A software platform for peptide synthesis QC by both LC-free MALDI-TOF and LC-ESI-QTOF molecular weight determination

ASMS 2019, WP 683

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Overview

- Synthetic peptides are used as drugs, in R&D, antibody generation and biological assays.
- Peptide synthesis requires careful quality control (QC) in order to, e.g., control unspecific reactions and side products such as truncated peptides or degradation products.
- We developed a workflow-driven software platform for routine peptide QC by molecular weight determination, which also facilitates work under compliant conditions.
- BioPharma Compass (BPC) software supports the routine analysis of biopharmaceuticals, both with **LC-ESI** and **LC-free MALDI** workflows.
- Traffic light reports can be generated to reduce analyst time and accelerate analysis return times.

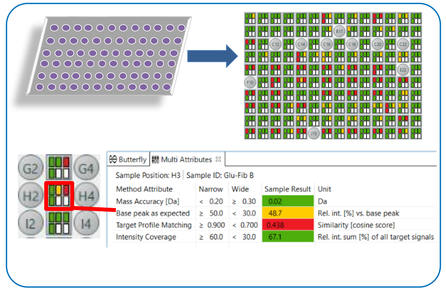

Fig. 1 Pass/fail results of the analysis are mapped directly to the position of samples on the MALDI plate or autosampler. Multiple Quality Attributes are reported simultaneously.

Methods

Peptides were synthesized by solid phase synthesis and purified using reversed phase chromatography.

MALDI samples were prepared on thin layers of HCCA matrix and acquired on a Bruker autofleX III MALDI-TOF in positive reflector ion mode, providing for isotopic resolution.

ESI spectra were acquired on a Bruker impact II QTOF using an HPLC system for sample injection of dissolved peptides without chromatographic separation.

Data were analyzed in BioPharma Compass 3.1 (BPC, Bruker), which provides a multi-attribute traffic light overview of each analysis and more details upon result selection. Mr values and intensities were obtained from ESI average spectra across eluting peaks and obtained by integration across the charge states z=1-6.

ESI spectra acquisition can be controlled within BPC and data directly stored in the database to warrant for data safety suitable for work under regulated conditions.

Position	Sample Name	Peptide Formula
B3	Leu-Enkephalin	C28H37N5O7
C3	Bradikinin fragment	C35H52N10O9
D3	Angiotensin III	C41H62N12O11
E3	Angiotensin II	C50H71N13O12
F4	Angiotensin I	C62H89N17O14
G3	Substance P	C63H98N18O13S1
H3	Glu-Fib B	C66H95N19O26
I3	Bombesin	C71H110N24O18S1
J3	Renin Substrate	C85H123N21O20
K3	ACTH clip 1-17	C95H145N29O23S1
L3	ACTH clip 18-39	C112H165N27O36

Fig. 2 A Sample Table provides the structural input to enable the spot specific interpretation of the acquired mass spectra.

MALDI

MALDI-MS is best suited for high throughput QC analysis and can easily acquire >60 spectra/min. The BPC processing time was negligible compared to acquisition with ~ 60 spectra analyses in 1 sec. Mass accuracy and susceptibility to oxidation at ambient air sample preparation present disadvantages.

Fig. 3 Example **MALDI** analyses:
Top Glu-Fib B represents a failed analysis requiring analyst inspection as the expected MH^+ peak has just 48.7% of the base peak intensity – due to loss of water (possible N-term pyro glutamylation).
Bottom Renin substrate was automatically validated with 4 green quality attributes. All side products (water loss, oxidation and salt adducts) were only marginally detected.

ESI

ESI works at lower throughput at ~5 min/sample with the advantage of 1-5 ppm mass accuracy and lower degree of oxidation artefacts.

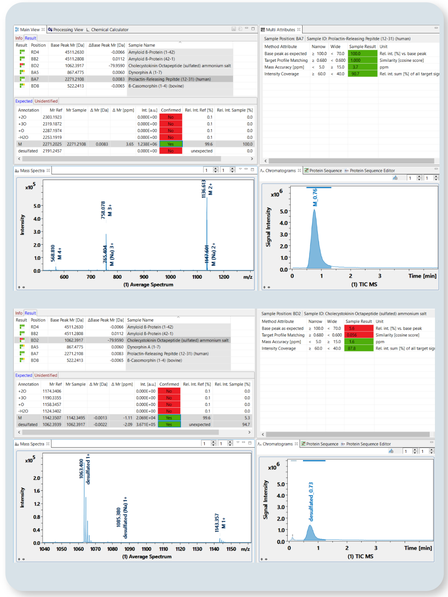

Fig. 4 Example **ESI** analyses:
Top PRP-12-31 was automatically validated based on the 2^+-4^+ states in the spectrum. 100 % of the spectra intensity was related to the target molecule.
Bottom COP sulfate was automatically marked as potentially failed analysis as the target mass amounted only to 5.3% of the spectra intensity. Base peak was the neutral loss of $-SO_3$(-80 Da), which is typical for sulfated peptides.

Mass Accuracy:
General QC parameter assessing the calibration quality of the dataset

Base Peak:
Indicates if the wanted target mass is base peak in the spectrum or at least greater than, say, 80% of it

Target Profile:
Expected, possibly wanted or unwanted, side products and artefacts can be defined in a quantitative profile. The similarity of the desired peak profile and the one in the spectrum is scored

Intens. coverage:
Scores the intensity of the peaks in the expected profile vs. all peaks in the dataset, well suitable to detect unexpected contaminations, early synthesis truncations etc.

Fig.5 The Quality Attributes and what they tell

Conclusions

- A software platform was developed and validated for the QC of synthetic peptides, both for MALDI-TOF as well as ESI-QTOF analysis
- Sample table and target profile control the automated interpretation of large numbers of peptides to be QCed even up to 100s or 1000s/per day
- They define target masses and modification or artefact profiles
- The traffic light reporting icons allow to speed up the oligo QC of large sample numbers
- CFR 21 part 11 compliant features, e.g., for data safety, available

BioPharma