Rapid Oligonucleotides QC with Automated High Resolution ESI-LCMS and BioPharma Compass

Oligonucleotides – sequences of nucleotides (RNA and DNA) – are vital research tools that have a wide range of applications in genetic testing, research, and forensics.

Abstract

Common applications include artificial gene synthesis, primers for polymerase chain reaction (PCR), DNA sequencing, molecular cloning and as molecular probes. Oligonucleotides are typically made in the laboratory by a solid-phase chemical synthesis process using phosphoramidite chemistry. Modern synthesis platforms and high-throughput automation systems support robust oligonucleotide manufacturing capabilities. Despite these advances, oligonucleotide synthesis is a complex process, involving hundreds of sequential chemical reactions. Quality control of precise and accurate synthesis is of high importance as it is ensuring that synthesis by-products or process contaminants are within acceptable limits.

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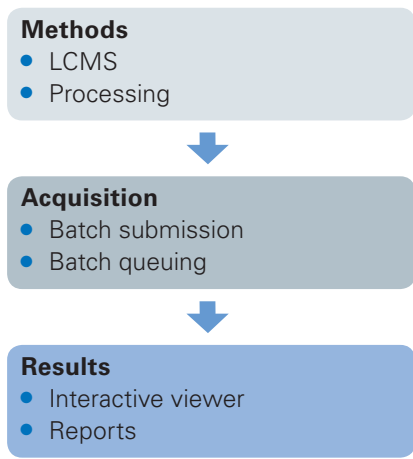
Steps involved in setting up the automated workflow

Mass Spectrometry (MS) is the technology of choice for analyzing oligonucleotides. It provides confirmation that the correct sequence has been synthesized with high mass accuracy and enables sensitive detection of low levels of by-products generated during synthesis process such as truncated or deleted sequences, depurination and incomplete removal of protecting groups. Mass spectrometry techniques and integrated software solutions facilitate oligonucleotide quality control by assessing oligonucleotide identity in a high-throughput manner.

Here we describe an automated UPLC-MS method for the quality control of synthetic oligonucleotides. The automated workflow is implemented using BioPharma Compass® software which encompasses data acquisition, processing and reporting using a seamless workflow solution.

Introduction

Dedicated software, such as BioPharma Compass (Bruker Daltonics), enables the routine analysis of biopharmaceuticals including oligonucleotides and offers LC-MS workflows with automated measurements and report generation. BioPharma Compass comprises a
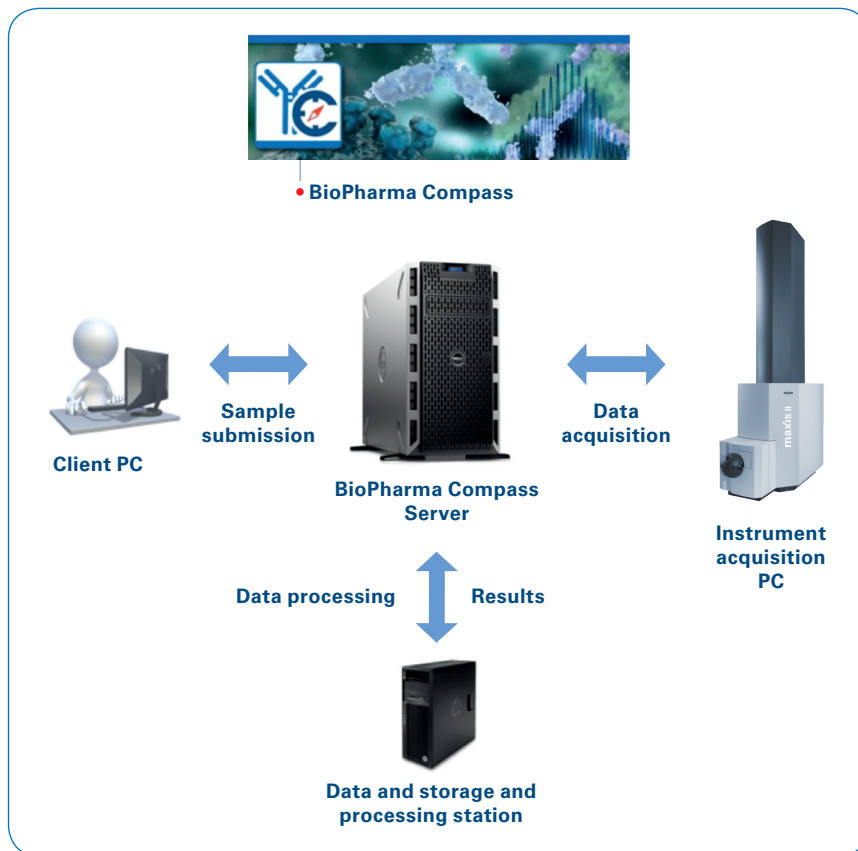

