

Evolution of Multi-Dimensional Chromatography from Research to Main Stream Pharmaceutical Analysis

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ABSTRACT

In the past decade, improvements in two-dimensional liquid chromatographic (2D-LC) instruments along with enhancement in software capabilities has transformed 2D-LC from the hands of experienced researchers to analytical laboratories around the world. 2D-LC offers novel solutions to problems ranging from complex samples requiring excessively large peak capacity to simple, yet difficult to resolve compounds. Recent developments in 2D-LC and 2D-LC-MS have clearly demonstrates the potential of this technique and is becoming an essential tool in modern day pharmaceutical industry.

The presentation will cover real world applications of 2D-LC-MS in assessing stability-indicating method for potential co-elution, monitoring on-column conversion, addressing dynamic range issue of one-dimensional chromatography, analysis of residual genotoxic impurities in the midst of main component differing significantly in their concentration ($>10^5$), simultaneous quantitative achiral-chiral analysis of chiral compounds. In addition, attributes critical for the progression of 2D-LC from research to quality control environment like linearity, accuracy, LOD & LOQ and precision will be discussed. In addition, design and application of 2D-LC-SFC in simultaneous achiral-chiral analysis will be presented.

BIOGRAPHY

C.J. Venkatramani is a senior scientist at Genentech USA and has over 20 years experience in the pharmaceutical industry. He was a key member of the Genentech technical team instrumental in the successful launch of Genentech's first small molecule *Erivedge*, leading from development to commercial. *Erivedge* is currently approved in several countries for the treatment of advanced basal cell carcinoma (BCC). He has several publications, national and international presentations on multidimensional separation. Over the years, he has successfully used multidimensional chromatography to address challenging problems commonly encountered in pharmaceutical industry. His areas of interest include 2D-LC, 2D-LC-SFC, ultra-trace analysis of genotoxic impurities and elemental impurities.

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SUMMARY

Senior Scientist with over 20 years of experience in major pharmaceutical companies providing analytical strategies and solutions to projects ranging from early stages of drug development to commercial products

EDUCATION

Ph. D. in Analytical Chemistry, Southern Illinois University, Carbondale, IL

Advisor: Late Professor John B. Phillips, Ph.D.

Thesis: Comprehensive Two-Dimensional Gas Chromatography

MS in Analytical Chemistry, University of Bombay, India

BS in Analytical Chemistry, University of Bombay, India

EXPERIENCE

GENENTECH, SOUTH SAN FRANCISCO, CA

Senior Scientist, SMPS

August 2013 - Current

GENENTECH, SOUTH SAN FRANCISCO, CA

Scientist, SMPS

2007 – July 2013

ABBOTT LABS, NORTH CHICAGO, IL

Research Investigator, Global Pharmaceutical R&D

SEARLE/PHARMACIA/PFIZER, SKOKIE, IL

Scientist, Chemical Development & Global Supply

MALLINCKRODT INC, ST LOUIS, MO

Analytical Research Scientist, Imaging R&D

THE PROCTER AND GAMBLE, CINCINNATI, OH

Summer Intern

DOSSIERS

NDA and MAA SUBMISSIONS

NDA & MAA authoring of Genentech's first small molecule *Erivedge* - Control and Stability Sections, Dossier Designer and Reviewer, Dossier PAI, Risk Mitigation Strategy and Regulatory Defense

PATENT APPLICATION

C. J. Venkatramani and Mohammad Al-Sayah, **“Systems and Methods for Two-Dimensional Chromatography (2D LC x SFC)”**, GNE Ref Number: P32332-US-PR; MoFo Ref: 14639-30281.00

WEBINAR

C. J. Venkatramani, **“Harnessing multi-dimensional chromatography in pharmaceutical analysis”** C&E News, World-wide broadcast, October 2015.

PUBLICATIONS

- 1) C. J. Venkatramani, **“What can 2D-LC offer the pharmaceutical industry”**, in-press, *LCGC Europe*, October (2018).
- 2) Kelly Zhang, Kenji L. Kurita, C.J. Venkatramani and David Russell, **“Seeking universal detectors for analytical characterizations”**, *Journal of Pharmaceutical and Bio-Medical Analysis*, 162, 192-204 (2019)
- 3) D.R. Stoll, C.J. Venkatramani, S.C. Rutan, **“Peak purity in liquid chromatography, Part III: Using two-dimensional liquid chromatography”**, *LCGC North America*, 36, 6, 356-361 (2018).
- 4) Daniel W. Cook, Sarah C. Rutan, C.J. Venkatramani, and Dwight R. Stoll, **“Peak purity in liquid chromatography, Part II: Potential of curve resolution techniques”**, *LCGC North America*, 36, 4, 248-255 (2018).
- 5) Sarah.C. Rutan, C.J. Venkatramani and Dwight.R. Stoll, **“Peak purity in liquid chromatography, Part I: Basic concepts, commercial software, and limitations”**, *LCGC North America*, 36, 2, 100-110 (2018).
- 6) Meenakshi Goel, Eli Larson, C.J. Venkatramani and Mohammad Al-Sayah, **“Optimization of a two-dimensional liquid chromatography supercritical fluid chromatography-mass spectrometry (2D-LC-SFC-MS) system to assess in-vitro inter-conversion of chiral drug molecules”**, *Journal of Chromatography B*, 1084, 89-95 (2018).

