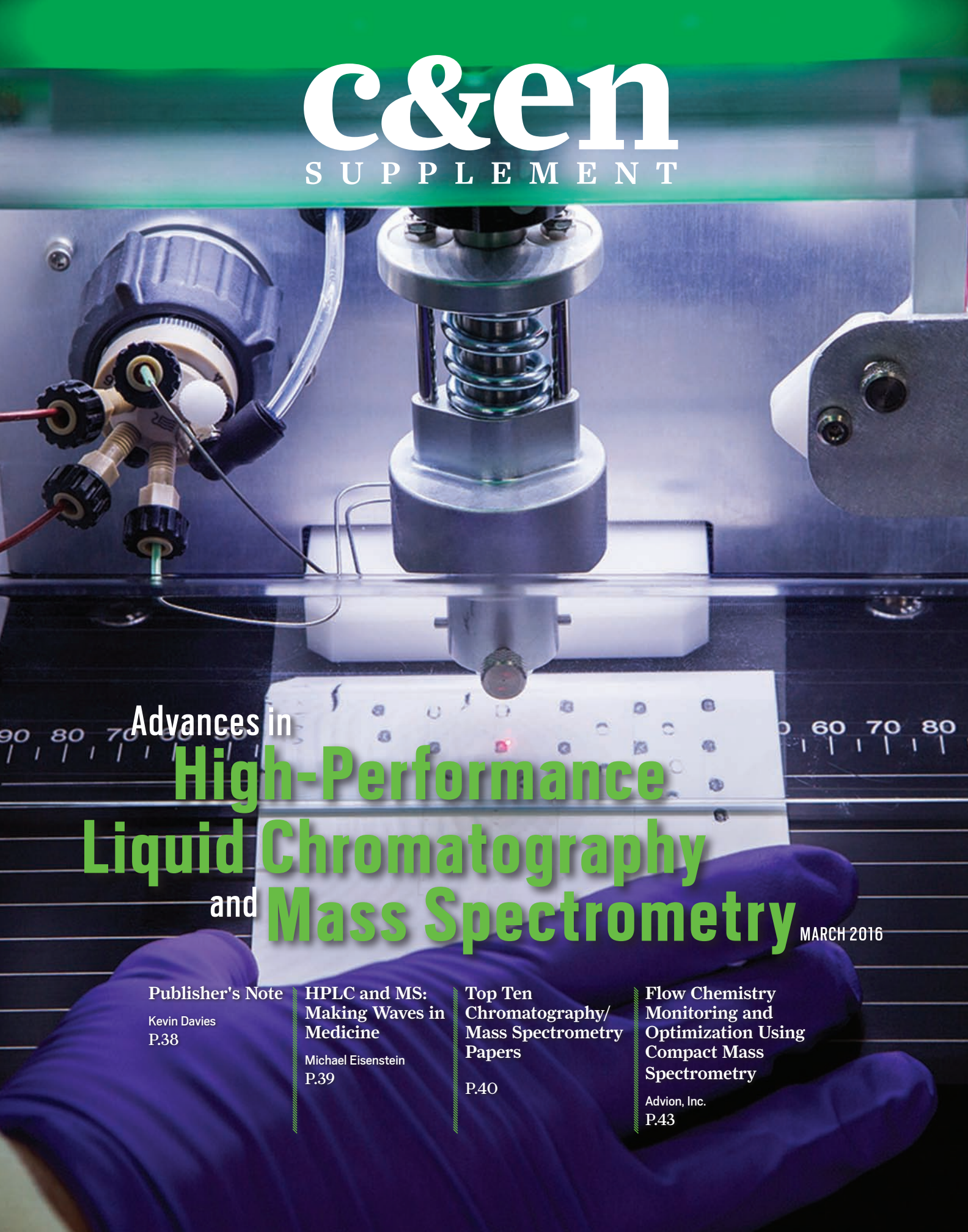
c&en
SUPPLEMENT



Advances in High-Performance Liquid Chromatography and Mass Spectrometry

MARCH 2016

A SUPPLEMENT TO
c&en

This C&EN Supplement is produced by C&EN, the flagship magazine of the American Chemical Society.

