CASSS AT Europe
Dublin, March 13th

Can low resolution MS generate high-quality MAM Data and replace high resolution MS for process development support?

Scott Corley, Sr. Scientist, Analytical Science, Boehringer Ingelheim Fremont, CA, USA

Boehringer Ingelheim

Our mission: Producing value to customers to help patients

- Family-owned global corporation
- Founded 1885 in Ingelheim, Germany
- Focus on Human Pharmaceuticals, Animal Health and Biopharmaceutical Contract Manufacturing
- Around 50,000 employees worldwide
- 181 affiliated companies worldwide
- Net sales of around EUR 18.1 billion

Status April 2018

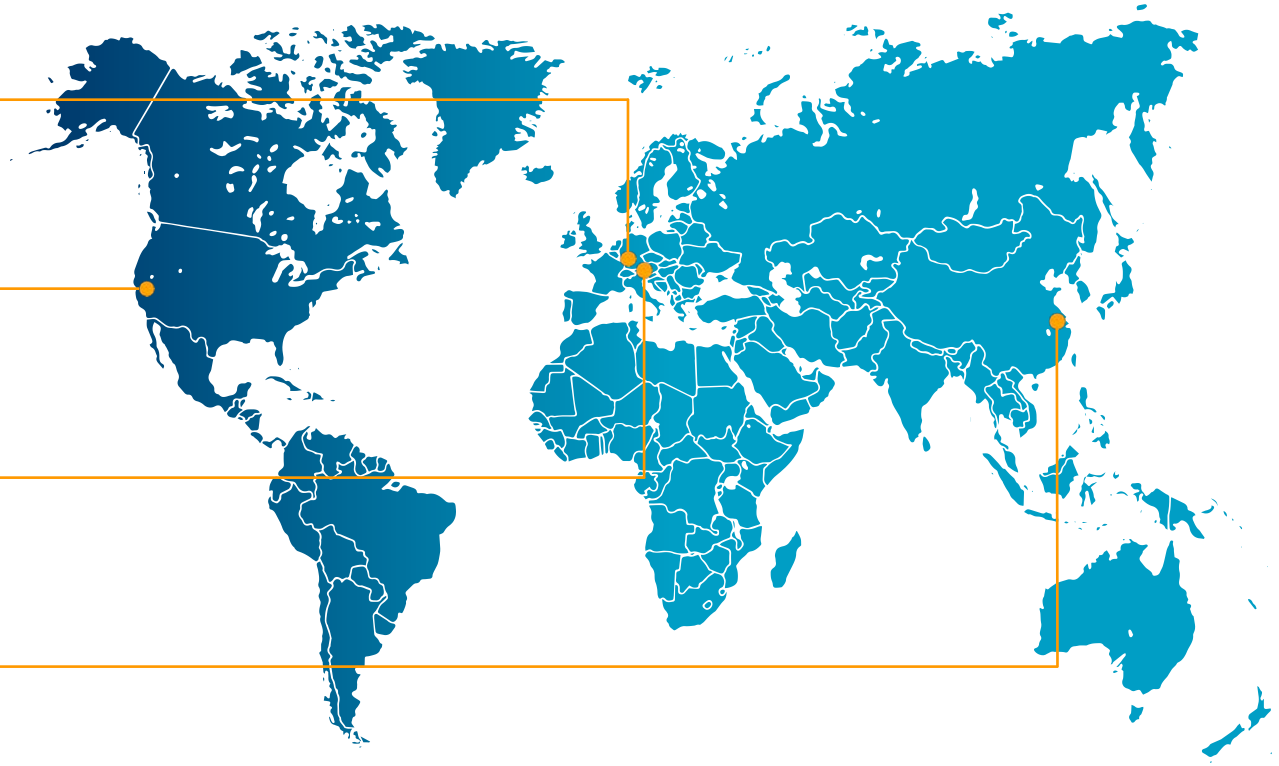
Our development and production network spans the globe

