A Robust and Versatile New Peak Detection Workflow

to Facilitate the Implementation of the LC-MS Multi-Attribute Method from Research to Commercialization

Patrick Sascha Merkle, PhD
Novartis Pharma AG, Basel, Switzerland

CASSS MS – Session VIII – MAM for Product and Process Characterization and Quality Control

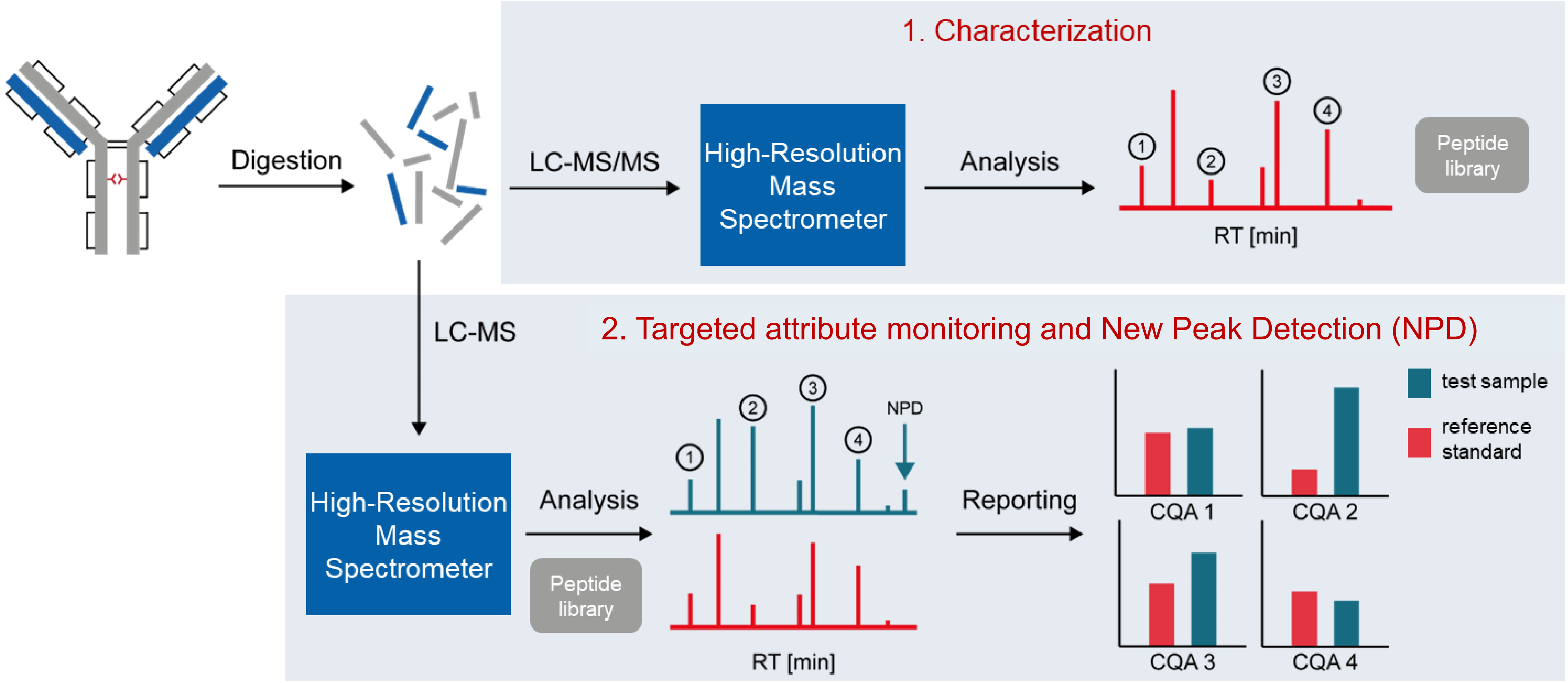
September 26th 2025, Costa Mesa, CA

NOVARTIS | Reimagining Medicine

Disclaimers

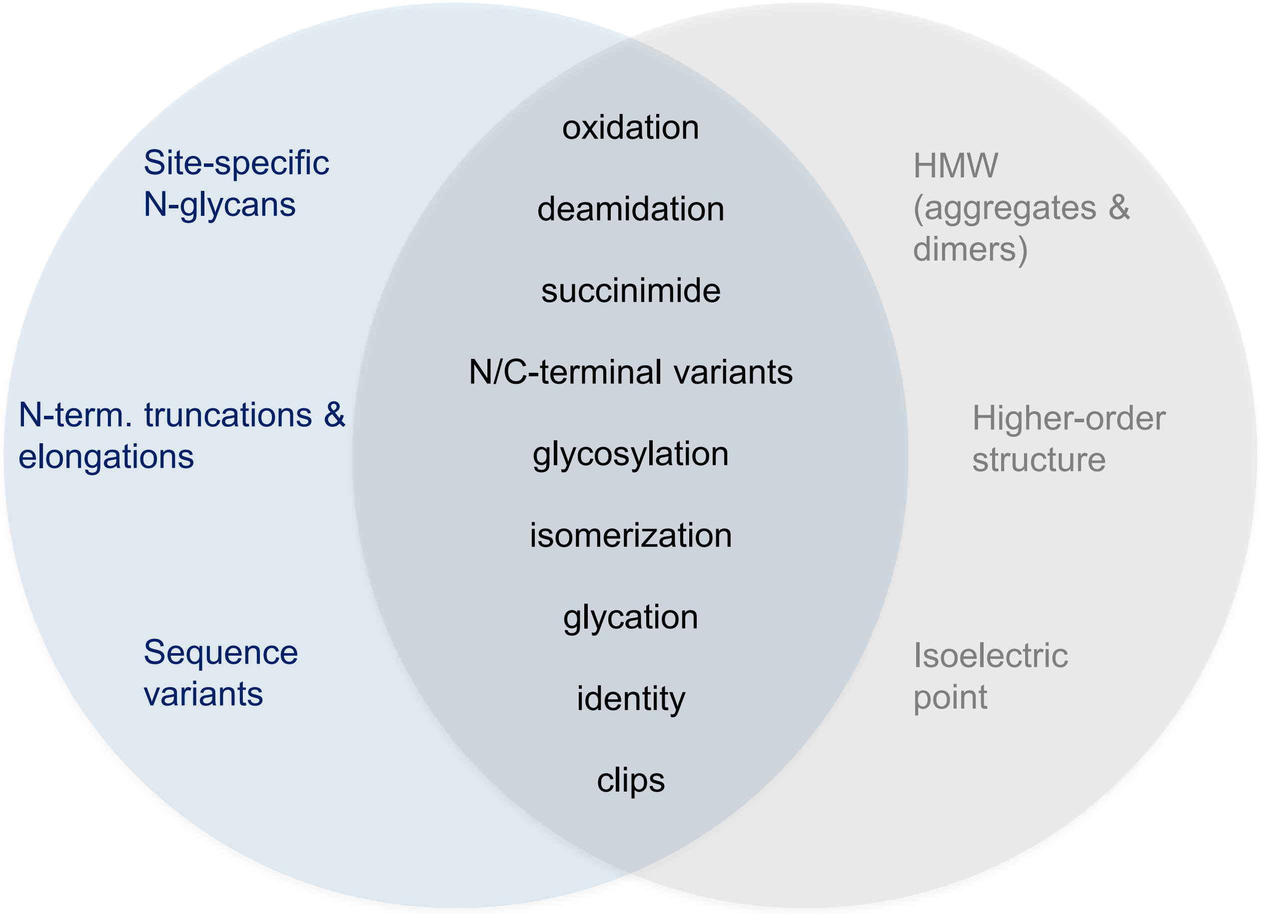
- The following slides are intended for educational purposes only and for the personal use of the audience.
- The following slides are not intended for wider distribution outside the intended purpose without presenter approval.
- The content of this slide deck is accurate to the best of the presenter’s knowledge at the time of creation.

