



Rapid automated analysis of oligonucleotides and peptides using the neofleX platform

Rapid automated analysis and batch post-processing of DNA and RNA oligonucleotides and peptides was successfully demonstrated on a novel neofleX MALDI-TOF/TOF platform. Automated run of a fully loaded MALDI plate in 384-format was accomplished in less than 4 minutes. Confirmation of the sequence and modifications of a 12mer RNA oligonucleotide was shown in TOF/TOF mode.

Introduction

Fast development of new therapeutic modalities in the past two decades required the concurrent development of automated mass spectrometry analysis methods to analyze drug substance, impurities, and degradants. One of the drug modalities that saw explosive growth in the past several years is RNA and DNA therapeutic oligonucleotides. Many of the therapeutic RNA oligonucleotides are modified at 2'-OH as 2'-O-methyl or 2'-fluoro derivatives to improve metabolic stability. Among 18 oligonucleotide therapeutics approved by the end of 2022, most single stranded oligonucleotides have 30 or fewer nucleotide residues, and double stranded have 23 or fewer nucleotides per strand. Seven out of these 18 therapeutic oligonucleotides were approved between 2020 and 2022 [1].

Keywords:
Automation, MALDI-TOF,
neofleX, RNA,
oligonucleotides, DNA,
peptides

MALDI MS is ideally suited to increase productivity with automated analysis. This study focuses on demonstrating automated analysis capabilities of the novel neofleX™ MALDI-TOF/TOF platform for DNA and RNA oligonucleotides and peptides.

Methods

Fibrinopeptide A and [Glu]-fibrinopeptide B were acquired from Millipore Sigma (St. Louis, MO). The 17mer and 21mer DNA oligonucleotides were purchased from Integrated DNA Technologies (Coralville, IA). The 12mer, 40mer and 60mer 2'-O-methyl RNA oligonucleotides were graciously provided by Axolabs (Kulmbach, Germany). The peptide samples were prepared using a 2 mg/mL solution of α -cyano-4-hydroxycinnamic acid in 85% acetonitrile, 0.1% TFA containing 1 mM ammonium dihydrogen phosphate on a BigAnchor MALDI plate. The BigAnchor plate was fully loaded with 384 samples. The DNA oligonucleotide samples were prepared using a 10 mg/mL solution of 3-hydroxypicolinic acid in 30% acetonitrile containing 2 mg/mL diammonium hydrogen citrate and spotted on an AnchorChip MALDI plate. The RNA oligonucleotides were prepared using a 15 mg/mL solution of 2,4,6-trihydroxyacetophenone in 30% acetonitrile containing 1.3 mg/mL diammonium hydrogen citrate on a polished steel MALDI plate.

The measurements were performed using a neoflex benchtop MALDI-TOF/TOF mass spectrometer (Bruker Scientific, Billerica, MA) equipped with a smartbeam 3D 10 kHz laser. The automated runs for peptide samples were acquired in reflector mode using 200 laser shots per spectrum. The automated runs for oligonucleotide samples were acquired in linear mode using 5000 laser shots per spectrum. The spectra were post-processed in batches using the multi-target screening MALDI workflow in BioPharma Compass[®] software. The MALDI-TOF/TOF spectrum of the 12mer RNA oligonucleotide was post-processed in BioTools, a part of BioPharma Compass software package.

Results

Rapid automated runs are relevant for a wide variety of analytes including peptides, intact proteins, DNA and RNA oligonucleotides, lipids, lipid nanoparticle components, and small molecules. They provide near instant mass verification and information about impurities and degradants, which is valuable in assay optimization, verification of substrates, and quality control.

Automation run setup in the acquisition software was intuitive and accommodated any run size from one sample to a complete MALDI plate. The automated run of a fully loaded MALDI plate containing 384 peptide samples took 3 min 57 s (Figure 1). The whole batch was post-processed using the multi-target screening MALDI workflow in BioPharma Compass software. The results were visualized on a plate level in traffic light style. Two out of six attributes including mass accuracy and target profile matching based on cosine score were selected for the workflow. All but one sample spot (383 green positions in the plate view) passed the selected screening criteria, while one spot passed marginally due to low S/N ratio (1 yellow position in the plate view) (Figure 1). When the number of laser shots per spectrum was increased all sample spots passed the screening criteria.