Biberach, Germany,
Mammalian Technology
Fill & Finish

Fremont, Ca, US
Mammalian Technology
Fill & Finish

Vienna, Austria
Microbial Technology
Fill & Finish

Shanghai, China
Mammalian Technology
Fill & Finish



Confidential & Privileged by Boehringer Ingelheim BioXcellence™

Outline

- Motivation and Strategic Goals
- Method development
- Proof of concept studies
- Challenges and future work

Motivation

At Boehringer Ingelheim Fremont, we are evaluating low resolution MS for a comprehensive assay to

- support method development
- improve process understanding
- conduct timely investigations

Method under evaluation for multi-attribute information

- routine monitoring of critical attributes
- to supplement/replace peptide map by UV, CEX, CE-SDS, HILIC glycan, where possible

Advantages of a low resolution mass spectrometer, e.g., the Waters QDa®

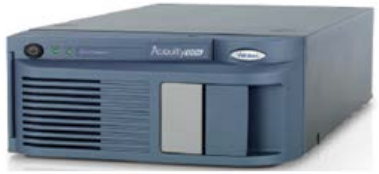
- facility / local knowledge fit with Waters HPLC/UPLC and Empower knowledge
- does not require a MS expert to run
- reduce support from HRMS and/or need for additional costly instrument to support development

MS systems utilized

www.waters.com

Waters Xevo® G2-S QTof

- 20 – 4000 mass range in resolving mode
- UNIFI Software 1.8
- Used for:
  - Routine peptide map analyses
  - Intact mass analyses
  - Disulfide Map



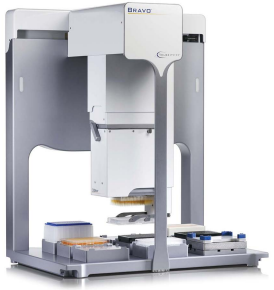
www.waters.com

Waters ACQUITY® QDa®

- Single Quadrupole, 50 – 1250 m/z range
- Single Ion Response (SIR) channels
- **Automated mass calibration**
- **Does not require a MS expert**; uses Empower
- Fits on top of UPLC

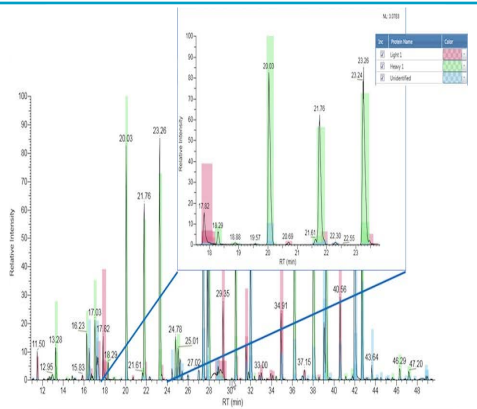
Workflow, utilizing Automation on the Agilent Bravo®

Aglient Bravo® Automated Liquid Handling Platform



www.agilent.com

www.waters.com



www.thermofisher.com

Sample Prep

- Denaturing
- Alkylating
- Digesting

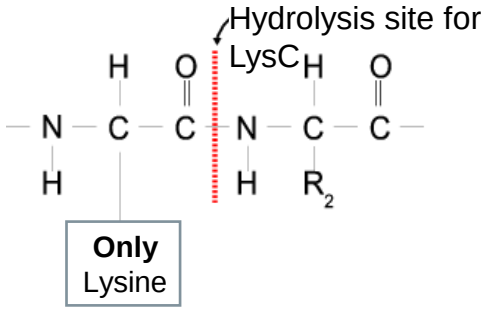
Testing

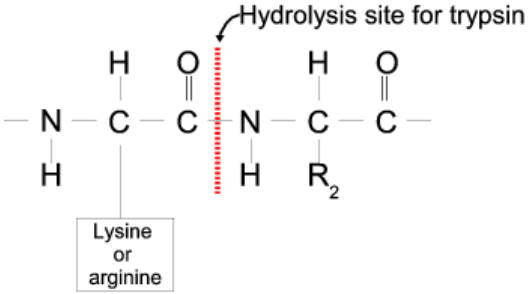
- Reverse Phase UPLC
- QDa® Mass Detector

Analysis

- Sequence Coverage
- PTM analysis

Lysyl Endopeptidase (LysC) vs. Trypsin





Lysyl Endopeptidase (LysC)

- Resistant to GnHCl and lower pH
- Cleaves mAb into manageable peptides
- Literature/ preliminary data promises glycan analysis

Trypsin

- Most common peptide for enzymatic digest
- Local knowledge, abundant data available
- Cost-effective

Confidential & Privileged by Boehringer Ingelheim BioXcellence™
Boehringer Ingelheim

Why Lys-C Digestion?

