A software platform for the quality control of synthetic oligonucleotides

ASMS 2019, ThP 601

Detlev Suckau[1], Sam Kyritsoglou[2], Yue Ju[3], Guillaume Tremintin[3], Anjali Alving[4], Michael Greig[3], Robert Kane[4]

[1] Bruker Corporation, Bremen, Germany
[2] Kaneka Eurogentec SA, Liège, Belgium
[3] Bruker Corporation, San Jose, CA, USA
[4] Bruker Corporation, Billerica, MA, USA

Overview

- The routine quality control (QC) of synthetic oligonucleotides currently faces challenges by the increasing demand for oligos in research as well as diagnostic and therapeutic applications. MS can address these needs, though with limitations:
- **MALDI-MS** can easily provide for the analysis of several 1000 samples per day, but its analysis success rate drops beyond the size of 30mers.
- **ESI-MS** can provide good quality data beyond the size of 100mers, but its throughput is limited to a few hundred samples per day.
- We have developed and applied a software platform for routine oligo QC that can be applied to both ESI and MALDI thus increasing throughput as well as success rate for the daily quality control requirements with reduced analysis return times.

Methods

Oligonucleotides were obtained by solid phase synthesis.

MALDI samples were prepared in 3-HPA/DAHC matrix using robotics on a 384 AnchorChip sample carrier and analyzed on a autoflex MALDI-TOF in linear positive ion mode (all Bruker).

ESI samples were on-line desalted using a C_{18} pre-column and DIEA as ion pairing agent. They were then eluted using acetonitrile to a microTOF Q III QTOF (Bruker) operated in negative ion mode.

Data were analyzed in BioPharma Compass 3.1 (BPC, Bruker), which provides a multi-attribute traffic light overview of each analysis which evaluates mass accuracy and sample purity. A detailed result of each analysis is available for reporting.

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.

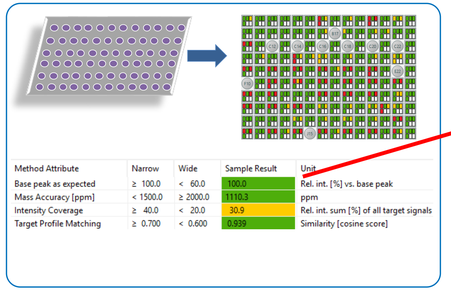

Fig. 1 Pass/fail results of the analysis are mapped directly to the position of samples on the MALDI plate or autosampler. Multiple Quality Attributes are reported simultaneously.

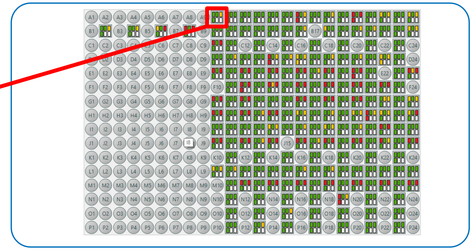

Fig. 2 MALDI sample plate result overview. Click on the reporting icon opens the more detailed view of the analysis as shown in Fig. 3 and 4.

MALDI

Automatic validation rate was >90% in the 20-30mer range (<12 kDa) but the failed analysis rate increased towards 20 kDa. The bulk of analyses was confirmed with MALDI acquisition times of 2 sec/sample and data analysis time of 5 sec for the ~200 sample set. The +1 charge states only were used for analysis.

ESI

ESI works at lower throughput (~1 min/sample), however with the advantage of 1-5 ppm mass accuracy and a high success rate of analysis even beyond 20 kDa oligonucleotides (>50mers). MaxEnt deconvolution is routinely applied for clear result representation.

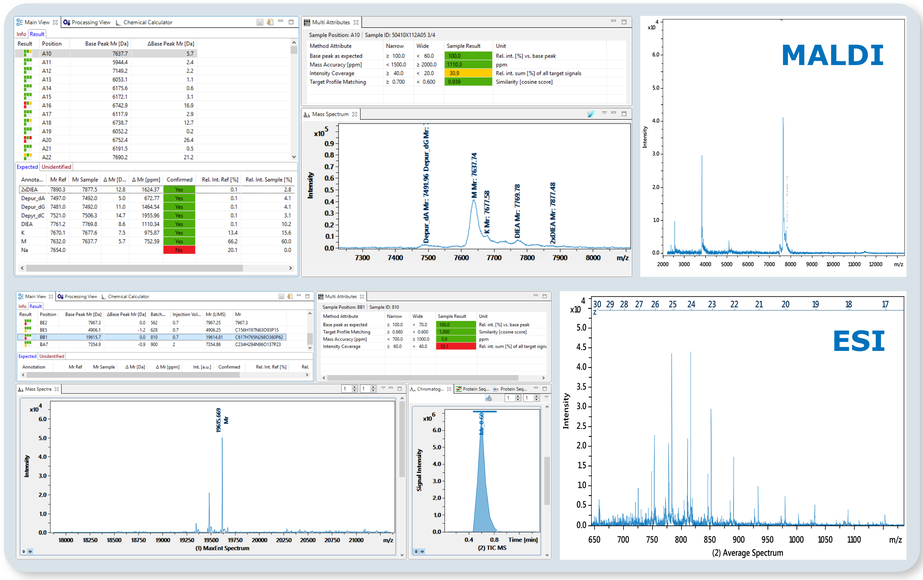

Fig. 3 (**Top**) Example **MALDI** analysis: Successful analysis, may require analyst inspection as the intensity coverage is somewhat low due to 2+ and 3+ charge states. Spurious depurination was observed as well.

Fig. 4 (**Bottom**) Example **ESI** analysis: Successful analysis of a large oligo. The intensity coverage is low as well, as some unexplained peaks at the low mass side of the target peak are present, which likely are early truncation products from oligo synthesis.

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 common software platform was developed and validated for the QC of synthetic oligonucleotides, both for MALDI-TOF as well as ESI-QTOF analysis
- Together, high throughput and success rate of QC analysis were achieved, which is not conceivable with one ionization technique alone
- The traffic light reporting icons allow to speed up the validation of large sample numbers
- CFR 21 part 11 compliant features available

BioPharma