. WE TOOK THE OPPORTUNITY OF THE COMPANY'S 10TH ANNIVERSARY TO CHAT WITH FELIX ABOUT THE LAST 10 YEARS.

Tosoh Bioscience (TB): Felix, congratulation to the 10th anniversary of your company. Let's look back to the start. Where does the name "Sebio" come from?
Felix Senn (FS): We started thinking about a suitable name in 2008. We wanted the company name to contain the name of the founder family and to indicate its relation to biotechnology. Furthermore, the name had to beshort and easy to visualize in the logo. And in the times of increasing internet usage, it was last but not least very important to find a memorable web address and e-mail domain, that was still available. All these prerequisites were achieved by combining "Senn" and "Biotechnology" in the name Sebio.

TB: How do you remember the first year of Sebio?
FS: My wife, Beatrice Senn–Müller, founded the company on 6th November 2008. I took over the business six months later. Daniele Di Girolamo was our first employee. We had to organize a lot of things but in February 2009 we could start to work as distributor of Tosoh Bioscience chromatography media and columns in Switzerland.

TB: How did Sebio GmbH develop over the first decade?
FS: In 2010, in our second year of business, we took the opportunity to present our company together with our partners at a large booth at ILMAC, the important Swiss tradeshow on process and laboratory technology, that takes place in Basel every three years. This was a good public kick-off that laid ground for the development of Sebio into a successful trading company in the Basel region. We can now look back on a very successful decade characterized by continual growth and constant development. Today, Sebio GmbH serves the Swiss biotech industry with 4 employees.

TB: Felix, we thank you for this interview and for the successful cooperation over the last 10 years. We wish you all the best for the future.
FS: We would like to say thank you! Our success is mainly down to you – our suppliers – and of course to our customers. We are delighted that you placed your trust in us and would like to thank you for your loyalty. We were only able to maintain and optimize the quality of our work through competence, continuity and a sense of camaraderie. I would therefore particularly like to thank our employees, who work tirelessly and reliably to serve our customers' needs.

SEBIO AND TOSOH BIOSCIENCE SHARING A BOOTH AT ILMAC

THE SEBIO TEAM AT THEIR OUTING DAY

07
PEOPLE BEHIND TOSOH

MANUELA SEVILLA, TOSOH BIOSCIENCE GMBH, GRIESHEIM, GERMANY

MANUELA SEVILLA IS THE NEWEST MEMBER OF THE TOSOH BIOSCIENCE'S TECHNICAL SUPPORT TEAM. MANUELA WORKS AS TECHNICAL SPECIALIST IN THE TEAM OF PD DR. EGBERT MÜLLER. WE ASKED MANUELA TO GIVE SOME INSIGHTS INTO HER LIFE AND HER ON-BOARDING TIME AT TOSOH BIOSCIENCE.

Tosoh Bioscience (TB): Manuela, you joined Tosoh Bioscience in September. How did you get to know about Tosoh?
Manuela Sevilla (MS): During my master studies I was searching for an internship in the pharmaceutical industry. Patrick Endres (Senior Laboratory Specialist) published in our university group, that Tosoh was looking for interns. I decided to apply for the internship and I had a great experience as an intern. I could learn a lot, so I also stayed at Tosoh Bioscience GmbH for my master thesis. And now I started as employee.

TB: What was the topic of your master thesis?
MS: The title of my thesis was "Purification of Antibody Drug Conjugate-Surrogates with Hydrophobic Interaction Chromatography". Tosoh developed an ADC-mimic, which consists of a non-toxic payload conjugated to an antibody. My task was to develop a suitable purification process. Our main focus was the separation of conjugates with multiple drug-to-antibody ratios (DARs). We started with a high throughput screening and selected the best resins and parameters. After that the separation was further tested at lab scale. ADCs are promising therapeutics in cancer therapy and I am really happy that I could contribute to such an important project (https://bioprocessintl.com/2018/august-2018/antibody-drug-conjugate-surrogate-purification-toyopearl-ppg-600m-hic-resin-for-dar-separation/).