Prototypical LC-MS MAM Workflow

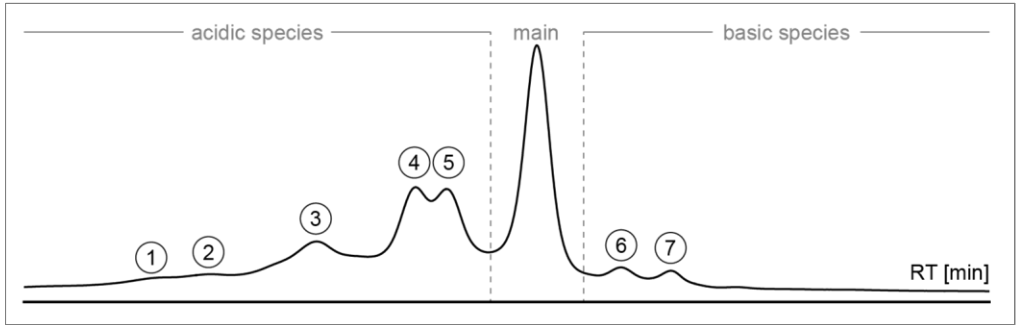


see also Rogers *et al.*, 2018; Rogstad *et al.*, 2019; Pohl *et al.*, 2023

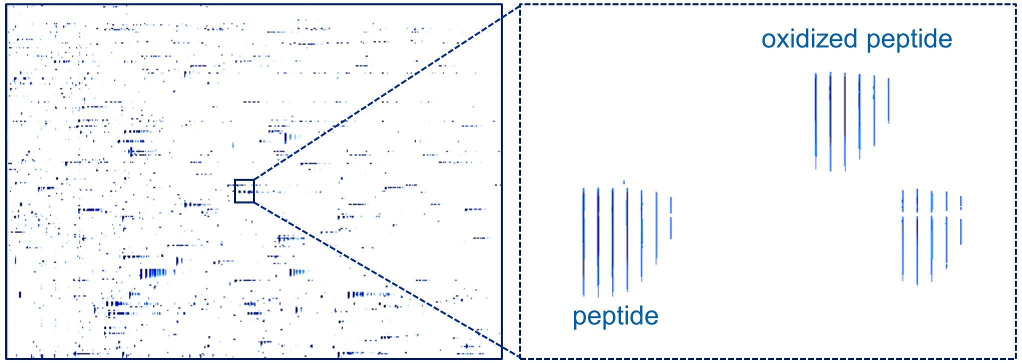
MAM vs. Traditional Separation Methods



Traditional separation methods (e.g., CEX)

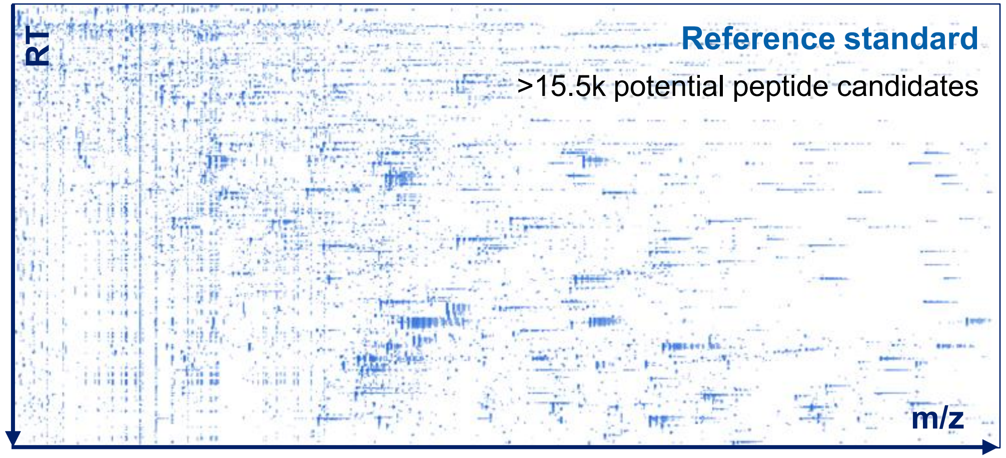


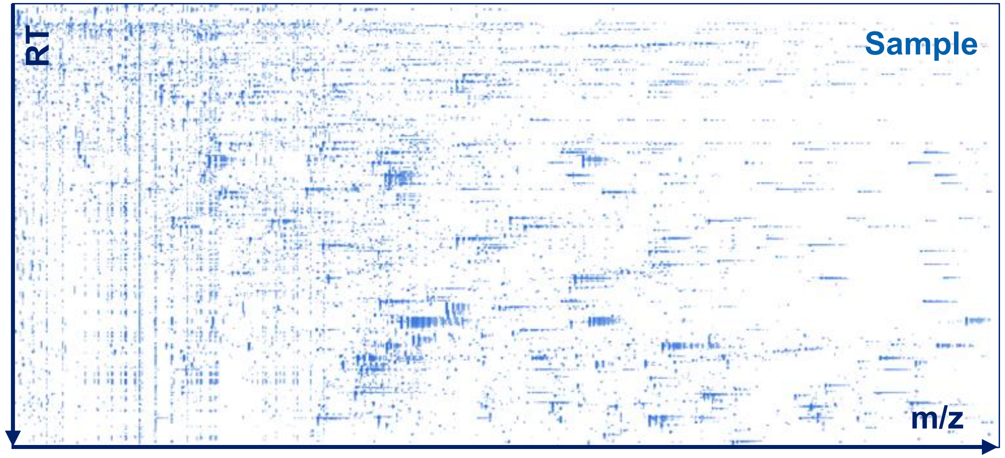
LC-MS Multi-Attribute Method (MAM)



MAM has the proven capacity to replace multiple conventional methods, while providing improved specificity.