American Chemical Society
1155 16th Street, NW
Washington, DC 20036
(202-872-4600; 800-227-5558)

C&EN Supplement Management

Kevin A. Davies
Publisher, C&EN

Bibiana Campos Seijo
Editor-in-Chief, C&EN

Michael Eisenstein
Managing Editor, C&EN Media Group

Stephanie Holland
Manager, Advertising Sales and Marketing

Renee Zerby
Manager, Digital Production & Imaging

Joseph B. Davis, Jr.
Lead Digital Production Associate

Krystal E. King
Lead Digital Production Associate

Marielyn Cobero
Digital Production Associate

ACS Management

Thomas M. Connelly, Jr.
CEO & Executive Director

Brian D. Crawford
President, ACS Publications

To advertise in C&EN Media Group products, contact Stephanie Holland at (202) 872-4536 (s_holland@acs.org) or go to CENMediaKit.org

Cover: Image courtesy of Advion, Inc.
Marielyn Cobero/C&EN

PUBLISHER'S NOTE

New Horizons for Separation Science

WELCOME TO THIS C&EN advertising supplement on **Advances in High-Performance Liquid Chromatography and Mass Spectrometry.**

These crucial analytical technologies continue to play a major role in chemical and environmental analysis, and we are witnessing many important new applications in a variety of areas. It is exciting to see these mature technologies evolve and move into scientifically and economically important new fields.

This year marks the 60th anniversary of The Chromatographic Society in the UK, and next year will be the 50th anniversary of mass spectrometry imaging. Many of these trends are featured in C&EN's in-depth coverage of the annual Pittcon event, which took place earlier this month, and will likely be included in upcoming editorial features on the Top 25 Instrumentation Companies (April 25) and Applications of Mass Spectrometry (May 30), to name just two.

I'm delighted to acknowledge the contributions of managing editor Michael Eisenstein, who will be working with the C&EN Media Group on various projects including the C&EN supplement series and our rapidly growing collection of sponsored white papers. Michael is a talented Philadelphia-based author and editor who contributes frequently to Nature Methods and other publications, and previously worked with the Seed Media Group.

Finally, I thank our advertisers and the team at Advion, which contributed an interesting application note for this latest supplement. We appreciate your support.

Kevin Davies, PhD

Publisher, C&EN

ADVERTISER INDEX

For the record: The editorial content in this supplement was created without direct involvement of C&EN reporters or editors.

HPLC AND MS: MAKING WAVES IN MEDICINE

Michael Eisenstein

IN A COMMENTARY published last year in *Clinical Laboratory News*, University of Utah pathologist Alan Lockwood jokingly revealed his vision for the future: "As a mass spectrometrist who has migrated into the realm of clinical chemistry, I frequently tell my clinical chemistry colleagues that mass spectrometrists want to take over the world."[1] He was clearly exaggerating, but MS is undoubtedly poised to become a transformative technology in the clinical world.

The initial clinical applications of MS have been as detection assays, offering a more sensitive and specific complement to immunoassays. Although far more expensive and complicated to use than an ELISA kit, MS can also readily distinguish closely-related molecules that might generate false-positive results in an antibody-based test, thereby achieving superior sensitivity and accuracy. For example, Lockwood cites the challenge of using immunoassays to detect the low concentrations of testosterone found in serum samples from women and children.

Clinical researchers have been steadily pushing the performance of the technology into exciting new frontiers, including broad profiling of the metabolome to identify useful prognostic or diagnostic indicators of disease states or drug response. Much of the work in this area has used a combination of liquid chromatography separation and tandem mass spectrometry (LC-MS/MS) to go after 'targeted' sets of known metabolites, but researchers are increasingly embarking on 'untargeted' screens that attempt to capture the full biomolecular diversity of patient specimens—according to one recent review, up to 5,000 distinct metabolites have been detected in humans to date[2].

Many metabolites undergo subtle chemical modifications that critically alter their function or bioavailability, but also make them challenging to identify via standard analysis. In one of the Top 10 Articles presented here—a collection of our most-read articles on the subject of LC and MS—the authors employ a variant of LC based on 'in-source collision-induced dissociation' (ISCID) to separate molecules from their modifications, which are then identified via specialized software. This made it possible to identify 900 different modified metabolites in urine samples from patients with liver cirrhosis[3].