Figure 1: BioPharma Compass data flow

LC gradient	
Time	**%B**
0	10
10	35
22	50
23	10
25	10

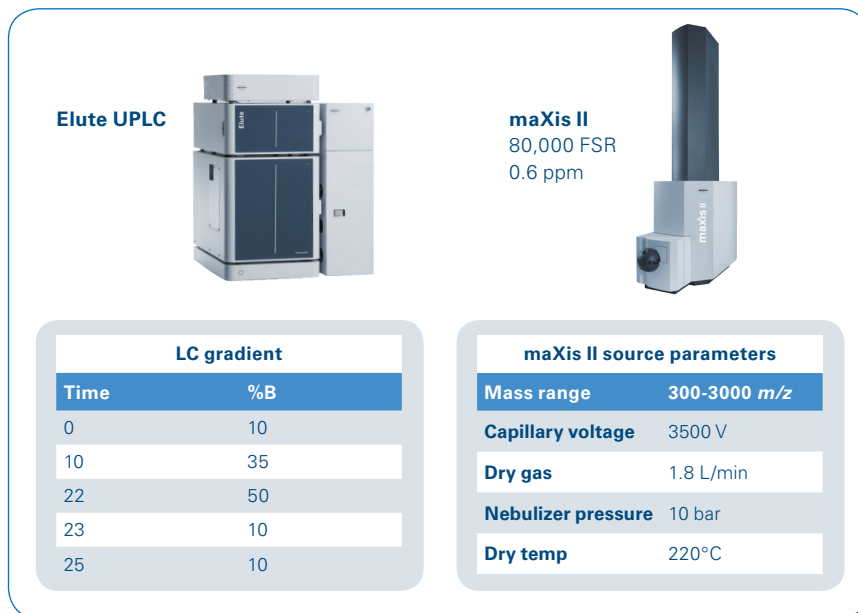| maXis II source parameters	
Mass range	300-3000 *m/z*
Capillary voltage	3500 V
Dry gas	1.8 L/min
Nebulizer pressure	10 bar
Dry temp	220°C

Figure 2: Elute HPLC gradient and maXis II parameters

secure server that communicates with the client PC and the instrument acquisition PC. The user can set up sample batches for acquisition; the data are acquired and can be automatically moved to the data storage and processing station to preserve the data integrity and security. The results are accessible to the user from the BioPharma Compass server. The entire workflow is fully supported by 21 CFR Part 11 compliance features.
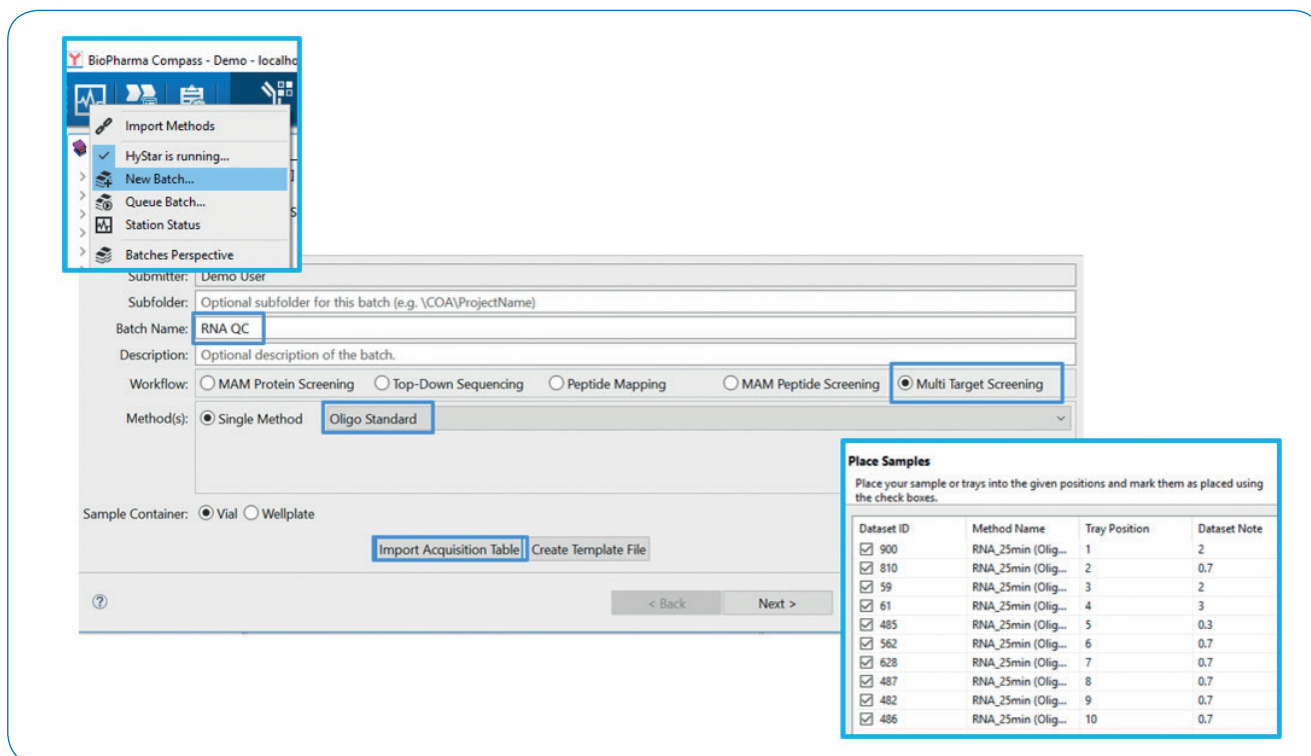
Figure 3: Automated acquisition set-up in BioPharma Compass