New Peak Detection (NPD) – General Characteristics





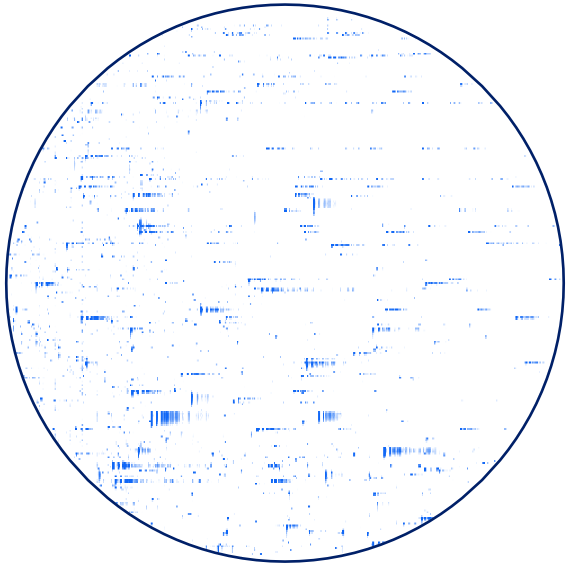
NPD Characteristics

- Process designed to identify and report on unexpected changes in product quality
- Pairwise comparison of sample data against a reference standard
- Detection of new, absent, and changed (increased or decreased) species relative to the reference standard

NPD as an essential tool to mitigate the risks of unexpected changes in product quality.

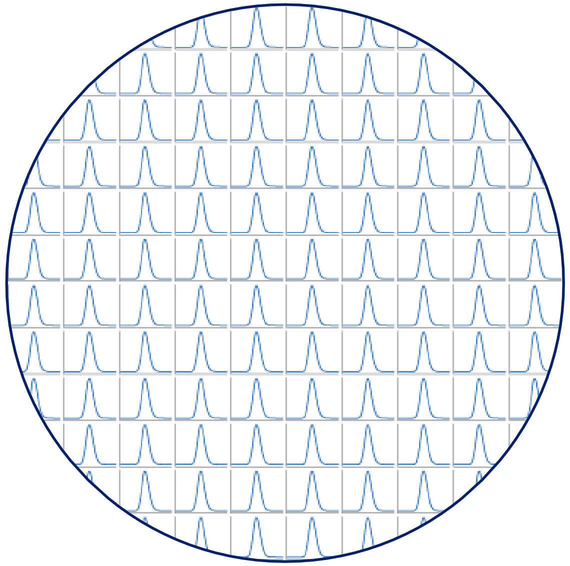
The Novartis-Genedata Partnership Project for NPD

April 2024 – March 2025



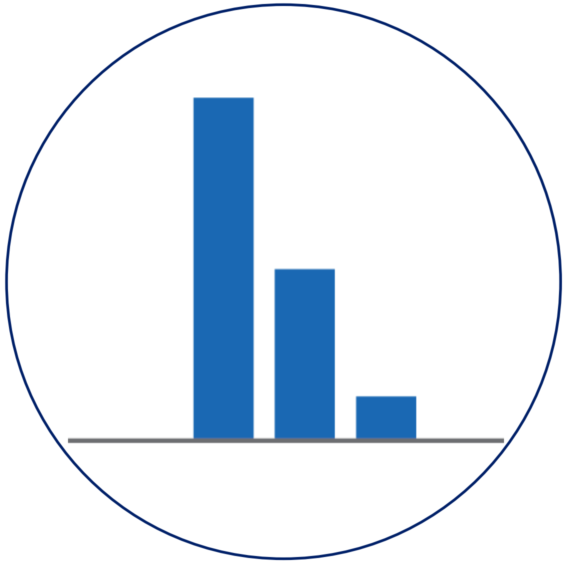
Tackle Data Complexity

Development of NPD-related core activities to tackle the complexity of LC-MS MAM data



Need for Speed

Development of an integrative workflow in Genedata Expressionist that can provide NPD results in a timely manner



Reduction of False Positives

Reduction of false positive NPD hits that derive from workflow variability and/or inherent method artifacts

Maximize Applicability

Maximize workflow applicability by designing an intuitive, synchronized, and informative NPD user interface

Blue sky vision: Development of a best-in-class NPD workflow in terms of sensitivity, speed, and robustness

Published Concepts to Reduce False Positives

Rogers et. al
NPD analysis as an impurity / purity method in the context of MAM
2017

Mouchahoir et. al
NPD performance metrics from the MAM consortium interlaboratory study
2021

Sadek et. al
Known Peak List (KPL) and pairwise analysis of MAM samples
2023

Zhang et. al
Single replicate statistics to detect rare differences between two LC-MS runs

Cao et. al
Reference replicates and respective statistical evaluation
2025

Pohl et. al (submitted)
Automatic artifact detection and consolidated NPD analysis

"Funnel strategy" to reduce the number of false positives during NPD analysis using a combination of automatic artifact detection, statistics, and KPL functionalities

Types of False Positives (according to Sadek et al., 2023)

Type	**Description**	**Solution in Genedata Expressionist**
Mass shift	e.g., faulty mass computation / clustering	SST *
Instrument noise	e.g., increase of baseline noise	Chemical noise detection
Gas-phase complex	e.g., ≥ 2 peptides form a complex in the ESI source	KPL
Peptide recovery	e.g., faulty sample handling / ESI source condition	SST, library validation *
Chromatography shift	e.g., identical peptides display different RT values	Library RT calibration *
Carry-over	e.g., peptides from preceding sample are introduced	KPL
Adduct ion	e.g., metal adducts incl. sodium, potassium, and iron	Co-elution detection
Missed cleavages	e.g., incomplete digestion during sample preparation	KPL

* custom activities not included in Genedata Expressionist 2025.1

Developed NPD Workflow in Genedata Expressionist 2025.1

False Positives

Preparing the Data
- Annotated .sbf files from targeted attribute monitoring
- Defining NPD reference sample(s)
- Charge state grouping

Artifact Flagging
- Detection of instrument noise, polymers, and adduct ions

Thresholding & Statistics
- Intensity thresholding
- Fold-change calculations
- Statistical evaluation

KPL & Filtering
- Known Peak List (KPL) search
- Filtering of insignificant species

Feature Change View
- Evaluation of NPD results with comprehensive visualization, filtering, review, and commenting functions

Updating & Reporting
- Updating the KPL (optional)
- Reporting

Selected Highlights

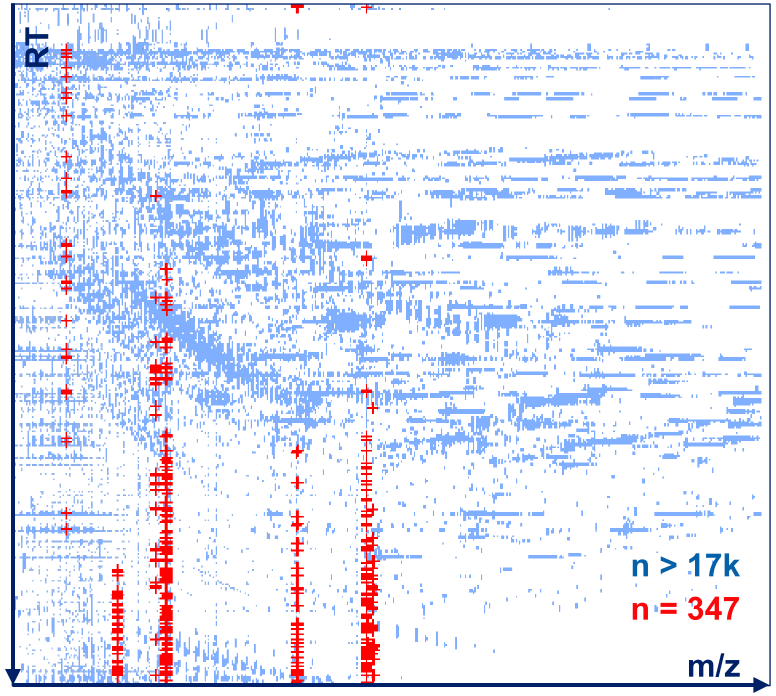
- Core NPD activities are made publicly available in Expressionist version 2025.1
- “Consolidated” NPD analysis reducing data analysis time to minutes without compromising workflow sensitivity
- Differentiation of true new peaks from false positives by means of automatic artifact detection
- Compatible with NPD datasets with or without replicate measurements