LC-MS/MS has been the standard method for metabolomics profiling for some time now, but other methods are also being explored. For example, ion mobility MS (IM-MS) uses an electrical field to separate ions based on properties such as size and shape prior to MS analysis. This can in turn be coupled with ultraperformance liquid chromatography (UPLC) to achieve superior separation and enhanced metabolite identification with a higher signal-to-noise ratio[4].

MS-based imaging is also opening up intriguing new possibilities, which were explored in a recent feature from *Analytical Chemistry*[5]. Typical experiments entail sequential collection of MS data at discrete points on the sample, generating site-specific metabolomic data that can be directly mapped to the histological features of the specimen. This could prove extremely useful for characterizing tumor heterogeneity or defining the borders of a malignancy, and can even offer insights into drug response at the cellular level.

All of these advances collectively suggest that a future is rapidly approaching in which MS-based techniques are a major component of the clinical diagnostics world. In that spirit, please enjoy this special focus on Advances in Chromatography and Mass Spectrometry.

References

1. https://www.aacc.org/publications/cln/articles/2015/february/future-of-mass-spec
2. Guma, M., Tiziani, S. & Firestein, G.S. Metabolomics in rheumatic diseases: desperately seeking biomarkers. *Nat. Rev. Rheumatol.* Published online 3 March 2016 (DOI:10.1038/nrrheum.2016.1)
3. Dai, W., Yin, P., Zeng, Z. et al. Nontargeted modification-specific metabolomics study based on liquid chromatography - high-resolution mass spectrometry. *Anal. Chem.* **2014,** 86, pp. 9146-9153 (DOI:10.1021/ac502045j)
4. Paglia, G., Williams, J.P., Menikarachchi, L. et al. Ion mobility derived collision cross sections to support metabolomics applications. *Anal. Chem.* **2014,** 86, 3985-3993 (DOI:10.1021/ac500405x)
5. Addie, R.D., Balluff, B., Bovee, J.V.M.G. et al. Current state and future challenges of mass spectrometry imaging for clinical research. *Anal. Chem.* **2015,** 87, 6426-6433 (DOI: 10.1021/acs.analchem.5b00416)

TOP TEN CHROMATOGRAPHY AND MASS SPECTROMETRY PAPERS

These are the most-read papers in high-performance liquid chromatography (HPLC)/mass spectrometry (MS) from *Analytical Chemistry* and *Journal of Proteome Research* over the past 12 months.

Mass-Spectrometry-Based Molecular Characterization of Extracellular Vesicles: Lipidomics and Proteomics

Simion Kreimer†, Arseniy M. Belov†, Ionita Ghiran||, Shashi K. Murthy†, David A. Frank⊥#, and Alexander R. Ivanov*†

†Northeastern University, Boston, Massachusetts, USA || Beth Israel Deaconess Medical Center, Boston, Massachusetts, USA ⊥ Dana–Farber Cancer Institute, Boston, Massachusetts, USA #Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts, USA

J. Proteome Res., **2015,** 14 (6), pp 2367–2384

DOI: 10.1021/pr501279t

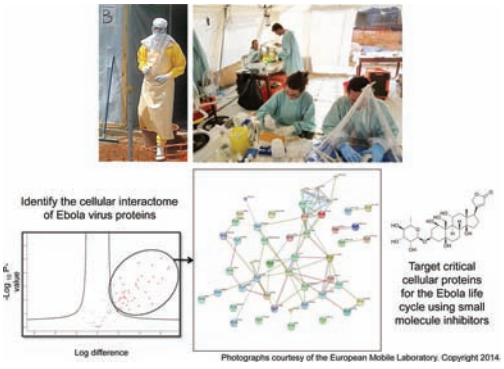
Elucidation of the Ebola Virus VP24 Cellular Interactome and Disruption of Virus Biology through Targeted Inhibition of Host-Cell Protein Function

Isabel García-Dorival†‡, Weining Wu†, Stuart Dowall§, Stuart Armstrong†‡, Olivier Touzelet†, Jonathan Wastling†‡, John N. Barr||, David Matthews⊥, Miles Carroll‡§, Roger Hewson*‡§, and Julian A. Hiscox*†‡

