Customer Testimonials & Presentations

Fusion QbD Software Platform

S-Matrix Corporation

www.smatrix.com

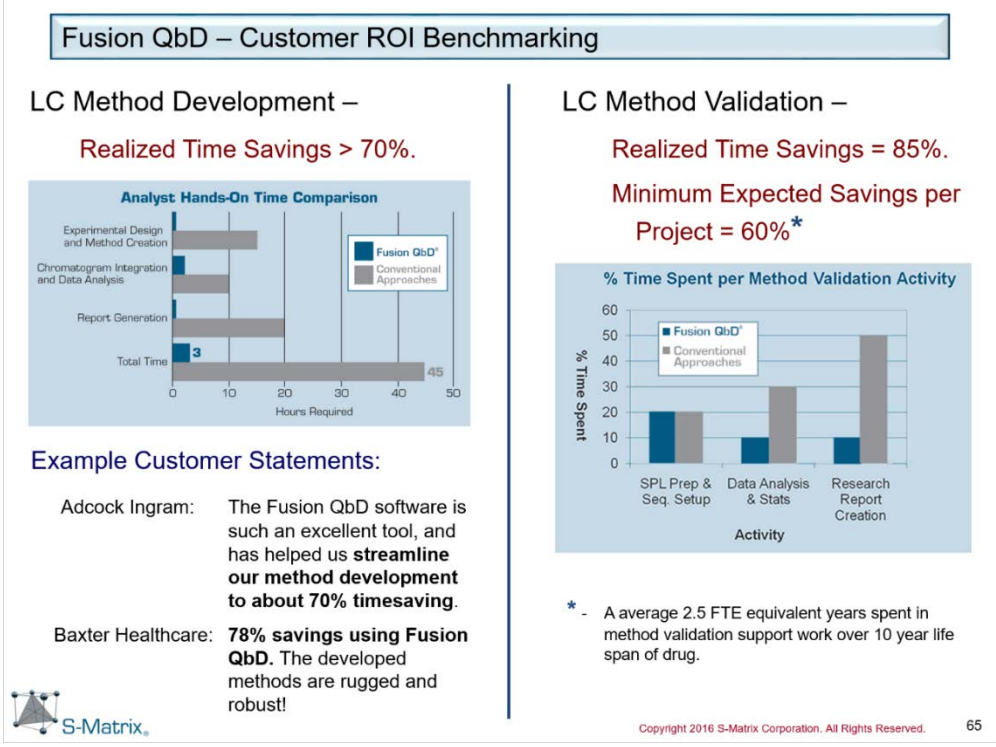
Contents

The Fusion QbD Software Platform (Fusion QbD®) has been used in the Pharmaceutical Industry for over 10 years. Analytical R&D customers have successfully applied the Fusion LC Method Development module (FMD) and the Fusion Method Validation module (FMV) to develop, optimize, qualify, and formally validate LC methods according to QbD guidelines. The software has been used successfully for a wide variety of sample types, including small molecules, peptides, proteins, and nucleotides, and supports a wide range of chromatographic techniques for these samples, including reversed phase, normal phase, ion exchange, HILIC and Chiral separations. Analytical R&D customers also gain dramatic increases in efficiency using the Fusion Product Development module (FPD) to develop robust Non-LC methods such as GC, CE, MS, and Dissolution. In QC the Fusion Inhaler Testing module (FIT) is saving customers many times the software's initial cost every year.

The following pages present some of the many public examples of customer successes achieved using Fusion QbD. In every case:

- Fusion QbD always dramatically improved method performance.
- Fusion QbD always profoundly reduces the development and validation timeline.

  [Note that in some cases herein the platform is referred to as "Fusion AE" – this is the previous product name for Fusion QbD.]



Large Molecule – used for both early chemistry system screening and method optimization with integrated robustness. Successful development and transfer of multiple methods. Method robustness proven on transfer to QC.