- 7) C.J. Venkatramani, Shu Rong Huang, Mohammad Al-Sayaha, Ila Patel, and Larry Wigman, **“High-resolution 2D-LC analysis of key linker drug intermediate used in antibody drug conjugates (ADCs)”**, *Journal of Chromatography A*, 1521, 63-72 (2017).
- 8) S. Stowers, A. Kumar, D. Carrera, C. Gu, P. Patel, C. Venkatramani, D. Stoianov, and L. Wigman, **“Analytical Methodology for Characterization of Reactive Starting Materials and Intermediates Commonly Used in the Synthesis of Small Molecule Pharmaceuticals”**, *American Pharmaceutical Review*, 20(5), 76-84 (2017).
- 9) I. Patel, C.J. Venkatramani, Andrew Stumpf and Larry Wigman, **“Trace Analysis of Potential Mutagenic Boronic Acids and Esters”**, *OPReD*, 21 (2), 182-186 (2017).
- 10) Remy Angelaud, Mark Reynolds, C. J. Venkatramani, Scott Savage, Hulderich Trefelet, Thomas Landmesser, Peter Demel, Oliver Ruha, Baerbel Rueckert and Heinz Jaeggi, **“Manufacturing Development and Genotoxic Impurity Control Strategy of the Hedgehog Pathway Inhibitor Vismidigib”**, 20, 8, 1509-1519, *OPReD* (2016).
- 11) C.J. Venkatramani, Mohammad Al-Sayah, Guannan Li, Meenakshi Goel, James Girotti, Lisa Zang, Larry Wigman, Peter Yehl and Nik Chetwyn, **“Simultaneous Achiral-Chiral Analysis of Pharmaceutical Compounds using Two-Dimensional Reversed Phase Liquid Chromatography x Supercritical Fluid Chromatography”**, *Talanta*, 148, 548-555 (2016).
- 12) Ila Patel, C. J. Venkatramani, Colin Medley, Kavita Mistry, Larry Wigman, **“Enhancing efficiency of trace metal analysis using a validated generic ICP-OES Method”**, *American Pharmaceutical Review*, 18(1) (2015).
- 13) C. J. Venkatramani and James Girotti, **“Assessing Stability-Indicating Methods for Co-elution by Two-Dimensional Liquid Chromatography with Mass Spectrometry”**, *Journal of Separation Science*, 37, 3214-3225 (2014).
- 14) C. J. Venkatramani and Mohammad Al-Sayah, **“Analytical Strategies for Genotoxic Impurities in the Pharmaceutical Industry, American Pharmaceutical Review”**, *American Pharmaceutical Review*, 17(5) (2014).
- 15) C. J. Venkatramani, Larry Wigman and Nik Chetwyn, **“Applications of Two-Dimensional Liquid Chromatography in Pharmaceutical Analysis”**, *Bio-Pharma Asia*, January-February (2014).
- 16) C. J. Venkatramani, Larry Wigman, Kavita Mistry and Nichols Chetwyn, **“Simultaneous, sequential quantitative achiral-chiral analysis by two-dimensional liquid chromatography”**, *Journal of Separation Science*, Vol. 35, p1748, 2012.
- 17) Yong Cui, Xiling Song, King Chuang, C. J. Venkatramani, Sueanne Lee, Gregory Gallegos, T.G. Venkateshwaran, Minli Xie, **“Variable selection in multivariate modeling of drug product formula and manufacturing process”**, *Journal of Pharmaceutical Sciences* (2012).
- 18) C. J. Venkatramani and Anurag Patel, **“Towards a comprehensive 2D-LC-MS separation”**, *Journal of Separation Science*, 29, 475 (2006).