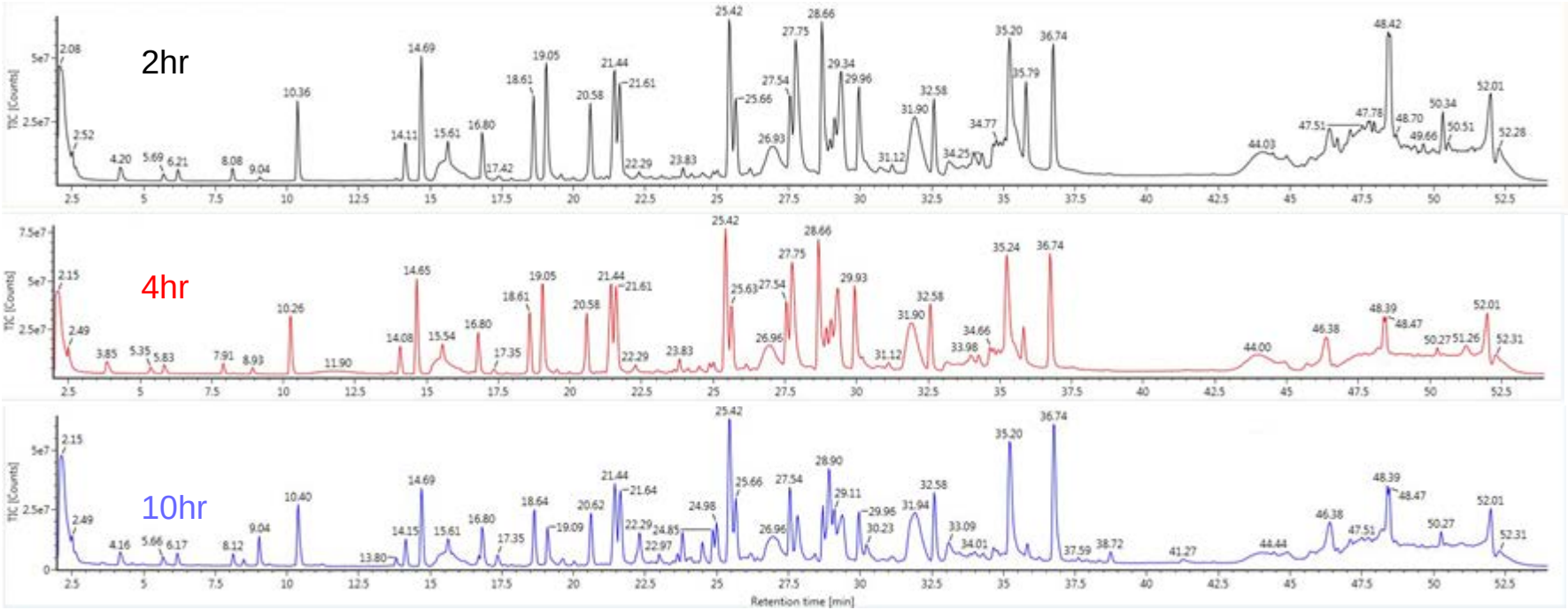
Lys-C

Glycan Species	Observed RT (min)	Response	Observed m/z	Charge	Percentages (%)
A2FG0	27.88	7764188	**981.8715**	**5**	69.8
A1FG0	27.87	1739986	**941.2555**	**5**	15.6
A2FG1	27.82	1209883	**1014.282**	**5**	10.9
Man5	28.04	407436	**936.2486**	**5**	3.7