- Internal benchmarking showed profound reduction in method development time with far superior results over other approaches.

ACS 2015: **Book Chapter on mAbs – all work done using Fusion QbD.**

State-of-the-Art and Emerging Technologies for Therapeutic Monoclonal Antibody Characterization Volume 2. Biopharmaceutical Characterization: The NISTmAb Case Study.

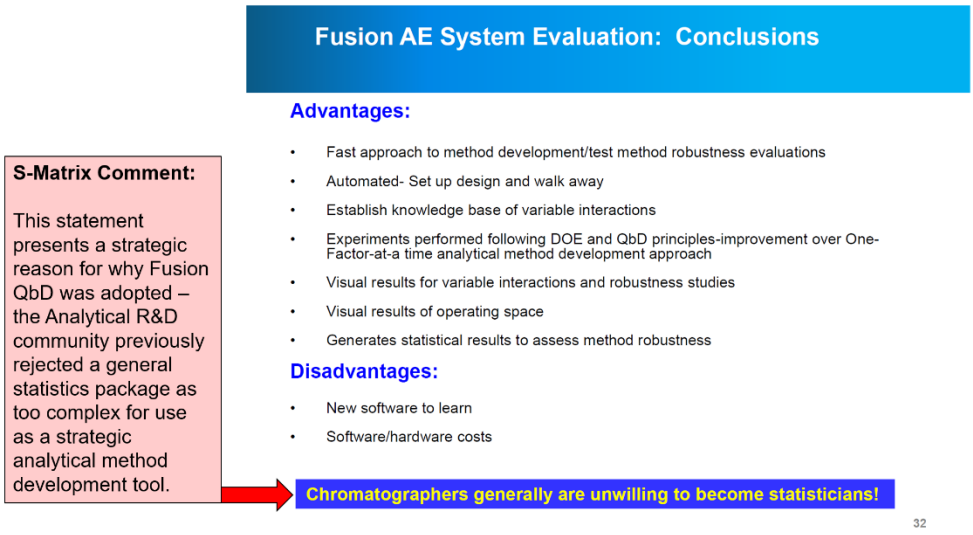
Editors: John Schiel, Darryl Davis, Oleg Borisov, Copyright © 2015 American Chemical Society

http://www.nist.gov/mml/bmd/nist-mab.cfm

Separation Methods and Orthogonal Techniques

http://pubs.acs.org/doi/abs/10.1021/bk-2015-1201.ch005

BPI 2013: **BioProcess International Meeting – Fusion AE Evaluation: A QbD Approach to Method Development and Robustness Studies (SEC-LC and CEX-LC)**

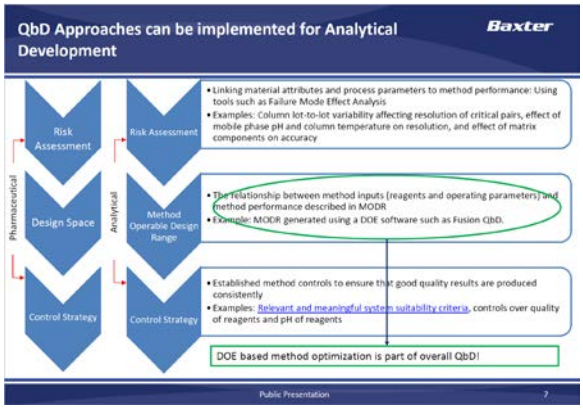


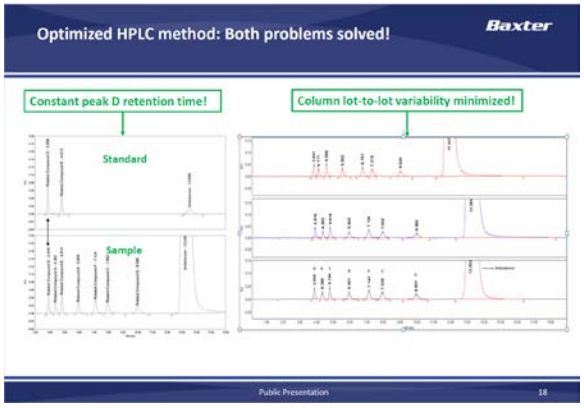
Key ongoing results of using Fusion QbD:

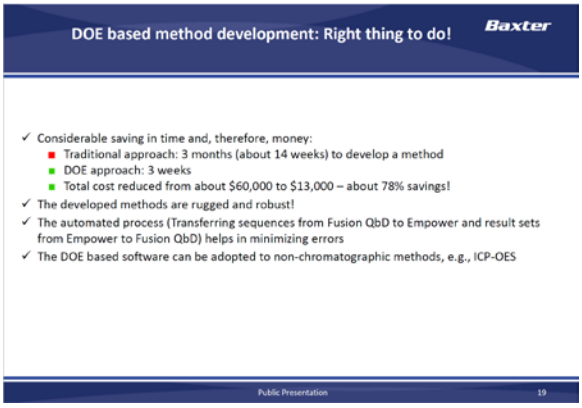
- Used for early chemistry screening and method optimization with integrated robustness.
- Successful development and transfer of nine (9) methods in a 2-year period. Method robustness proven on transfer to QC.

No method performance problems from any of these methods.

Pittcon 2015: (Organized Session) – A review of applying QbD concepts for analytical development for Pharmaceutical Drug Products







Pittcon 2012: **QbD Approach to Rapid LC Method Development for Pharmaceuticals Using Automated Screening and Design of Experiments**

Total Time to Develop using Fusion QbD ~ 30 Hours.

Quality by Design (QbD) Approach to Rapid LC Method Development for Pharmaceuticals Using Automated Screening and Design of Experiments (DOE)

Catharine Johnson and Shaun Mendonsa
Analytical Development
Boehringer Ingelheim Pharmaceuticals, Ridgefield, CT
Pittcon 2012, Orlando, FL

Boehringer Ingelheim

Overcoming QbD Method Development Challenges

Boehringer Ingelheim

Manual LC QbD

- Software 1 - Generate DOE
- Software 2 - Write the instrument methods and sequences
- Software 3 - Graph data to study how method parameters interact
- Manual - Select final method conditions

← 2 to 4 weeks

Automated LC QbD

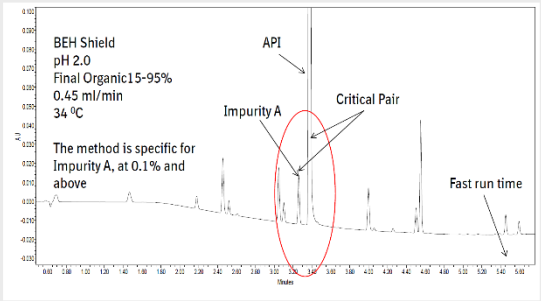
- Software 1 – LC Specific - Fusion AE (S-Matrix Corp, Eureka, CA)
  - Generate DOE
  - Translate DOE to LC methods and sequences
  - Graph method parameters for visualization
  - Sort chromatographic data
  - Select and test final method

← 2 to 4 days

Verify Predicted Final Method

Boehringer Ingelheim

Ensure target performance profile and performance criteria are met



Total QbD LC Method Development Time with Automation

Boehringer Ingelheim

QbD Method Development Task	Time (Hours)
Generate DOE screening design: Multiple columns, pHs and gradient conditions	0.5
Export design to Empower (CDS) and execute screening exp	15 (unattended)
Integrate peaks and automatically transfer results to Fusion	0.5
View automatically generated 2D and 3D surface plots to study critical factors	0.5
Sort results and find general conditions that meet method objectives	0.5
Perform fine optimization	0.5
Export design to Empower (CDS) and execute optimization exp	9.0 (unattended)
Integrate peaks and automatically transfer results to Fusion	0.5
Assess chromatographic performance characteristics: Automatically compute and visualize factors affecting method robustness, select final method	2.0
Total QbD method development (not counting sample/buffer prep)	~30 hrs