Genedata Webinar: Enabling MS-Based MAM in QC Through a Robust New Peak Detection Workflow

info@genedata.com

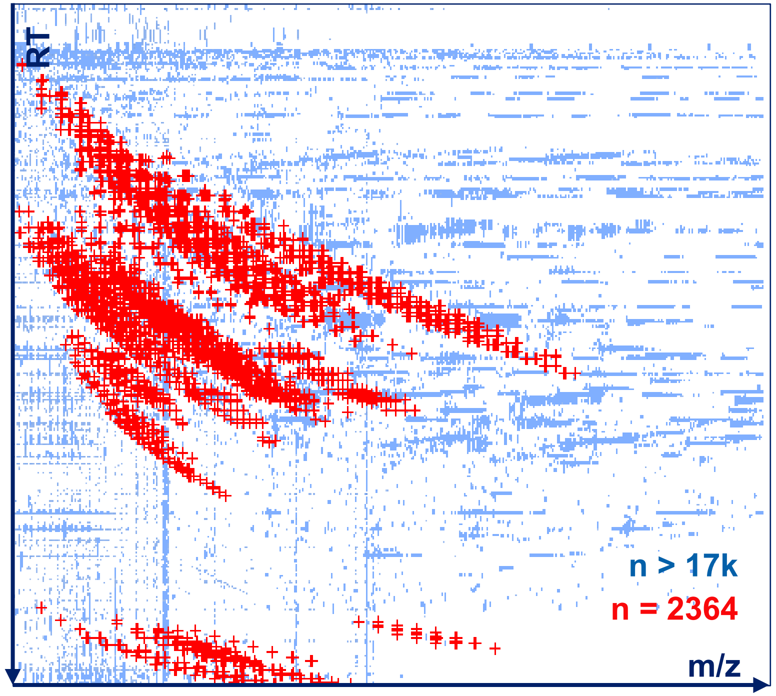
Automatic Artifact Detection

Chemical noise detection



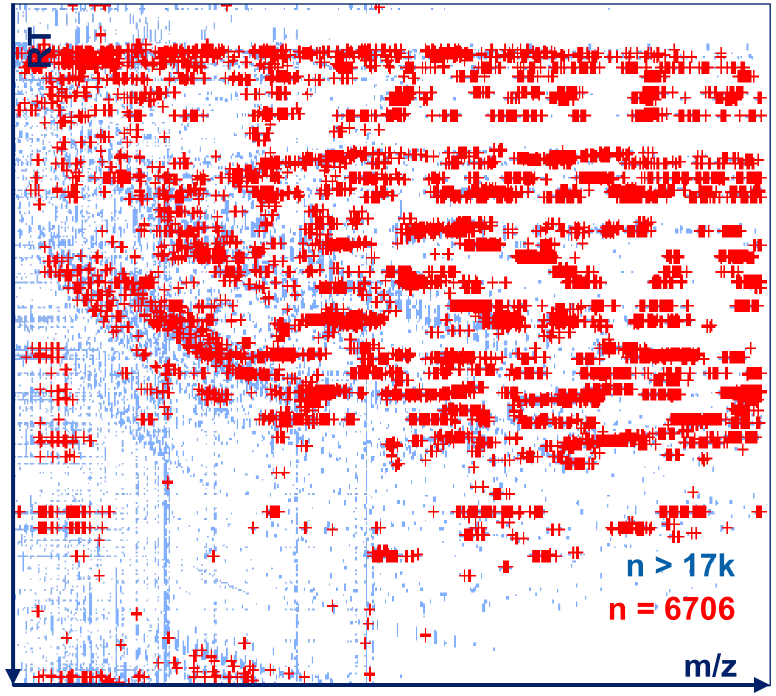
Chemical noise is detected and flagged by consolidating features with the same m/z (± mass tolerance window) that exceed a user-defined minimum feature count threshold

Polymer detection



Polymer species are identified and flagged based on repeating mass differences, a user-defined minimum feature count threshold as well as m/z and RT tolerance windows

Adduct ion detection

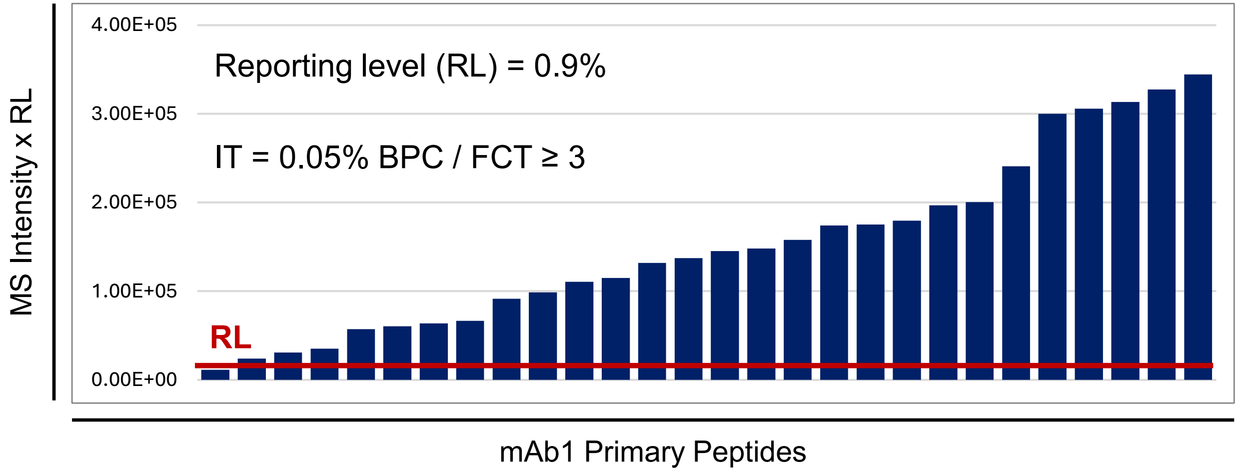


Adduct ions are detected and flagged within a user-defined mass range based on co-elution with a primary feature (most intense signal) ± user-specified RT tolerance window