† University of Liverpool, Liverpool, United Kingdom ‡ NIHR Health Protection Research Unit in Emerging and Zoonotic Infections, Liverpool, United Kingdom § Public Health England, Salisbury, United Kingdom || University of Leeds, Leeds, United Kingdom ⊥ University of Bristol, Bristol, United Kingdom

J. Proteome Res., **2014,** 13 (11), pp 5120–5135

DOI: 10.1021/pr500556d



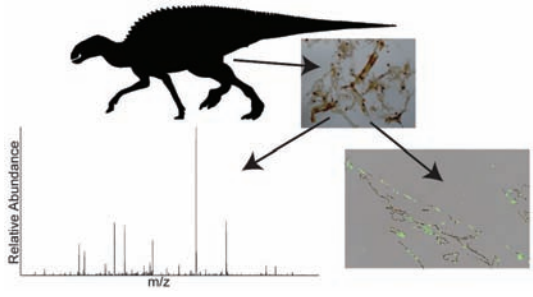
Mass Spectrometry and Antibody-Based Characterization of Blood Vessels from *Brachylophosaurus canadensis*

Timothy P. Cleland*†, Elena R. Schroeter†, Leonid Zamdborg§, Wenxia Zheng†, Ji Eun Lee§||, John C. Tran⊥, Marshall Bern#, Michael B. Duncan∇○, Valerie S. Lebleu∇○◆, Dorothy R. Ahlf⊥, Paul M. Thomas⊥, Raghu Kalluri∇○◆¶, Neil L. Kelleher⊥, and Mary H. Schweitzer†□

† North Carolina State University, Raleigh, North Carolina, USA § University of Illinois, Urbana, Illinois, USA || Korea Institute of Science and Technology, Seoul, Republic of Korea ⊥ Northwestern University, Evanston, Illinois, USA # Protein Metrics, San Carlos, California, USA ∇ Beth Israel Deaconess Medical Center, Boston, Massachusetts, USA ○ Harvard Medical School, Boston, Massachusetts, USA ◆ University of Texas MD Anderson Cancer Center, Houston, Texas, USA ¶ Harvard University, Cambridge, Massachusetts, USA □ North Carolina Museum of Natural Sciences, Raleigh, North Carolina, USA

J. Proteome Res., **2015,** 14 (12), pp 5252–5262

DOI: 10.1021/acs.jproteome.5b00675



Toward Merging Untargeted and Targeted Methods in Mass Spectrometry-Based Metabolomics and Lipidomics

Tomas Cajka† and Oliver Fiehn*†‡

† University of California Davis, Davis, California, USA ‡ King Abdulaziz University, Jeddah, Saudi Arabia

Anal. Chem., **2016,** 88 (1), pp 524–545

DOI: 10.1021/acs.analchem.5b04491

Advances in Ultrahigh-Pressure Liquid Chromatography Technology and System Design

Jelle De Vos, Ken Broeckhoven, and Sebastiaan Eeltink*

Vrije Universiteit Brussel, Brussels, Belgium

Anal. Chem., **2016,** 88 (1), pp 262–278

DOI: 10.1021/acs.analchem.5b04381

Core–Shell, Ultrasmall Particles, Monoliths, and Other Support Materials in High-Performance Liquid Chromatography

Nobuo Tanaka† and David V. McCalley*

†GL Sciences Inc., Saitama, Japan * University of the West of England, Bristol, U.K.

Anal. Chem., **2016,** 88 (1), pp 279–298

DOI: 10.1021/acs.analchem.5b04093

Novel Approach for Analysis of Bronchoalveolar Lavage Fluid (BALF) Using HPLC-QTOF-MS-Based Lipidomics: Lipid Levels in Asthmatics and Corticosteroid-Treated Asthmatic Patients

Yun Pyo Kang†, Won Jun Lee†, Ji Yeon Hong†, Sae Bom Lee†, Jeong Hill Park†, Donghak Kim‡, Sunghyouk Park†, Choon-Sik Park§, Sung-Woo Park*§, and Sung Won Kwon*†