QbD Summary

Boehringer Ingelheim

- OFAT approach to LC method development:
  - Does not provide a true understanding of the method
  - May not provide true optimum method
  - Lengthy process
- QbD approach
  - Determines how parameters interact
  - Leads to a defendable, robust LC method
- QbD LC automation is key!
  - LC specific QbD software (i.e. Fusion AE)

Conclusions

Boehringer Ingelheim

Automated QbD Results in:

- High quality robust methods
- Fast development
- Meaningful SST criteria
- QbD LC method development can be performed by analysts with minimal statistical knowledge

Cambridge Isotope Laboratories, Inc.

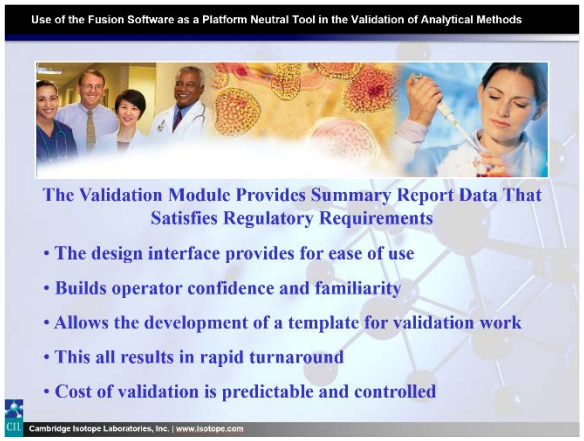
Key ongoing results of using Fusion QbD:

- 100% ROI achieved almost immediately.
- Consistently achieves a minimum of 70% reduction in time to develop and validate methods.
- Enables standardized approach to method validation across instrument platforms.
- Dramatically reduces risk and enhanced productivity by eliminating most sources of transcription, calculation, and reporting errors.

Consistently achieve at least 70% time reduction to develop & validate methods.

Pittcon 2014: **Use Fusion QbD as a platform-neutral tool in the validation and development of analytical methods for Quantitative NMR, HPLC, and GC/MS**

Speaker: Tim Eckersley, PhD., Director of Quality Control, Cambridge Isotope Laboratories, Inc.



Pittcon 2015: **A Perfect Storm of Technologies Drives QbD-aligned LC Method Development (Authorized Use of Lilly Data for QbD Case Study)**

- Natural Product: 14 Compounds – 2 APIs, 11 related impurities, and 1 process impurity
- Image A Chromatogram – no usable result after a 6 month effort using trial and error.
- Image B Chromatogram – result **3 days development using Fusion QbD.**

Image A – 6 Months Trial and Error

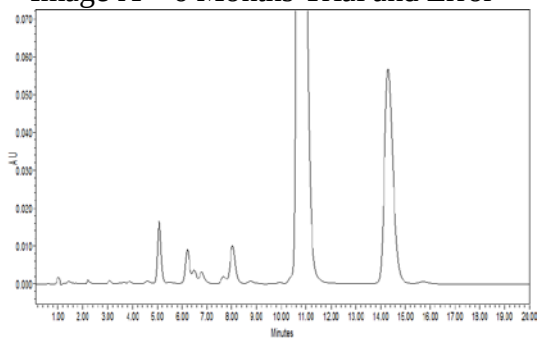
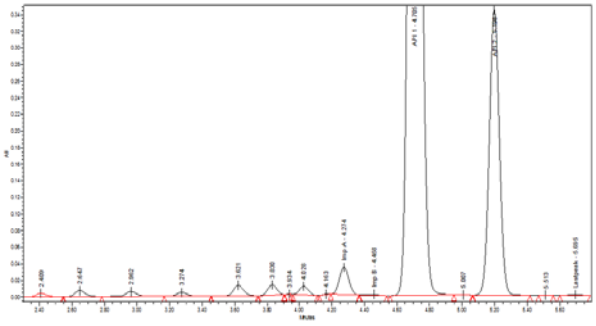