Efficient flagging of method artifacts and polymer species for exclusion in downstream analysis

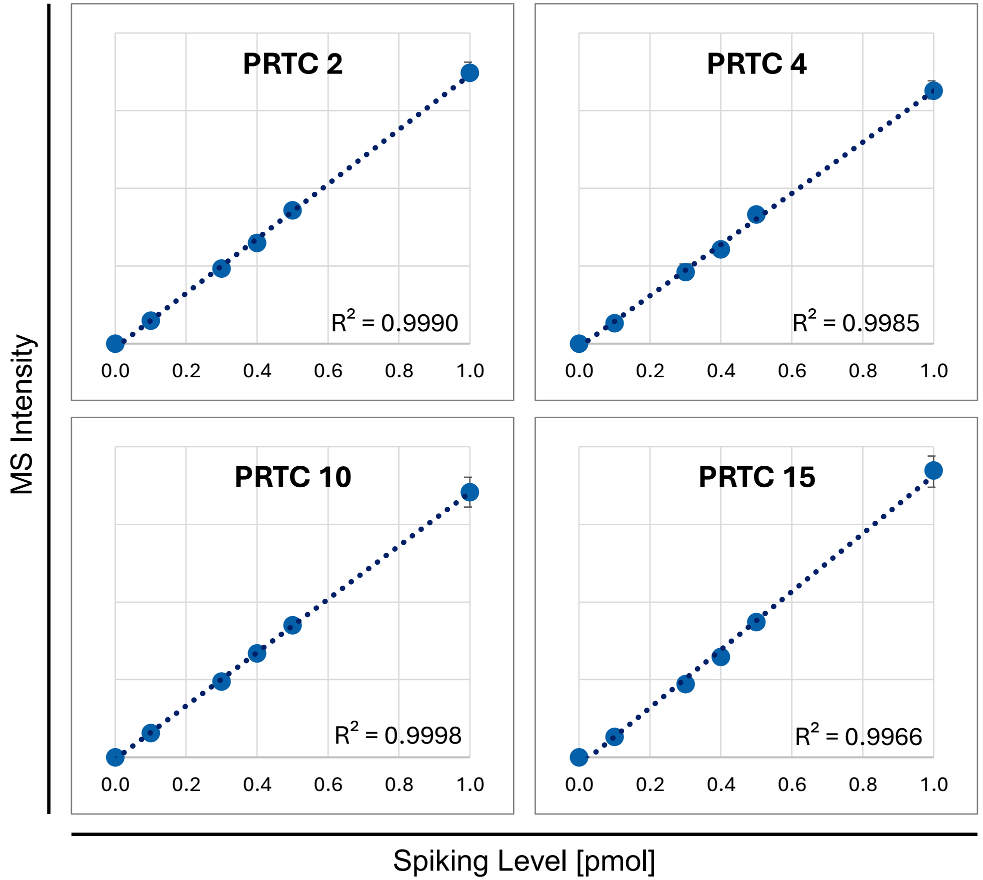
Validation of the NPD Workflow for mAb1

Rational Design of Intensity & Fold-Change Thresholds (IT / FCT)



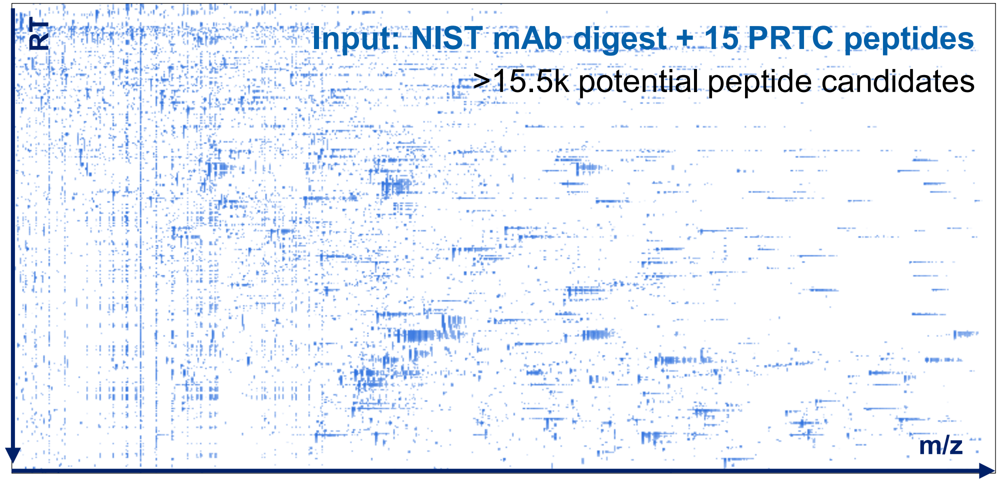
- IT was defined based on the intensity distribution of mAb1 peptides
- FCT was defined based on the variability of mAb1 peptide intensities
- Validation of the NPD workflow as limit test according to ICH Q2 (LOD, LOQ, and specificity) using the Pierce Retention Time Calibration (PRTC) standard for impurity spiking

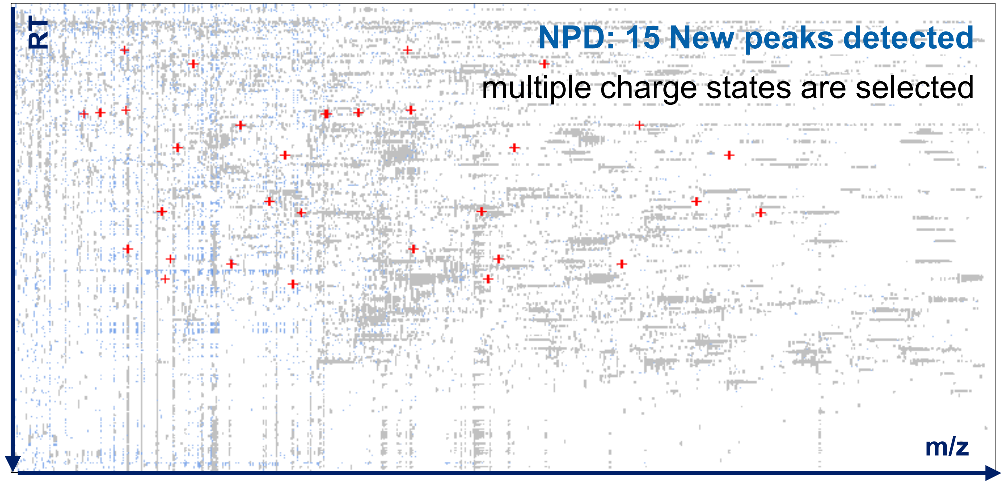
PRTC Spiking & Linearity Assessment



Determined LOQ values of PRTC peptides were below the intensity threshold and defined reporting level. Specificity confirmed through absence of interferences (no false positive assignments)

Analytical Control Strategy and Customized Reporting





Sample Overview

Name	NPD role
04_NISTmAb_01	Reference
11_NISTmAb_RTcalPepMix_0p5pmol_1	Positive control
26_NISTmAb_04	Negative control