† Seoul National University, Seoul, Republic of Korea ‡ Konkuk University, Seoul, Republic of Korea § Soonchunhyang University Bucheon Hospital, Bucheon, Republic of Korea

J. Proteome Res., **2014,** 13 (9), pp 3919–3929

DOI: 10.1021/pr5002059

Miniature and Fieldable Mass Spectrometers: Recent Advances

Dalton T. Snyder, Christopher J. Pulliam, Zheng Ouyang, and R. Graham Cooks*

Purdue University, West Lafayette, Indiana, USA

Anal. Chem., **2016,** 88 (1), pp 2–29

DOI: 10.1021/acs.analchem.5b03070

High-Pressure Open-Channel On-Chip Electroosmotic Pump for Nanoflow High Performance Liquid Chromatography

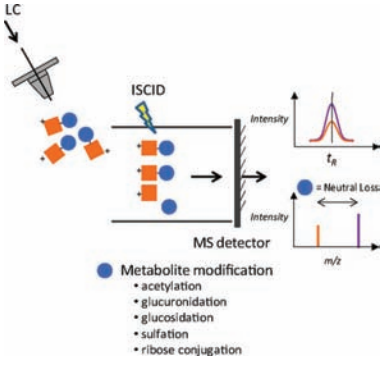
Wei Wang†, Congying Gu†, Kyle B. Lynch†, Joann J. Lu†, Zhengyu Zhang†, Qiaosheng Pu*‡, and Shaorong Liu*†

† University of Oklahoma, Norman, Oklahoma, USA ‡ Lanzhou University, Lanzhou, P.R. China

Anal. Chem., **2014,** 86 (4), pp 1958–1964

DOI: 10.1021/ac4040345

Nontargeted Modification-Specific Metabolomics Study Based on Liquid Chromatography–High-Resolution Mass Spectrometry



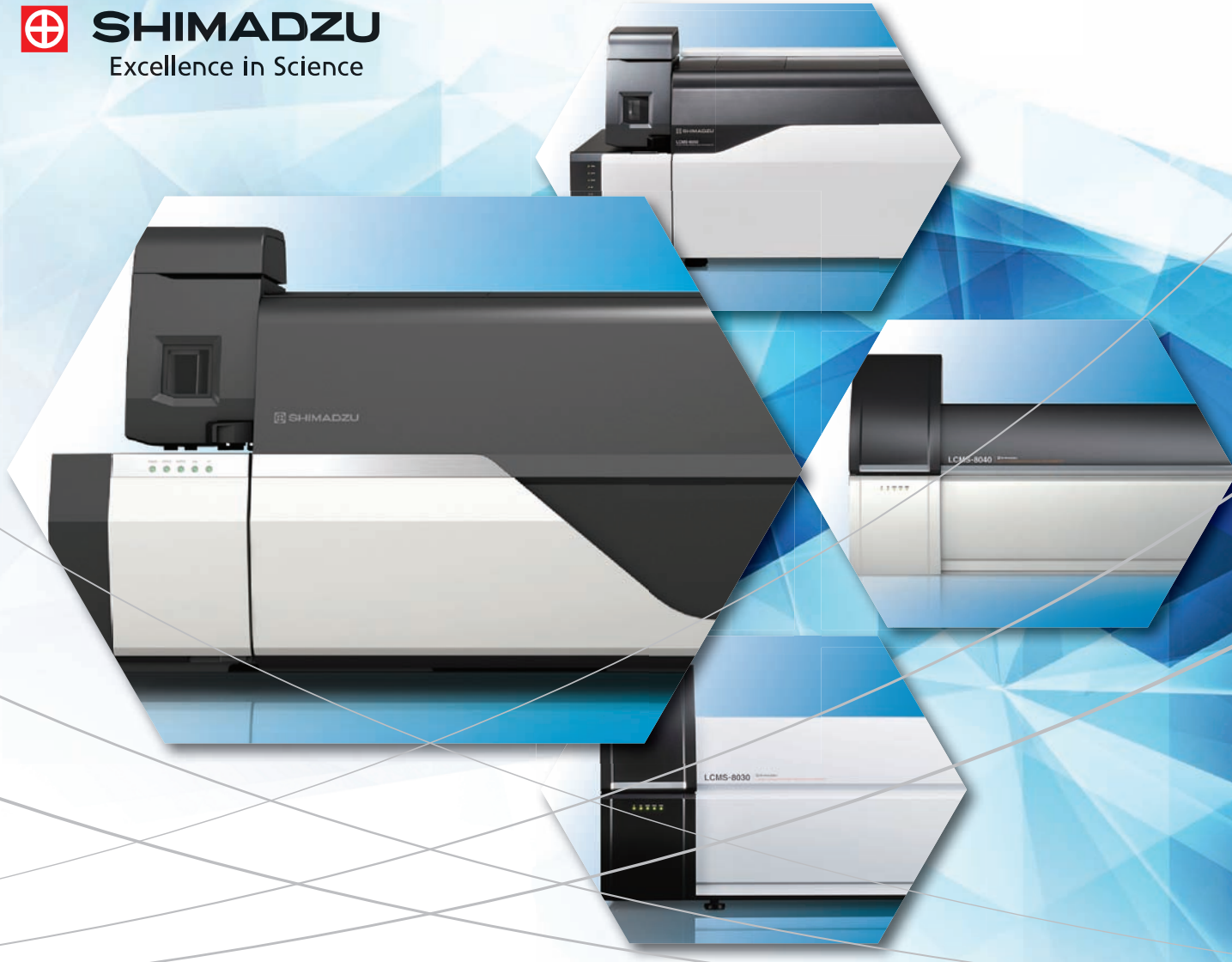
Weidong Dai†, Peiyuan Yin†, Zhongda Zeng†, Hongwei Kong†, Hongwei Tong‡, Zhiliang Xu†, Xin Lu†, Rainer Lehmann*§∥⊥, and Guowang Xu*†