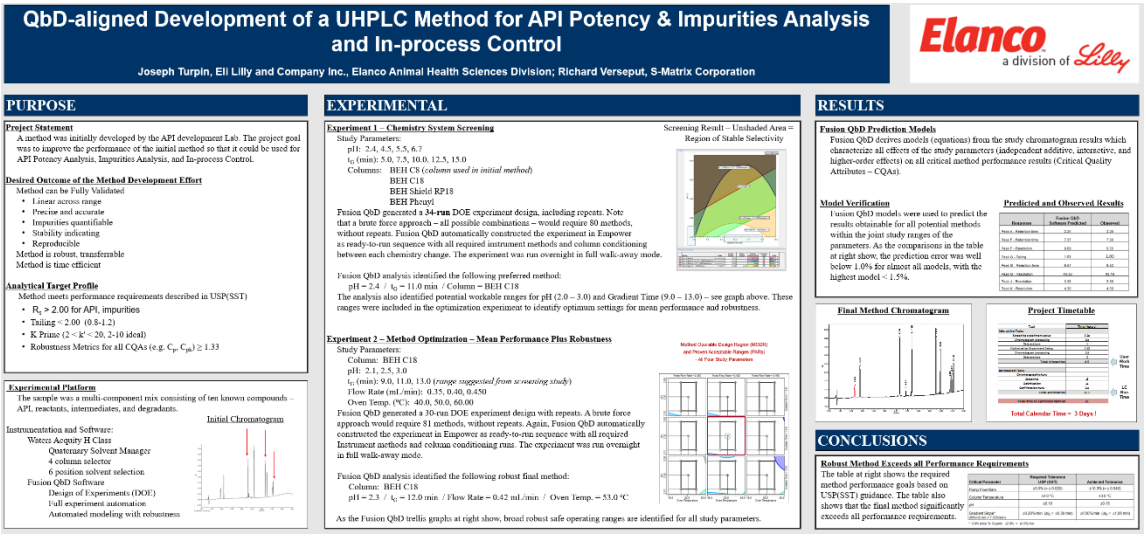
Image B – 3 Days using Fusion QbD



Pittcon 2014: **Pursuing the "Perfect" Method Using Quality by Design**

- Natural Product: 10 Compounds – API, Intermediates, Reactants, Degradants
- Generic Method for Potency Assay, Impurities Assay, and In-process Control.

Total Time to Develop using Fusion QbD – 30 Hours.



IFPAC 2015: Screening and Optimization Designs to Improve Method Performance and Robustness

- Initial Effort: 3 months using trial and error. No acceptable results.
- Fusion QbD: First overnight screen separated Sildenafil and all 5 analogs.

1 week from start to final method.

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Screening and Optimization Designs to Improve Method Performance and Robustness

John F. Kauffman, Ph.D.
Daniel J. Mans, Ph.D.
FDA Division of Pharmaceutical Analysis
IFPAC 2015

Disclaimer:
The findings and conclusions in this presentation have not been formally disseminated by the Food and Drug Administration and should not be construed to represent any Agency determination or policy.

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Research Problem Statement

- FDA will develop a method using the QbD paradigm, and transfer the method to an EMA lab.
  - Begin with a harmonized compendial method and apply QbD concepts to improve the method
  - Method: HPLC analysis of sildenafil and analogues of sildenafil

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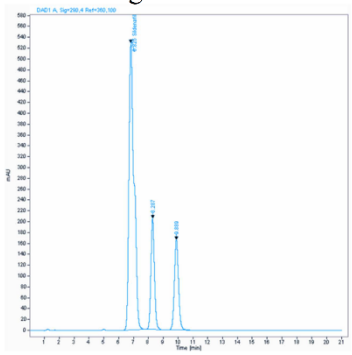
Starting Point: USP Method for Sildenafil