Positive Control

Expected Peaks	Detected Peaks	Pass/Fail
15	15	Pass

Negative Control

Unexpected Peaks	Pass/Fail
0	Pass

Positive Control
Expected Peptides

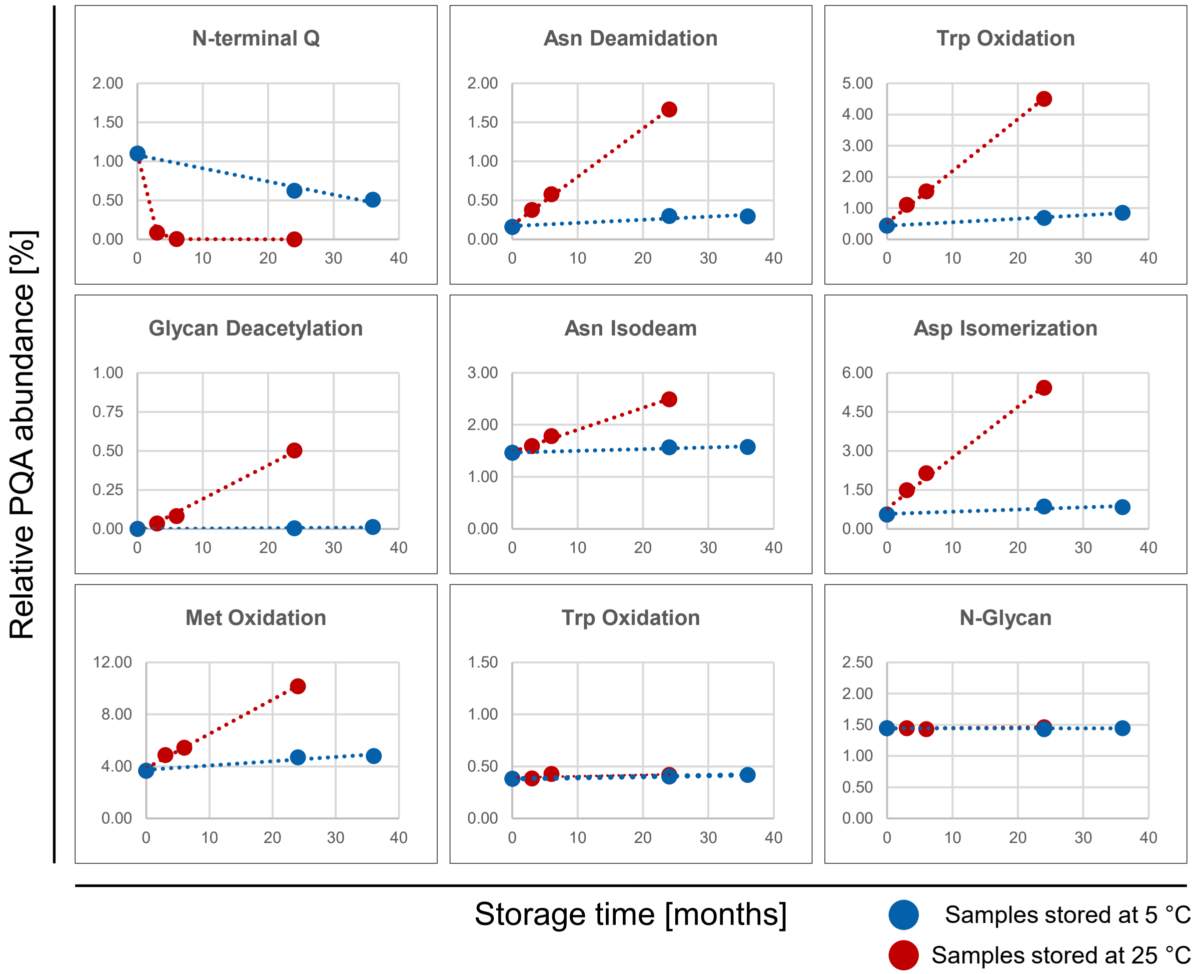
Name	Peptide	Peptide (Library)	Mass	Fold Change	Change Type	Integrated Max. Int.	Pass/Fail
Group_04161	SAAGAFGPELSR	SAAGAFGPELSR	1171.59	4.33	Increase	411140.38	Pass
Group_04849	LSSEAPALFQFDLK	LSSEAPALFQFDLK	1572.83	Infinity	New	49379.70	Pass
Group_04929	GILFVGSGVSGGEGAR	GILFVGSGVSGGEGAR	1600.81	Infinity	New	178098.56	Pass
Group_26629	IGDYAGIK	IGDYAGIK	843.46	Infinity	New	505838.53	Pass
Group_27222	DIPVPKPK	DIPVPKPK	900.55	Infinity	New	254079.31	Pass
Group_29205	SSAPPPPPR	SSAPPPPPR	985.52	Infinity	New	241043.60	Pass
Group_29267	HVLTSIGEK	HVLTSIGEK	990.56	Infinity	New	181322.62	Pass
Group_29411	LTILEELR	LTILEELR	995.59	Infinity	New	147921.14	Pass
Group_31063	GLILVGGYGTR	GLILVGGYGTR	1114.64	Infinity	New	151983.69	Pass
Group_31213	NGFILDGFPR	NGFILDGFPR	1144.59	Infinity	New	101499.09	Pass
Group_31494	GISNEGQNASIK	GISNEGQNASIK	1224.62	Infinity	New	187745.56	Pass
Group_31995	ELASGLSFPVGFK	ELASGLSFPVGFK	1358.73	Infinity	New	103271.73	Pass
Group_32078	TASEFDSAIAQDK	TASEFDSAIAQDK	1389.65	Infinity	New	116145.41	Pass
Group_32292	SFANQPLEVVYSK	SFANQPLEVVYSK	1488.77	Infinity	New	285242.44	Pass
Group_32427	ELGQSGVDTYLQTK	ELGQSGVDTYLQTK	1545.78	Infinity	New	206920.69	Pass

* report generated with custom activiy, not included in Genedata Expressionist 2025.1

All PRTC peptides must be found at 0.5 pmol spiking level (eq-uivalent to validated RL). Absence of new / changed species for neagtive control sample.

