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Supporting Bioprocess Development and Method Transfer using Multi Attribute Monitoring Methods

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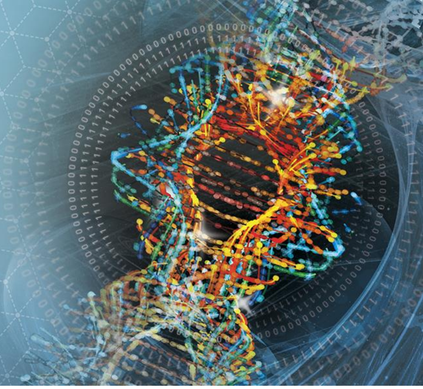


PURPOSE

As part of a sound pharmaceutical quality system (ICH Q10), an increasing trend of MS-based techniques in lab settings traditionally associated with optical-based assays has been observed in an effort to improve the body of knowledge surrounding a drug product. This has facilitated the introduction of LC-MS-based methods for semi-targeted monitoring of biotherapeutic protein attributes or "Multi-Attribute Monitoring" in an effort to reduce redundant assays and increase productivity. Challenges associated with these approaches include data-mining expansive data sets, determining screening parameters, and transferring methods.

Our purpose here is to demonstrate a compliance-ready LC-MS workflow that can be readily deployed for multi-attribute monitoring and Quality by Design (QbD)-based process and method development, as well as stability analysis and QC release testing.

OBJECTIVE(S)

Our objective in this study was to generate a data set representative of a process development setting, and to demonstrate how multiple critical quality attributes (CQA's) can be monitored simultaneously using a single compliance-ready CDS platform with MS control and data processing capabilities.

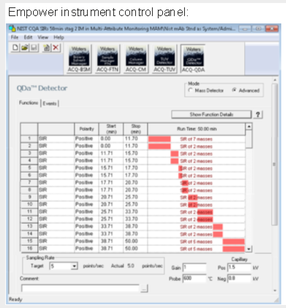
METHOD(S)

The ACQUITY® QDa was chosen for this study for its ability to easily integrate into existing LC platforms across industry to facilitate a peptide-based MAM workflow. The straightforward user interface combined with disposable source elements minimizes training and maintenance for daily operation and improved productivity in the lab.

Time (min)	Flow (mL/min)	%A	%B	Curve
Initial	0.200	99.0	1.0	Initial
2.00	0.200	99.0	1.0	6
52.00	0.200	65.0	35.0	6
58.00	0.200	15.0	85.0	6
62.00	0.200	15.0	85.0	6
67.00	0.200	99.0	1.0	6
80.00	0.200	99.0	1.0	6

Gradient Table. A 50 minute separation gradient was deployed to demonstrate MAM-based workflows can be deployed in high-throughput environments with robust results.

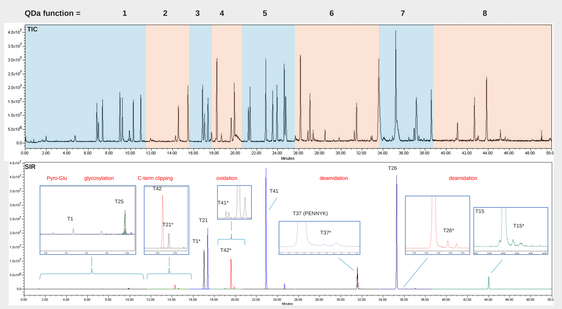
RESULT(S)