† Chinese Academy of Sciences, Dalian, China ‡ The First Affiliated Hospital of Zhejiang Chinese Medical University, Hangzhou, China § University Hospital Tuebingen, Tuebingen, Germany∥ Institute for Diabetes Research and Metabolic Diseases of the Helmholtz Centre Munich at the University of Tuebingen, Tuebingen, Germany ⊥ German Center for Diabetes Research, Tuebingen, Germany

Anal. Chem., **2016,** 86 (18), pp 9146-9153

DOI: 10.1021/ac502045j

SHIMADZU
Excellence in Science



Redefining Mass Spectrometry

Shimadzu's Triple Quad LCMS Instruments Provide Solutions to the Most Challenging Problems

The ever-changing demands of each unique industry require not only the right people but also the best technology. Companies require speed, sensitivity, and the flexibility to adapt to meet new challenges and requirements. For years, Shimadzu has been known as the leader in HPLC; now Shimadzu is being recognized as the leading manufacturer of triple quadrupole mass spectrometers. Discover Shimadzu's portfolio of **LC-MS/MS** instrumentation for the ultimate in ultrafast analysis without compromising sensitivity.

Learn more about Shimadzu's LC-MS/MS family.
Call (800) 477-1227 or visit us online at
www.ssi.shimadzu.com/LCMS

Order consumables and accessories on-line at http://store.shimadzu.com
Shimadzu Scientific Instruments Inc., 7102 Riverwood Dr., Columbia, MD 21046, USA

Shimadzu's LC-MS/MS instrumentation includes:

- **LCMS-8060:** offers an incomparable fusion of LC-MS/MS speed and sensitivity to achieve new levels of data quality and throughput
- **LCMS-8050:** High-sensitivity LC-MS/MS incorporating patented ultrafast technologies to provide excellent performance no matter the matrices
- **LCMS-8040:** An LC-MS/MS system redesigned for enhanced sensitivity for complex matrices and difficult analytes
- **LCMS-8030:** A rugged LC-MS/MS instrument built for routine quantitative analysis in challenging matrices

No longer settle for anything less than the best.