Case Study I: Long-Term Stability Study of mAb1

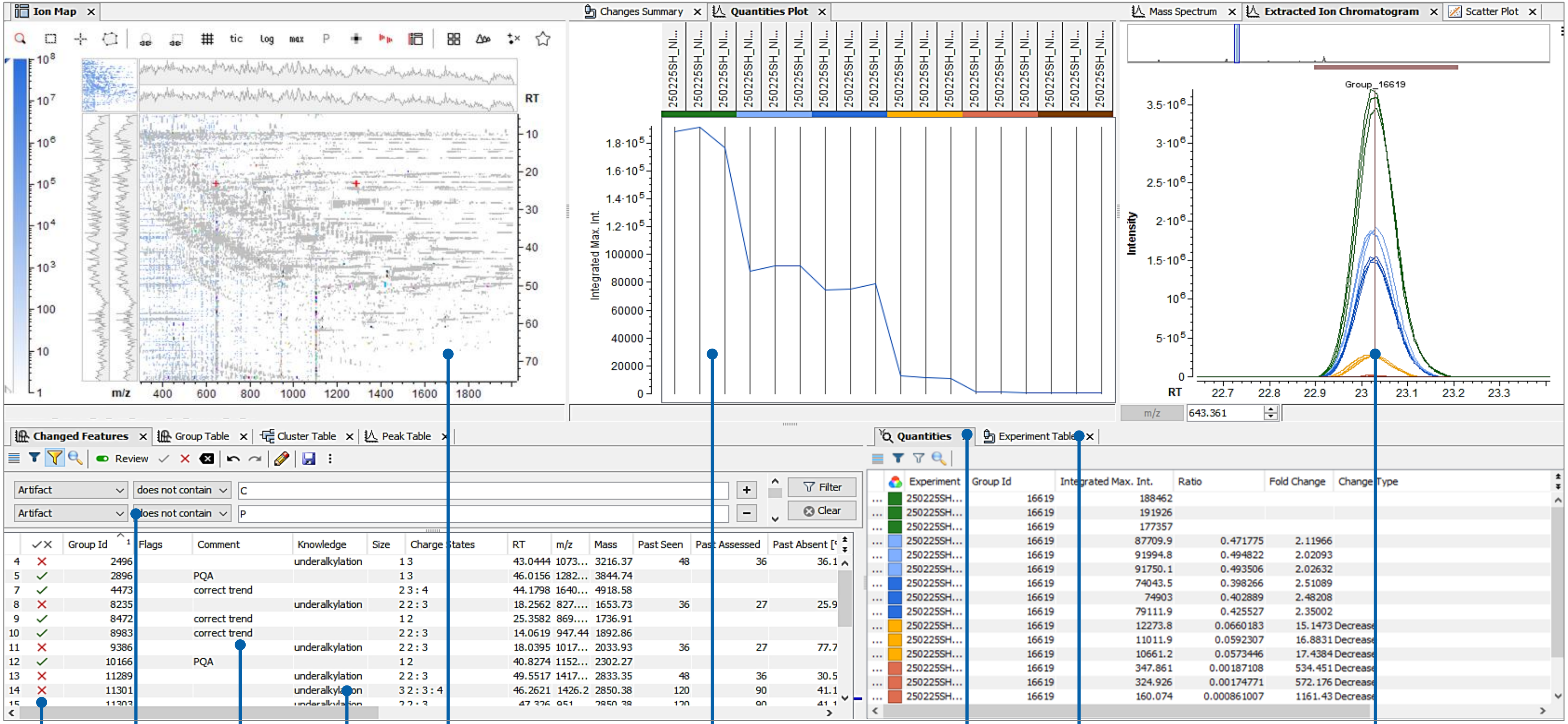
Targeted PQA Monitoring



- mAb1 samples from DP long-term (5 °C) and accelerated (25 °C) stability studies.
- Triplicate measurements of 6 time points including T0 (defined as reference during NPD analysis)
- MAM peptide library contains approx. 170 species of common post-translational modifications

mAb1 DP is stable under intended long-term storage conditions. Accelerated stability conditions trigger mAb1 degradation.

NPD User Interface – Comprehensive Information at Your Fingertips



NPD review

User comments

Ion map

NPD trending & replicate assessment

Sample selection

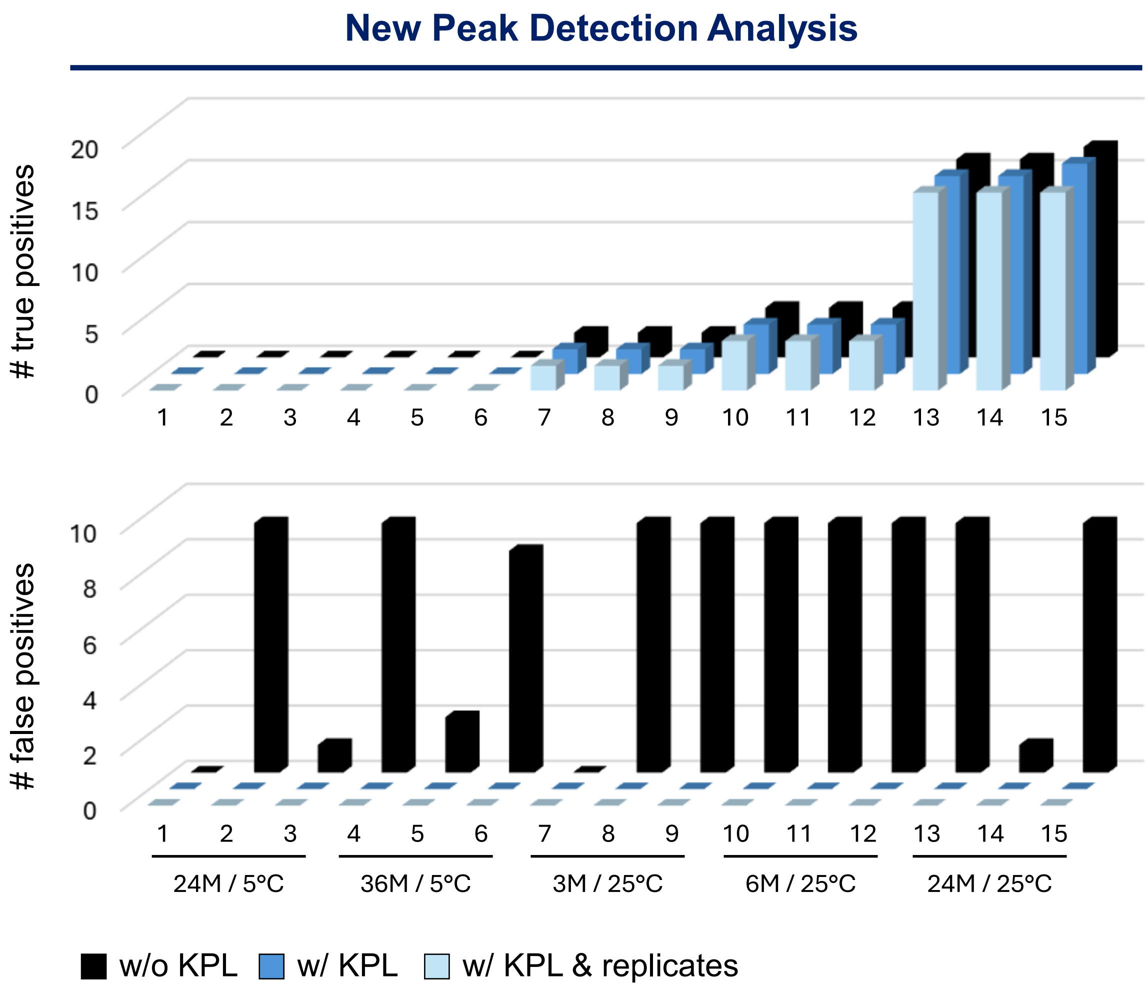
Advanced MS data visualization
(MS spectra, XIC, scatter plot)