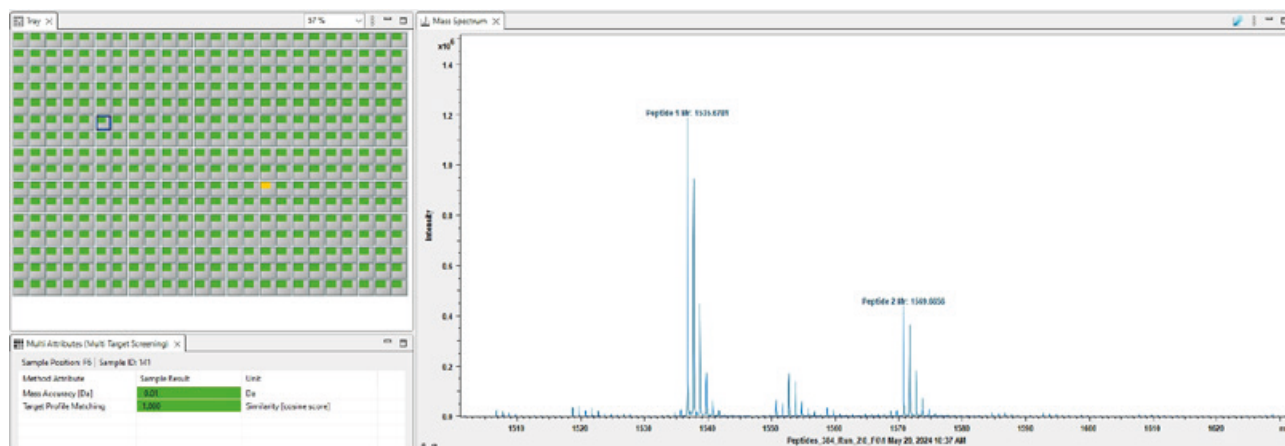


Figure 1

Automated run and batch post-processing results of a peptide mixture from a MALDI plate in 384-format using the multi-target screening MALDI workflow in BioPharma Compass software

The screening of a mixture of 17mer (Mr 5228.4) and 21mer (Mr 6372.1) DNA oligonucleotides was carried out using an identical attribute definition in the same BioPharma Compass workflow (Figure 2). The run was acquired from 24 identical sample spots, and all of them passed the screening criteria. RNA oligonucleotides (40mer and 60mer) were screened using mass accuracy and intensity coverage, and all sample spots in each of the two batches passed these criteria (Figures 3, 4).

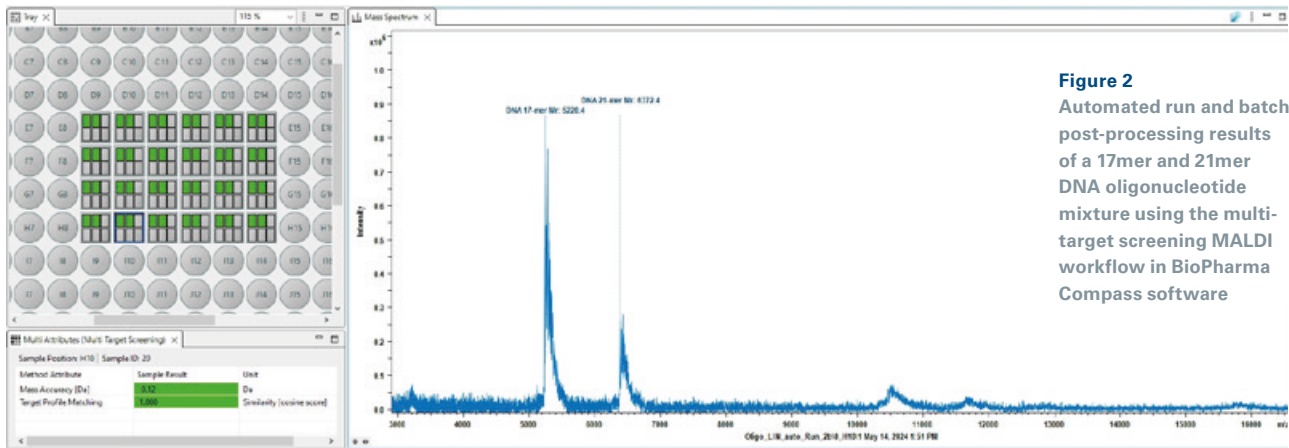


Figure 2
Automated run and batch post-processing results of a 17mer and 21mer DNA oligonucleotide mixture using the multi-target screening MALDI workflow in BioPharma Compass software