- 19) C. J. Venkatramani and Yuri Zelechonok, **“2D-LC with mixed mode stationary phases”**, *Journal of Chromatography*, 66, 47 (2005).
- 20) C. J. Venkatramani and Yuri Zelechonok, **“An Automated orthogonal two-dimensional liquid chromatograph”**, *Analytical Chemistry*, 75, 3484 (2003).
- 21) C. J. Venkatramani, Jingzhen Xu, and John B. Phillips, **“Separation orthogonality in temperature programmed comprehensive two-dimensional gas chromatography”**, *Analytical Chemistry*, 68, 1486 (1996).
- 22) C. J. Venkatramani and John B. Phillips, **“Comprehensive two-dimensional gas chromatography applied to the analysis of complex mixtures”**, *Journal of Micro column Separations*, 5, 511 (1993).
- 23) J. D. Pinkston, C. J. Venkatramani, L. J. Tulik, D. J. Bowling, and K. R. Wehmeyer, **“Evaluation of capillary supercritical fluid chromatography with mass spectrometric detection for the analysis of a drug (mebeverine) in a dog plasma matrix”**, *Journal of Chromatography*, 622, 209 (1993).
- 24) C. J. Venkatramani and John B. Phillips, **“Comprehensive two-dimensional gas chromatography applied to the analysis of complex mixtures”**, *Fifteenth international symposium on capillary chromatography*, Riva-del-Garda, Italy (1993).
- 25) K. R. Wehmeyer, C. J. Venkatramani and P. A. Rodrigues, **“Simple modification of a commercially available gas chromatography to perform automated programmed temperature vaporization injection”**, *ACS*, Ohio, USA (1992).

ORAL PRESENTATIONS

- 1) C. J. Venkatramani and Anne Kraft, **“Novel application of 2D-LC-MS in assessing enantiomeric purity of complex linker drug intermediates with multiple chiral centers used in ADCs”**, *HPLC2018, Washington DC, 2018*.
- 2) C. J. Venkatramani, Mohammad Al-Sayah, Meenakshi Goel and Gunnan Li, **“Simultaneous achiral-chiral analysis of pharmaceutical compounds”**, *Pittcon2018, Orlando, 2018*.
- 3) C. J. Venkatramani, Ila Patel and Shu Rong Huang **“Towards a detailed characterization of linker drugs using 2D-LC-MS”**, *Pittcon2017, Chicago, 2017*.
- 4) C. J. Venkatramani, Mohammad Al-Sayah, Ila Patel, Larry Wigman, Jacob Kay, Meenakshi Goel and Shu Rong Huang **“High-resolution analysis of linker drugs used in ADC’s by 2D-LC-MS; Transition of 2D-LC-MS from research to main stream pharmaceutical analysis”**, *HPLC2017.Prague, Czech Republic, 2017*.

- 5) C. J. Venkatramani, Ila Patel and Larry Wigman, **“Analytical strategies for genotoxic impurities”**, *Knect365, Prague, Czech Republic, 2017.*
- 6) C. J. Venkatramani, Rolf Schulte Oestrich and Andrew Marriott, Round Table Discussions on **Genotoxic Impurities**, *Knect365, Prague, Czech Republic, 2017*
- 7) C. J. Venkatramani, Mohammad Al-Sayah, Ila Patel, Larry Wigman, Jacob Kay, Meenakshi Goel and Shu Rong Huang **“Applications of Multidimensional Chromatography in Real World Pharmaceutical Analysis”**, *EAS2017, Plainsboro, NJ, 2017.*
- 8) C. J. Venkatramani, **“Applications of Multi-Dimensional Chromatography in Pharmaceutical Analysis”**, *HPLC 2016, San Francisco, USA, 2016.*
- 9) C. J. Venkatramani, **“Applications of Multi-Dimensional Chromatography in Pharmaceutical Method Development”**, *Informa2016, Berlin, Germany, 2016.*
- 10) C. J. Venkatramani, Mohammad Al-Sayah, Guannan Li, Meenakshi Goel, James Girotti and Lisa Zang, **“Design of a true orthogonal multi-dimensional chromatographic system and its applications in pharmaceutical analysis – 2D LCxSFC”**, *HPLC 2015, Geneva, Switzerland.*
- 11) C. J. Venkatramani, Mohammad Al-Sayah, Guannan Li, Meenakshi Goel James Girotti and Lisa Zang, **“Harnessing Multi-dimensional chromatography in Pharmaceutical Analysis”**, *CoSMos, San Diego, 2015.*
- 12) C. J. Venkatramani, **“Multi-dimensional technologies in real world pharmaceutical analysis”**, Agilent Technologies, *Santa Clara, October 2015.*
- 13) C.J. Venkatramani and Mohammad Al-Sayah, **“Extending Capabilities of Multi-Dimensional Chromatography in Pharmaceutical Analysis – 2D LCxSFC”**, Roche AHM, *Ireland, 2014.*
- 14) C. J. Venkatramani, **“Applications of multi-dimensional chromatography in pharmaceutical analysis”**, Agilent Technologies, *Santa Clara, 2014.*
- 15) C. J. Venkatramani, Ila Patel, Larry Wigman and James Girotti, **“Analytical strategies for ultra-trace analysis of genotoxic impurities”** *Informa2014 GTI conference, Berlin, Germany, 2014.*
- 16) C. J. Venkatramani, Larry Wigman, Nik Chetwyn, and James Girotti, **“2D-LC-MS: Transitioning from Research Laboratories to main Stream Pharmaceutical Analysis”**, *HPLC2014, New Orleans, USA, 2014.*
- 17) C. J. Venkatramani, Larry Wigman, and James Girotti, **“Method development strategies for pharmaceutical analysis using 2D-LC and 2D-LC-MS”**, *Pittcon2014, Chicago, 2014.*