Trypsin

A2G0	5.49	71212	**1244.496**	**2**	0.90
A2FG0	5.47	4038897	**1317.521**	**2**	51.23
A1FG0	5.47	2652483	**1215.981**	**2**	33.65
A2FG1	5.31	689437	**932.7008**	**3**	8.75
A1FG1	5.3	175214	**1297.006**	**2**	2.22
Man5	5.27	255997	**1203.464**	**2**	3.25

2hr Digestion



→ 96 % coverage of HC, 100% of LC

Peptide digestion manual

Denaturation / reduction: 6 M GnHCl, pH 7, DTT, 37°C, 30 mins

⇩

Alkylation: (IAM), 20°C for 30 mins (dark)

⇩

Buffer exchange: NAP-5, to 100 mM Tris (pH **7.6**)

⇩

Digestion: LysC addition, (protein/enzyme 25:1 by ug), 37°C, **2hr**

⇩

Quench: Formic acid to 0.5% final concentration

Monitoring PTMs

- Methionine Oxidation
- Deamidation

Methionine oxidation

- Forced oxidation; 0.3% H_2O_2, room temperature, 12 hrs

Peptide	Modifiers	Response	RT (min)	Control (%)		Forced Oxidation	
				QToF	QDa®	QToF	QDa®
HC_K1	control	98523008	28.69	99.34	100		
	Oxidation M [34]	656257	27.03	**0.66**	**0**	**8.19**	**7.9**
HC_K4	control	43863384	21.59	99.72	99.3		
	Oxidation M [7]	124855	18.66	**0.28**	**0.7**	**7.83**	**6.2**
HC_K14	control	150148640	25.44	97.61	97.2		
	Oxidation M [4]	3679532	24.13	**2.39**	**2.8**	**99.21**	**96.5**
HC_K23	control	62552828	15.42	99.47	99.0		
	Oxidation M [18]	335108	13.68	**0.53**	**1.0**	**92.6**	**91.6**
HC_K28	control	72748120	19.06	99.71	99.5		
	Oxidation M [14]	213808	15.97	**0.29**	**0.5**	**99.62**	**98.8**

- Results comparable

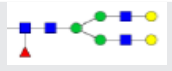
Asn Deamidation

- Forced deamidation; pH 10, 12 hrs, 37 °C
- +3 or +4 charge state, 0.33 or 0.25 m/z difference

Peptide	Modifiers	Response	RT (min)	Control (%)		Forced Deamidation	
				QToF	QDa®	QToF	QDa®
HC_K5	control	33184612	29.08	98.1		98.9	
	Q [17]	380241	30.12	**1.7**	**N/A**	**1.1**	**N/A**
HC_K15	control	42216892	21.41	100		99.7	
	N [12]	0	N/A	**0**	**N/A**	**0.3**	**N/A**
HC_K25	control	28573212	25.62	99.6		96	97.3
	N [14]	116288	25.99	**0.4**	**N/A**	**4**	**2.7**
LC_K6	control	72062728	29.31	99.8		99.6	
	N [11 or 12]	118745	30.36	**0.2**	**N/A**	**0.4**	**N/A**

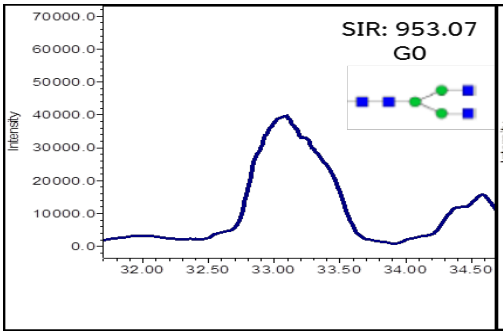
Monitoring N-linked Glycans with Lys C digest