TB: Could you please tell our readers a little bit about yourself and what you do for Tosoh now?
MS: I work at Tosoh as a Technical Specialist. Currently I spend the most of my time in the lab, where I am testing columns or resins and generating application data with our products. I am also involved in the supervision of the students, I really like showing them, what we do and how our lab works. In the future, I will start working directly with customers and I will attend conferences and present my work internally and externally. I am looking forward to these new challenges.

TB: What part of the work do you like most?
MS: What I like the most about Tosoh is that you have the opportunity to learn something new every day. There are always new projects or new prototypes to test. Also there are always new focuses to consider when developing a process, which makes the work more challenging. I also really like the work atmosphere. We have a multicultural team and I enjoy learning new things from my colleagues.

TB: What were the highlights during your time at Tosoh?
MS: One of the highlights during my time at Tosoh was when I learned that there was a vacancy as Technical Specialist. Knowing that I had the opportunity to stay was really exciting. I applied for the job and when I received the offer I was very happy about starting my professional career with Tosoh.

TB: What are your interests besides Chromatography?
MS I really enjoy to travel, I am always interested in getting to know new countries and new cultures. I try to travel to different cities and to get in contact with the people to better get to know their culture and habits. I enjoy being spontaneous when I travel, e.g. I only book a flight to my destination, sometimes also the first hotel, but then I let the people or the city lead me. I also love to dance, I come from Ecuador and we say that "The Latinos have rhythm in their blood".

MANUELA AT WORK

MANUELA'S HOME COUNTRY ECUADOR

08
WHAT'S HAPPENING
HIC/DSP CONFERENCE 2019

DISCUSS THE LATEST BIOSEPARATION DEVELOPMENTS

THE 11TH HIC/DSP BIOSEPARATION CONFERENCE WILL BE HELD ON FEBRUARY 18-21, 2019 AT THE HOTEL ROYAL-ST. GEORGE IN INTERLAKEN, SWITZERLAND. WE RECEIVED MANY EXCITING ABSTRACTS AND ARE CURRENTLY COMPILING A STRIKING PROGRAM ON CUTTING EDGE DOWNSTREAM PROCESSING SOLUTIONS.

The HIC/DSP conference is providing a platform to better understand the chromatographic isolation and purification of biological targets. From basic theory to industrial scale purification, recognized professionals will share their expertise and experiences on all aspects of chromatographic separation and process design for biomolecules. The practical elements of chromatographic development and implementation will be balanced by discussions about novel approaches and theories.

Professor Dr. Alois Jungbauer, University of Natural Resources and Applied Life Sciences (BOKU), Vienna, Austria will be heading the scientific committee. We are excited to announce that Roman Necina from Shire will give a keynote lecture about how complex biopharmaceuticals drive implementation of novel and smart technologies.

REGISTER TODAY FOR THE 11TH HIC/DSP BIOSEPARATION CONFERENCE ON THE CONFERENCE WEBSITE WWW.HIC-DSP.ORG

NEWS & EVENTS | MEET TOSOH BIOSCIENCE

MEET TOSOH AT TRADESHOWS AND CONFERENCES

UPCOMING EVENTS

NOV	4	7	2018	ISPPP	BERLIN	[GERMANY]
NOV	20	22	2018	BIOPROCESS UK	EDINGBURGH	[UK]
NOV	29		2018	HPLC PRAXISTAG	BERLIN	[GERMANY]
DEZ	13	14	2018	BIOSEPARATION FORUM	STRASBOURG	[FRANCE]
JAN	20	FEB 1	2019	SCM-9	AMSTERDAM	[THE NETHERLANDS]
FEB	6	7	2019	BILS 2019	BERLIN	[GERMANY]
FEB	18	21	2019	HIC/DSP CONFERENCE	INTERLAKEN	[SWITZERLAND]
MAR	12	14	2019	ARABLAB	DUBAI	[UAE]

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