- 18) C. J. Venkatramani, James Girotti, Larry Wigman and Nik Chetwyn, **Applications of two-dimensional liquid chromatography in pharmaceutical analysis**, *HPLC2013, Amsterdam, Netherlands, 2013*.
- 19) Chunang (Christine) Gu, Kate Comstock, Hongxia (Jessica) Wang, Larry Wigman, C. J. Venkatramani, Alan Deese, **Quantitative analysis of potential genotoxic impurities by high-resolution mass spectrometry**, *ASMA, Vancouver, BC, Canada, 2012*.
- 20) C. J. Venkatramani, Nicholas Chetwyn and Kavita Mistry, **“Application of 2D-LC in pharmaceutical analysis”**, *Pacificchem2010, Honolulu, Hawaii, 2010*.
- 21) C. J. Venkatramani, Anurag patel and Yuri Zelechonok, **“Comprehensive two-dimensional liquid chromatography in pharmaceutical analysis”**, *APC2002, Michigan, 2002*.
- 22) C. J. Venkatramani and John Polta, **“HPLC analysis of non-chromophoric amines by pre-column derivatization with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC)”**, *Pittcon-1999, Orlando, USA, 1999*.
- 23) C. J. Venkatramani and John B. Phillips, **“Towards a complete analysis of petroleum product mixtures by comprehensive multidimensional gas chromatography”**, *Pittcon1994, Chicago, USA, 1994*.
- 24) C. J. Venkatramani, Liling Zhang Patricia O' Neal and John B. Phillips, **“High-speed comprehensive two-dimensional gas chromatography”**, *FACCS1993, Detroit, USA, 1993*.
- 25) K. R. Wehmeyer, C. J. Venkatramani and P.A. Rodriguez, **“Simple modification of a commercially available gas chromatography to perform programmed temperature vaporization injection”**, *ACS, Ohio, USA, 1992*.

POSTER PRESENTATIONS

- 1) Ila Patel, C. J. Venkatramani, Andy Stumpf, Larry Wigman, **“Determination of cyclopropyl boronic acid (CPBA) a genotoxic impurity in Pharmaceutical compound by ICP-MS”**, *AAPS, San Diego, 2015*
- 2) C. J. Venkatramani, Larry Wigman, James Girotti and Ila Patel, **“Analytical strategies for genotoxic impurities”**, *HPLC 2014, New Orleans, 2014*.
- 3) C. J. Venkatramani, Larry Wigman, Kavita Mistry and Nichols Chetwyn, **“Simultaneous achiral-chiral analysis by two-dimensional liquid chromatography”**, *HPLC 2012, Anaheim, CA, 2012*.
- 4) C. J. Venkatramani, James Girotti, Larry Wigman and Nichols Chetwyn, **“Quantitative analysis of reactive acid chloride, a potential genotoxic impurity”**, *HPLC 2012, Anaheim, CA, 2012*.
- 5) C. J. Venkatramani and Yury Zelechonok, **“Two-dimensional liquid chromatography with the same mixed mode stationary phase in both dimensions”**, *HPLC2003, Nice, France, 2003*.
- 6) C. J. Venkatramani, Anurag Patel and Yuri Zelechonok, **“Comprehensive two-dimensional**

liquid chromatography in pharmaceutical analysis”, HPLC 2002, Montreal, Canada, 2002.

7) C. J. Venkatramani and John B. Phillips, **“Comprehensive two-dimensional gas chromatography applied to the analysis of complex sample mixtures”**, *The 15th international symposium on capillary chromatography, Riva-del-Garda, Italy, 1993.*

8) John B. Phillips, Z. Liu, C. J. Venkatramani and V. Jain, **“Comprehensive two-dimensional gas chromatography”**, *The 13th international symposium on capillary chromatography, Riva-del-Garda, Italy, 1993.*