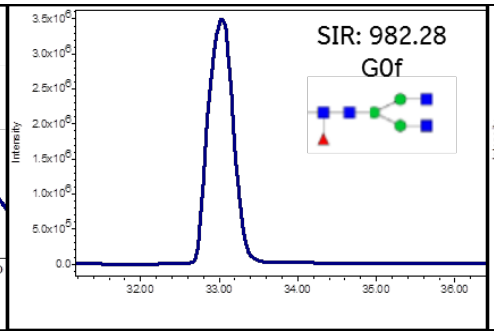
- N-linked glycopeptide is on a conserved region of the mAb
- + 5 charge state was used for glycan monitoring

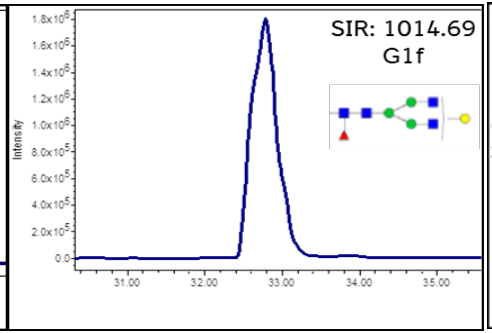
Glycan	Structure	$(M + 4H^+) / 4$	$(M + 5H^+) / 5$
nG		865.95	692.97
G0		1190.57	952.66
G0F-Gn		1139.80	912.04
G0F		1227.09	981.87
G1F		1267.60	1014.28
G2F		1308.11	1046.69
M5		1170.06	936.25
M6		1210.57	968.66
M7		1251.09	1001.07

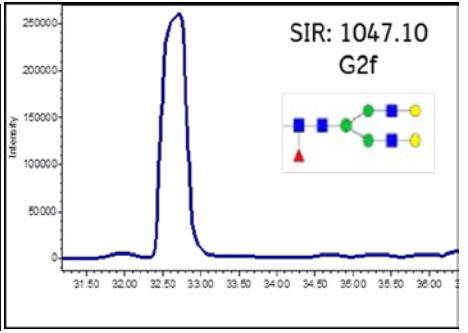
Monitoring Major Glycans

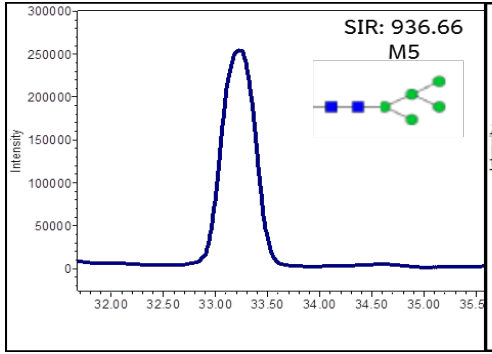
QDa® SIR channels of N-linked Glycans

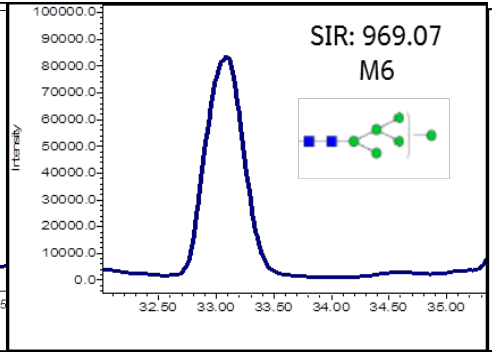


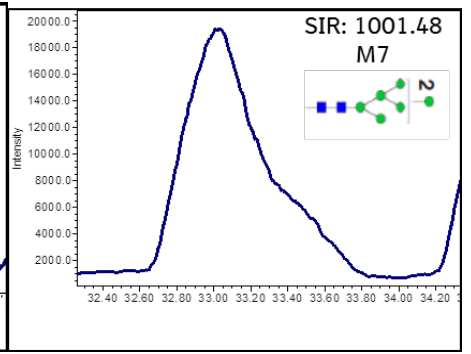












Glycan Profile

Modifiers	Response	RT (min)	Observed m/z	Charge	QToF
nG	334944	29.25	692.9649	5	0.44
G0F	48088500	27.64	981.8723	5	63.20
G0F-Gn	16201595	27.63	941.2565	5	21.29
G1F	8763085	27.55	1014.282	5	11.52
G2F	329510	27.51	1046.692	5	0.43
Man5	2372267	27.73	936.25	5	3.12