Custom filtering

KPL knowledge

Quantitative information & statistics

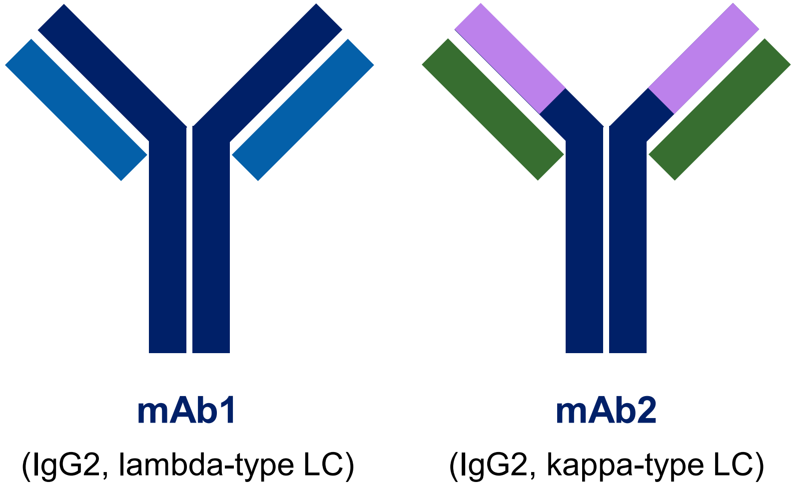
Case Study I: NPD Analysis of mAb1 Stability Samples



- IT = 0.05% BPC and FCT ≥ 3
- KPL (based on reference standard injections) included 40 species (e.g., underalkylation)
- Advanced filtering based on artifact flags, significance, KPL annotations, and charge state characteristics
- Required NPD data analysis time: ≤ 20 min

NPD results mirror the findings from targeted attribute monitoring. No false positive species reported.

Case Study II: Impurity Detection



NPD settings

IT (% BPC)	0.05%
FCT	≥ 3
# Rep.	3

Spiking level [%mol/mol]	**mAb2 LC peptides** detected / expected	**mAb2 HC peptides** detected / expected	**# False positives**
0.1	1 / 16	0 / 10	0
0.5	11 / 16	7 / 10	0
1.0	13 / 16	9 / 10	0
2.0	18 / 16[1]	11 / 10[2]	0

[1] two additional isoD variants of mAb2 LC peptides

[2] one additional miscleaved mAb2 HC peptide

- Spiking of mAb2 (IgG2, kappa LC) into mAb1 (IgG2, lambda LC) at patient-relevant levels (0.1 – 2.0%)
- Unique mAb2 peptides: 16 LC and 10 HC peptides (not including small and/or hydrophilic peptides)
- Unspiked mAb1 samples (n=3) served as references for NPD analysis

Results confirm specificity and sensitivity of the NPD workflow to detect unknown impurities at patient-relevant levels

Potential application of NPD in the context of protein characterization

Summary and Conclusions

- The developed Genedata Expressionist activities enable a robust and sensitive NPD workflow. The core NPD activities have been made publicly available in Expressionist version 2025.1.
- “Funnel strategy” to reduce the number of false positives during NPD analysis using a combination of automatic artifact detection, statistics, and KPL functionalities.
- Consolidated NPD analysis reduces the time investment for data analysis to minutes and allows for trending of new and changed species across a given dataset.
- The NPD workflow was validated as a limit test according to ICH Q2 guidelines. Results from individual case studies confirm workflow specificity and sensitivity to detect new and changed peptide species at patient-relevant levels.
- An informative and synchronized NPD user interface has been designed. Relevant and comprehensive data are displayed at your fingertips for data evaluation and informed decision making.

Acknowledgements

TRD Bx Switzerland

Thomas Pohl
Francois Griaud
Victor Le
Sonja Hudelmaier
Michael Schleicher
Haiyan Zhang
Michel Starck
Guillaume Rey
Joachim Ritter
Christoph Bächler
John den Engelsman

TRD Bx Austria

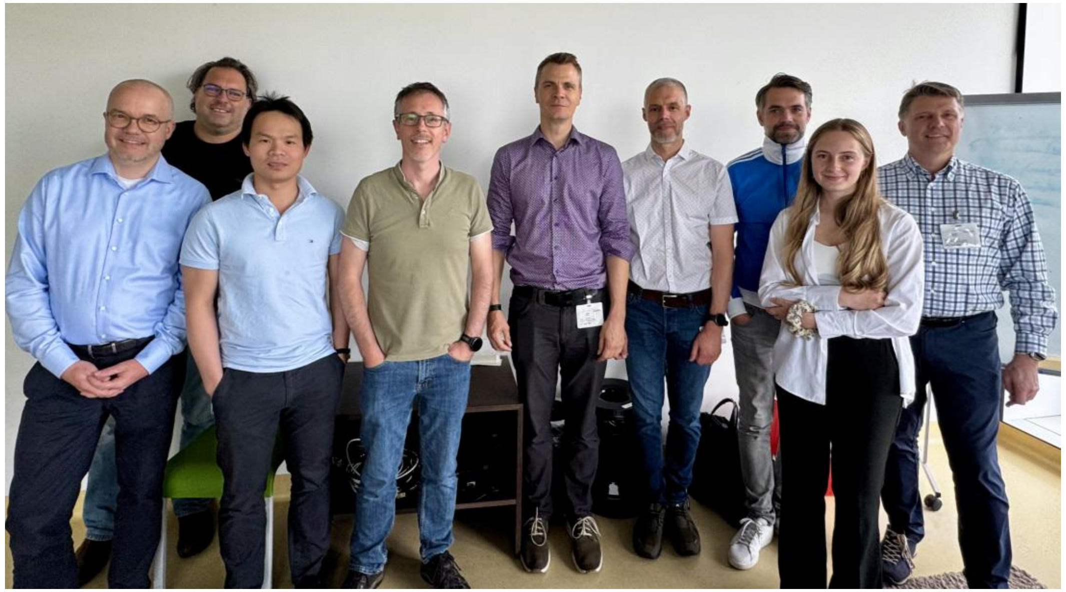
Stefan Mittermayr
Jens Pettelkau
Shah Hussain
Christian Kirchler

TRD Bx Slovenia

Maja Semanjski Curkovic
Aljaz Bertalanic
Nina Pirher
Eva Kranjc
Tilen Vidmar

Genedata

Claudio Schmid
Dominik Mertens
Marlis Zeiler
Andrei Starikov
Edward Waterman
Nick DeGraan-Weber
Michelle English
Carsten Soenksen
Reto Ossola
Petra Gutenbrunner



From left to right: Thomas Pohl, Michael Schleicher, Victor Le, Francois Griaud, Dominik Mertens, Reto Ossola, Patrick Merkle, Sonja Hudelmeier, and Claudio Schmid

Thank you

Contact information:

Patrick Sascha Merkle, PhD
patrick.merkle@novartis.com

Thomas Pohl, PhD
thomas.pohl@novartis.com