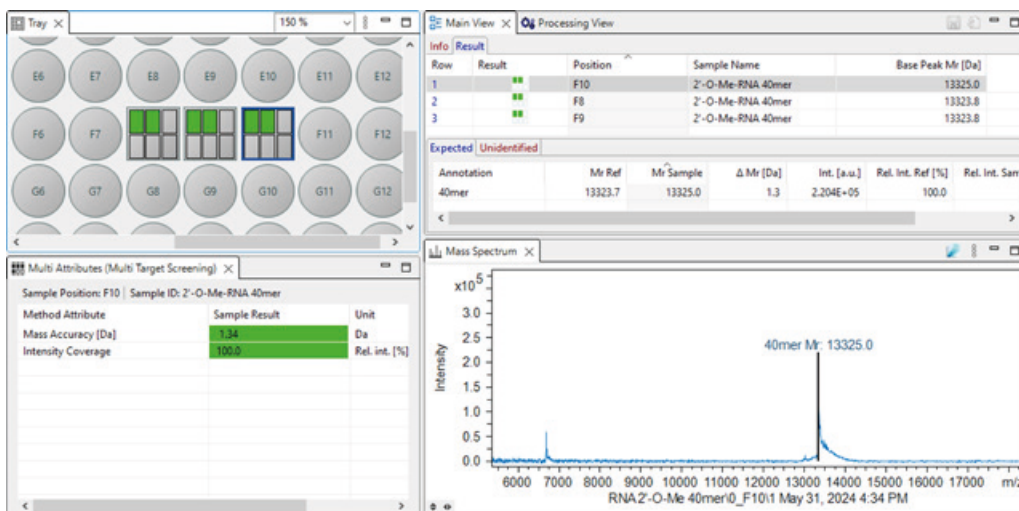


Figure 3
Automated batch processing results of 2'-O-methyl 40mer RNA oligonucleotide

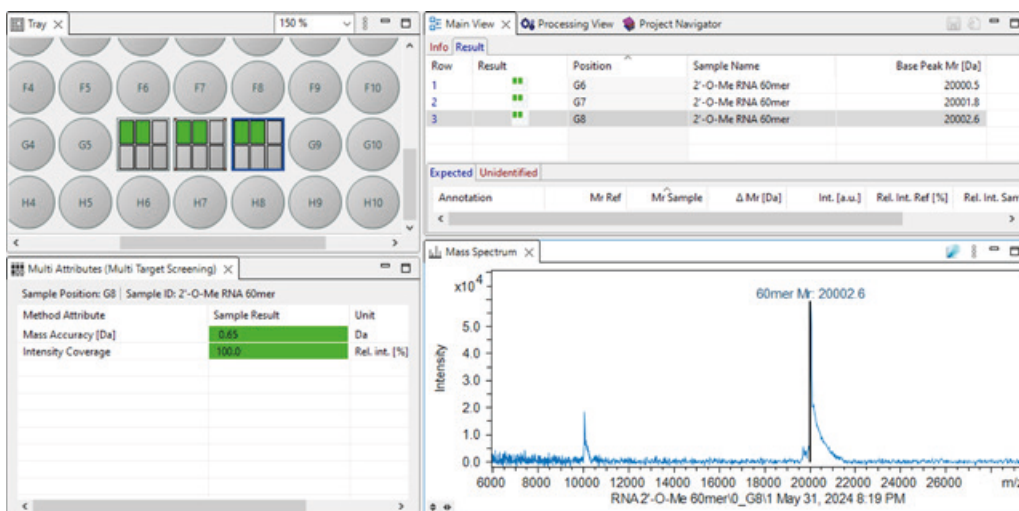


Figure 4
Automated batch processing results of 2'-O-methyl 60mer RNA oligonucleotide