Average G0F is 2.2% by HILIC-UPLC oligo map

Glycosylation, by QDa®

After correcting for in-source fragmentation of G0F to G0F-Gn, data of abundant glycans are comparable

	mAb 1					
	Dev. Std. Lot 1			Dev. Std. Lot 2		
	QTof (%)	QDa® (%)	HILIC (%)	QTof (%)	QDa® (%)	HILIC (%)
G0F	74.9	74.0	75.1	74.3	74.7	75.0
G1F	11.7	13.0	9.7	13.5	13.1	12.3
G2F	0.43	0.55	0.37	-	-	-
Man5	4.0	3.5	5.7	3.6	3.6	4.3

Case study:

- The effect of glucose limitation was investigated as a potential process improvement by our upstream partners
- Product quality was monitored
- CEX and HILIC glycan were used to determine PQ
- Our method was evaluated for comparison

Effect of culture conditions on high Mannose (Man 5)

- High Mannose is a known complication of glucose limitation due to limit of GlcNaC production
- MS was used to corroborate glycan determination by HILIC

	Current Fed-batch Process			Reduction of Glucose on Fed-batch		
	mAb 1 _DS2	mAb 1_BDS	mAb 1 _ctrl	mAb 1 (75%)	mAb 1 (95%)	mAb 1 (95% Day1-5; 115% Day 5 -13)
HILIC	4.3	5.7	4.2	22.6	16.3	12.3
QTof	3.6	4	2.9	29.2	19.9	11.1
QDa®	3.6	6.2	4.8	27.0	19.8	13.9

Effect of culture conditions on presence of C-terminal Lysine

- High % BPG (by CEX) correlated with severity of glucose limitation
- Contribution of C-term Lys to high BPG investigated by Carboxypeptidase B digest
- C-term Lysine investigated by QDa®
  - Conserved C-term peptides (SLSLSPGK and SLSLSPG)
  - SLSLSPK is more likely to have +2 charge state than SLSLSPG
  - QDa® SIRs were monitored for the +1 and + 2 charge states of each peptide
  - Potential for ionization efficiency differences; not controlled

Method	Current Fed-batch Process	Reduction of Glucose on Fed-batch	
	mAb 1 (control conditions)	mAb 1 a (most restricted)	mAb 1 b (restricted)
C-term Lysine estimate	6.5%	16.8%	13.3%
QDa® (C-term Lys)	19.0%	37.0%	28.5%

- QDa® sensitive to C-term Lysine; overestimates value data compared to CEX
  - Correction factors will be considered

What We Achieved:

Developed a multi-attribute assay which:

- can monitor PTM's, major glycan species
- good agreement with HRMS and orthogonal techniques

Advantages of approach

- leverage of automated sample prep to save hands-on time
- can support in place of HRMS, eliminate need for additional instrumentation and/or MS SME time

Potential as a strong tool during process development

- support process monitoring
- conduct investigations, e.g., potential to replace C-term Lys investigation with CP-B

Acknowledgements

BI Fremont Analytical Science:

- Alex Chen
- Tanu Priya
- Chris Warner
- Eike Zimmermann
- Yuan Guo
- Paulina Tau
- Guifeng Jiang
- Kenji Furuya

Confidential & Privileged by Boehringer Ingelheim BioXcellence™

Boehringer Ingelheim Biopharmaceuticals GmbH

Binger Straße 173, 55216 Ingelheim am Rhein, Germany

Phone +49 (0) 6132 770
Fax +49 (0) 6132 720

bioxcellence@boehringer-ingelheim.com
www.bioxcellence.com

For more information have a look at

www.bioxcellence.com

© Boehringer Ingelheim Biopharmaceuticals GmbH 2019

The concepts, statements and data elaborated in this presentation are the intellectual property of Boehringer Ingelheim Biopharmaceuticals GmbH and are subject to current copyright law. Complete or partial reproduction and passing on to third parties is not permitted.

Boehringer Ingelheim