- Isocratic: 57/28/15 Buffer/Methanol/CH_3CN
(Buffer = Phosphoric acid, pH 3 with triethylamine)
- C18 column
- 30 °C
- Poorly separated: 6 compounds → 3 peaks

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Summary and Conclusion of Initial Screen

- 6 columns screened (4 C18, 2 PFP): Results did not conform with theoretical expectations
- Varied combinations of mobile phases and gradient times
- Began to investigate pH effects: 4.5 vs. 3.0
→ affords separation of the 6 components but does not meet criteria of the ATP

❖ Time consuming and tedious one-variable-at-a-time conventional approach. Difficult to keep track of numerous generated method files.

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A Systematic QbD Approach

- Develop screening designs to evaluate diverse method options
- Use DOE methodology to predict optimal conditions
- Use statistical analysis to determine ranges of acceptable operating parameters - Robustness

- Implemented using S-Matrix Fusion QbD Software

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Optimal Conditions

- Phenylhexyl is the best column
  - Literature methods use C18
- Acetonitrile gives best peak shape and resolution.
  - MeOH/Phenylhexyl can support a method that meets the ATP. This is extremely useful information for method understanding
- Gradient time, pH, column temperature have been optimized

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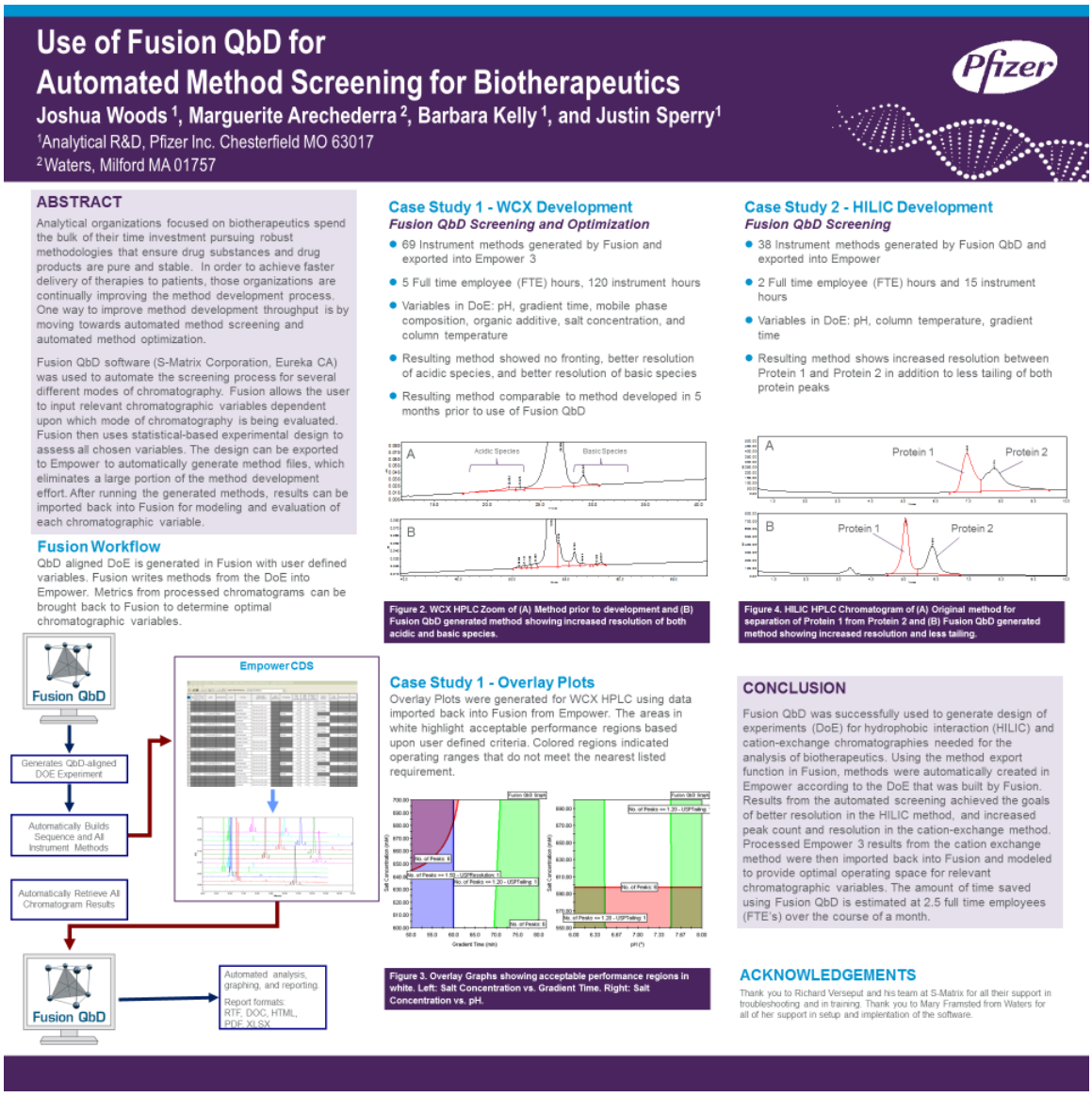
The poster below was presented at HPLC 2016. It presents results of two of the benchmarking studies which resulted in corporate licensing of the Fusion QbD Platform.