MS/MS measurements allow quick confirmation of an oligonucleotide sequence and modifications. The MALDI-TOF/TOF spectrum of the 12mer RNA oligonucleotide at m/z 3922.733 confirmed the sequence GCACGGCUCGTT and verified methylation of every nucleotide residue as well as loss of phosphate from the 5' terminus (Figure 5). Sequence validation coverage of 100% was achieved for this RNA oligonucleotide.

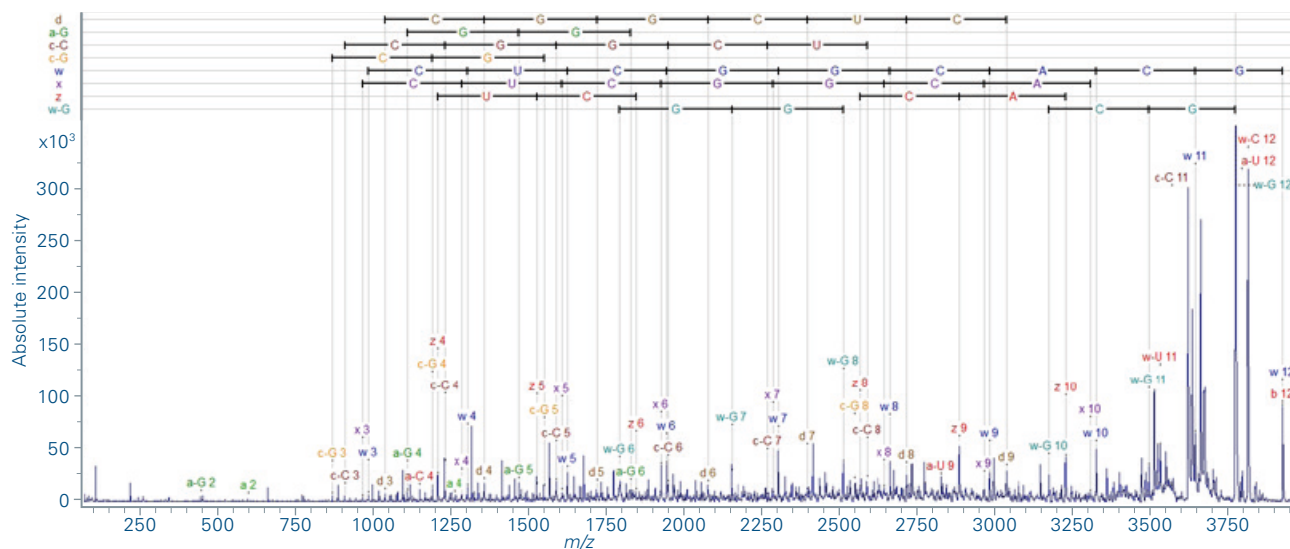


Figure 5
MALDI-TOF/TOF spectrum of 2'-O-methyl 12mer RNA oligonucleotide at m/z 3922.733

Conclusion

- Automated runs on a neoflex MALDI-TOF/TOF platform allowed performing rapid analysis of a MALDI plate in 384-format in less than 4 minutes.
- Batch post-processing in BioPharma Compass software provided flexibility to screen using multiple attributes with customizable acceptance criteria and result visualization on a plate level in traffic light style.
- This robust workflow is applicable to a wide range of modalities including RNA and DNA oligonucleotides and peptides.
- Confident oligonucleotide sequence confirmation and verification of modifications was achieved using TOF/TOF measurements.

References

- [1] Egli M, Manoharan M (2023). *Nucleic Acids Res.* **51**(6):2529. doi: 10.1093/nar/gkad067.

For Research Use Only. Not for use in clinical diagnostic procedures.

Bruker Switzerland AG

Fällanden · Switzerland
Phone +41 44 825 91 11

Bruker Scientific LLC

Billerica, MA · USA
Phone +1 (978) 663-3660