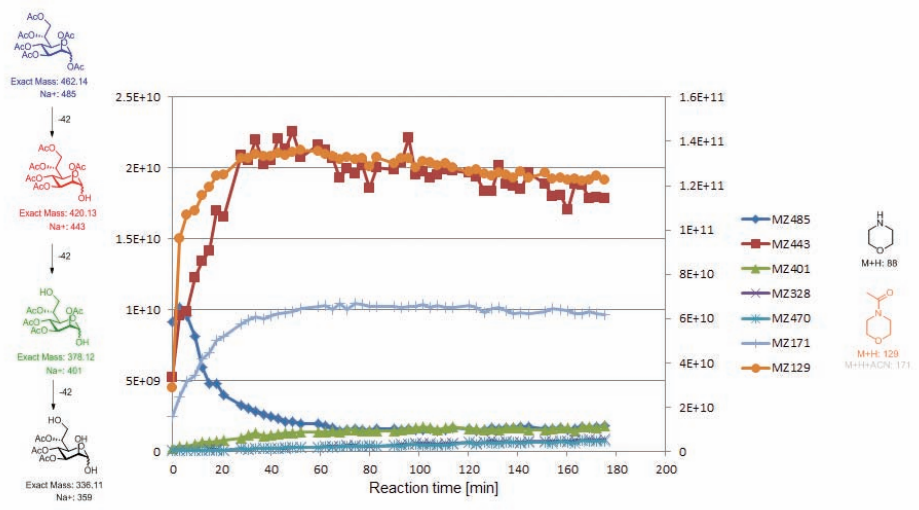
FLOW CHEMISTRY MONITORING AND OPTIMIZATION USING COMPACT MASS SPECTROMETRY

ADVION, INC

Introduction

A vital component of developing flow chemical synthesis is the ability to monitor the reactions in real-time. Techniques such as liquid chromatography/mass spectrometry (LC/MS) and gas chromatography/mass spectrometry (GC/MS) can take too long, whilst techniques such as infrared (IR) and near-infrared (NIR) do not have the specificity required to obtain detailed information on the reaction.

This note covers work carried out at Leeds University with co-workers from Durham University in the area of flow chemical synthesis using the expression Compact Mass Spectrometer (CMS) from Advion of Ithaca, NY.



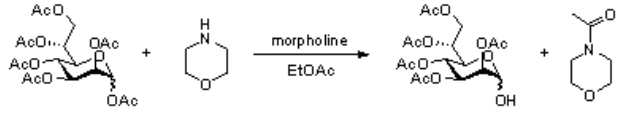


Figure 1
Anomeric deacetylation reaction.

Instrument Set-up

A syringe was used such that the reaction mixture was infused into the mass spectrometer via a valve. Data from the expression CMS were then fed into the reaction optimisation and data processing software suite.

Results

Data obtained for the anomeric deacetylation reaction are shown in Figure 1, which illustrates monitoring, in real-time, of the simultaneous increase in product and decrease in starting material. Intermediates and impurities are also observed, thus providing valuable information about the reaction. This information gives the chemist an advantage in reaction/process understanding that is not available from other techniques.

The detailed data obtained mean that reaction progress is understood to a much higher degree, enabling further optimization, which is vital for process development, and can provide increased mechanistic understanding that can be vital in developing the chemistries further.

Conclusions

- The expression CMS is an ideal mass spectrometer for integration with flow chemistry systems.
- The input and output options available on the expression give it a uniquely flexible interfacing capability.
- MS provides detailed and real-time information about reactions often not possible from other analytical techniques (e.g., chromatography, NMR, IR/NIR, ultraviolet spectroscopy).
- Advion is experienced in the integration of the CMS into novel synthetic chemistry solutions.

Acknowledgments

Thank you to Ian Baxendale and Christian Stanetty at Durham University as well as Richard Bourne and Nicholas Holmes at Leeds University. ■

Additional information:
http://www.advion.com/applications/expression-applications/reaction-monitoring-by-fia/

Advion, Inc
10 Brown Road, Suite 101
Ithaca, NY 14850
Tel: (607) 266-9162
Email: info@advion.com
Website: http://www.advion.com/

Raptor™ LC Columns

SPP speed. USLC® resolution.
A new species of column.

- Drastically faster analysis times.
- Substantially improved resolution.
- Increased sample throughput with existing instrumentation.
- Dependable reproducibility.

Choose Raptor™ SPP LC columns for all of your valued assays to experience *Selectivity Accelerated.*

www.restek.com/raptor

RESTEK®
Pure Chromatography

www.restek.com/raptor