- Key Findings:
  - Case Study 1 – reduced development time from five (5) months to under 2 days.
  - Case Studies 1 and 2 – Overall Conclusion:

    Estimated time savings equivalent to 2.5 full time employees per month.



Use of Fusion QbD for Automated Method Screening for Biotherapeutics

Joshua Woods [1], Marguerite Arechederra [2], Barbara Kelly [1], and Justin Sperry[1]
[1]Analytical R&D, Pfizer Inc. Chesterfield MO 63017
[2]Waters, Milford MA 01757

ABSTRACT

Analytical organizations focused on biotherapeutics spend the bulk of their time investment pursuing robust methodologies that ensure drug substances and drug products are pure and stable. In order to achieve faster delivery of therapies to patients, those organizations are continually improving the method development process. One way to improve method development throughput is by moving towards automated method screening and automated method optimization.

Fusion QbD software (S-Matrix Corporation, Eureka CA) was used to automate the screening process for several different modes of chromatography. Fusion allows the user to input relevant chromatographic variables dependent upon which mode of chromatography is being evaluated. Fusion then uses statistical-based experimental design to assess all chosen variables. The design can be exported to Empower to automatically generate method files, which eliminates a large portion of the method development effort. After running the generated methods, results can be imported back into Fusion for modeling and evaluation of each chromatographic variable.

Fusion Workflow

QbD aligned DoE is generated in Fusion with user defined variables. Fusion writes methods from the DoE into Empower. Metrics from processed chromatograms can be brought back to Fusion to determine optimal chromatographic variables.

Case Study 1 - WCX Development

Fusion QbD Screening and Optimization

- 69 Instrument methods generated by Fusion and exported into Empower 3
- 5 Full time employee (FTE) hours, 120 instrument hours
- Variables in DoE: pH, gradient time, mobile phase composition, organic additive, salt concentration, and column temperature
- Resulting method showed no fronting, better resolution of acidic species, and better resolution of basic species
- Resulting method comparable to method developed in 5 months prior to use of Fusion QbD

Figure 2. WCX HPLC Zoom of (A) Method prior to development and (B) Fusion QbD generated method showing increased resolution of both acidic and basic species.