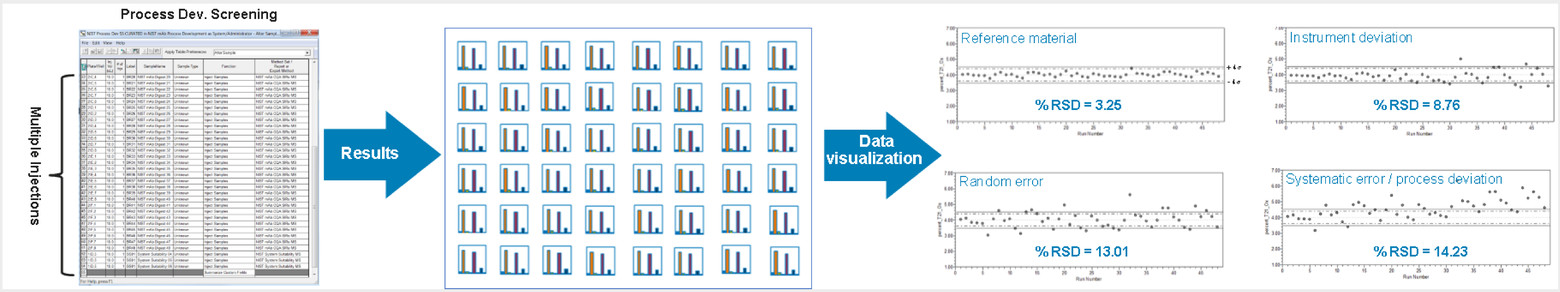
QDa function table — Charge states to be monitored

QDa	Frag	Modification	m/z
1	T25	Glycopeptides	FA2-879.2, FA2G1-933.2, FA2G2-987.3, A2-830.5, M5-803.1, FA1G1-865.5, FA1G1Gc1-726.2
2	T1	Pyro-Glu (native)	616.7, 308.9
3	T21	Oxidized	852.0, 426.5
4	T42	C-term Lysine (native)	788.9, 395.0
5	T21	Native	836.0, 418.5
6	T1	Pyro-Glu (mod)	599.7, 300.4
7	T42	C-term Lysine clip	660.7, 330.9
8	T41	Oxidized	705.5, 564.6
9	T41	Native	701.5, 561.4
11	T37	PENNYK (deamidated)	849.6, 637.4
12	T37	PENNYK (Native)	849.2, 637.2
13	T26	Deamidated	905.5, 604.0
14	T26	Native	905.1, 603.7
15	T15	Deamidated	1120.6, 960.6, 840.7, 747.4, 672.7
16	T15	Native	1120.4, 960.5, 840.6, 747.3, 672.6

Monitoring (P)CQAs. Programming the ACQUITY QDa can be done in a straightforward manner through the instrument control panel. Using the HRMS characterization data (data not shown), a list of PCQA's was generated and programmed through Empowers instrument control panel to track individual charge states associated with each PCQA's native and modified form. In this data set selected ion recording (SIR) is used in a staggered acquisition fashion to maximize dwell time spent on acquiring signal for increased sensitivity.



Increasing throughput. A benefit of MS-based detection in MAM-based workflows is the increased specificity afforded by the mass detector. In this example The SIR functionality of the QDa allows attributes of interest to be monitored using separate acquisition channels from non-critical species while shortening assay run times. For this method 50 minutes was found to offer sufficient resolution between critical pairs such as the oxidized form of T41 and the deamidated species of the T37 (PENNYK) peptide.



Data management. Development of proper control strategies involve performing the same assay with high frequency on large sample sets in a short amount of time (e.g. glycosylation profiles, cell line selection, stability testing, bio-reactor monitoring). Subtle differences across assays may be overlooked during manual curation of data, especially for MAM-based workflows that are monitoring multiple attributes in one assay. As shown in this example, %CV can be used as a holistic metric to identify potential process and instrument deviations such as random errors or systematic errors or a combination of both. As part of Empower's system suitability license, users have access to summary plots such as these to help in data visualization and assist in making informed decisions.

CONCLUSION(S)

Collectively, this user case demonstrates how Empower's integrated features can be used to streamline MAM-based workflows through visualization and data management and help reduce some of the burden associated with the deployment and migration of methods from process development to manufacturing and QC lot release through:

· Increased productivity by monitoring multiple attributes in a single acquisition

· Facilitation of fast decision making during process development

· Ease of deployment in non-regulated and regulated environments

· Greater accessibility and easy adoption for global QC deployment

· Straightforward method transfer and lifecycle management

Empower's support of the ACQUITY line of LC instruments and mass detectors such as the QDa provide a scalable Multi-Attribute Monitoring option that can support development and manufacturing processes through a drug products lifecycle with benefits such as:

Empower/QDa Benefits	
Affordable equipment	✓
Simple Yes/No answer	✓
Validated	✓
Tech transferable	✓
long-term use (>10 years)	✓
Generalist operators	✓
High throughput	✓

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