Case Study 2 - HILIC Development

Fusion QbD Screening

- 38 Instrument methods generated by Fusion QbD and exported into Empower
- 2 Full time employee (FTE) hours and 15 instrument hours
- Variables in DoE: pH, column temperature, gradient time
- Resulting method shows increased resolution between Protein 1 and Protein 2 in addition to less tailing of both protein peaks

Figure 4. HILIC HPLC Chromatogram of (A) Original method for separation of Protein 1 from Protein 2 and (B) Fusion QbD generated method showing increased resolution and less tailing.

Case Study 1 - Overlay Plots

Overlay Plots were generated for WCX HPLC using data imported back into Fusion from Empower. The areas in white highlight acceptable performance regions based upon user defined criteria. Colored regions indicated operating ranges that do not meet the nearest listed requirement.

Figure 3. Overlay Graphs showing acceptable performance regions in white. Left: Salt Concentration vs. Gradient Time. Right: Salt Concentration vs. pH.

CONCLUSION

Fusion QbD was successfully used to generate design of experiments (DoE) for hydrophobic interaction (HILIC) and cation-exchange chromatographies needed for the analysis of biotherapeutics. Using the method export function in Fusion, methods were automatically created in Empower according to the DoE that was built by Fusion. Results from the automated screening achieved the goals of better resolution in the HILIC method, and increased peak count and resolution in the cation-exchange method. Processed Empower 3 results from the cation exchange method were then imported back into Fusion and modeled to provide optimal operating space for relevant chromatographic variables. The amount of time saved using Fusion QbD is estimated at 2.5 full time employees (FTE's) over the course of a month.

ACKNOWLEDGEMENTS

Thank you to Richard Verseput and his team at S-Matrix for all their support in troubleshooting and in training. Thank you to Mary Framstad from Waters for all of her support in setup and implentation of the software.

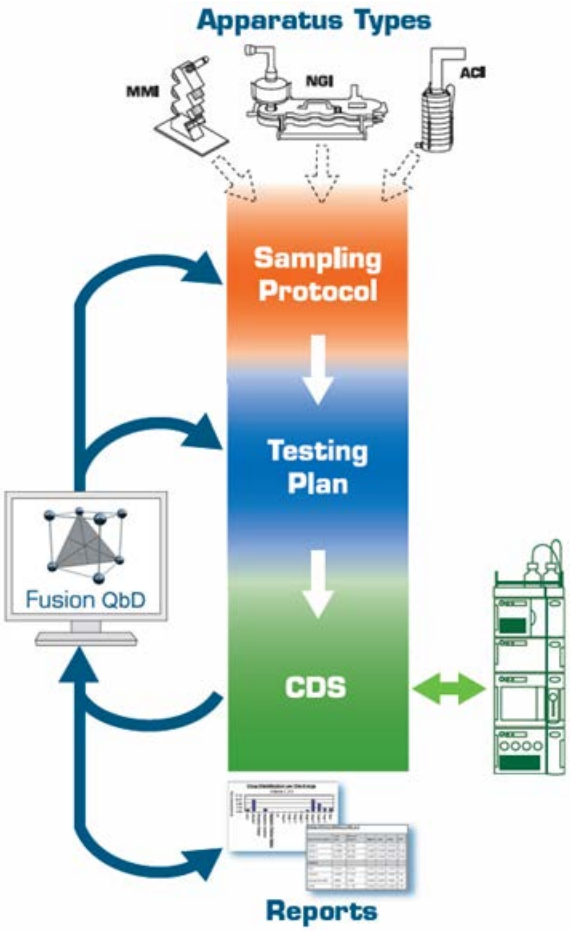
Since 2011 Teva has consistently realized a savings of two days per week per analyst using the Fusion Inhaler Testing software module (FIT).

40% reduction in direct cost for the work translates into excellent annual savings.

The following simple calculation illustrates the ongoing value of FIT:

Using an estimated TOTAL annual cost per analyst of USD* $75,000.00 –

Direct Savings = USD $30,000.00 per Analyst per Year Every Year!



(* – United States Dollars)