Experimental

A fully automated acquisition and processing workflow for rapid oligonucleotide QC was defined in BioPharma Compass. The LCMS method set included the LC separation and the detection by mass spectrometry. The separation was carried out on a Bruker Elute UPLC system (Bruker Daltonics) equipped with a BEH C4 1.7 µm 1x100 mm column at a flow rate of 500 µL/min and a temperature of 80°C. The mobile phases were A – 1% HFIP, 0.1% Hexylamine in water and B – 50% ACN + 50% MeOH. One µg of synthetic oligonucleotide samples dissolved in water were injected on column. The detection was performed on a Bruker maXis II ETD UHR QTOF operated in negative ion mode. The LC gradient and MS source parameters are shown in Figure 2.

The Automated Acquisition button in the blue BioPharma Compass Ribbon allowed straightforward setting up of sample batches for acquisition. The simple interface required minimal input from the user for a batch name, workflow and method as seen in Figure 3. A LIMS generated worklist can be directly imported. Once the samples are placed in the autosampler positions, the acquisition of the queued batch is initiated.

The processing view in BioPharma Compass allows monitoring of the submitted queue. As the data is acquired, the data mover feature automatically moves it to the data storage and processing station preventing direct user access, thus preserving data integrity and maintaining security. Spectra were obtained across the chromatographic peaks and charge deconvoluted using a Maximum Entropy algorithm yielding neutral average mass molecular weights.


Figure 4: Processing view shows status of queued batch

Results

BioPharma Compass has an interactive user-friendly interface that allows users to see the results at-a-glance. Several views highlight different aspects of the data such as the result tables, chromatograms and spectra. Screening of batches is facilitated through similarity scores and mass accuracy attributes which are the basis for an automatic pass/fail assessment. Reports utilize color coding of results and charts for rapid reviewing.

The Result table summarizes the analyses in the sample table including the MAM reporting icon 📊, base peak of the spectrum and mass difference to the base peak. Clicking one of the rows in the results table shows the expected table view seen in Figure 5 with the detailed modifications for that specific analysis. The relative intensities of the sample versus the reference allow comparison of the measured profile to the reference profile.
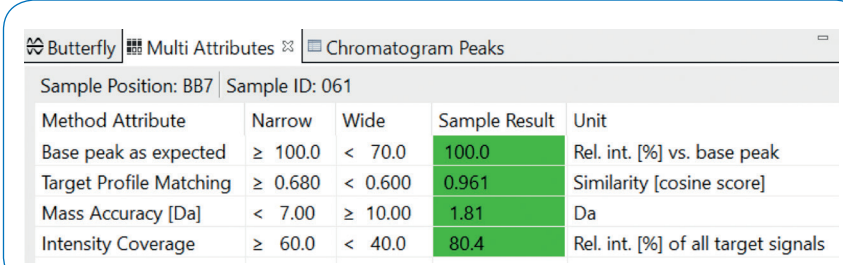

Figure 5: Results summary table and detailed expected results table showing fragmentation products and adducts resulting from the chromatographic additives.

Figure 6: Multi-attribute table provides a legend to each individual sample result.

Figure 7: Chromatogram and spectrum views

Figure 8: Report from an example dataset

The Multi-Attributes window (Figure 6) shows that this sample has the right base peak, the target profile matches expectations, good mass accuracy and intensity coverage.

The chromatogram window shows the highlighted TIC and details about the Rt, area and intensity. The spectra windows allow a detailed inspection of the raw spectrum and the Maximum Entropy deconvoluted spectrum.

A report was generated, summarizing the workflow acquisition and processing parameters with details about the chromatogram, spectra, summary tables and multi-attribute pass-fail criteria.

Conclusion

- BioPharma Compass enabled straightforward automated acquisition and processing of oligonucleotide QC batches in a seamless workflow that included LCMS acquisition methods, batch submission and queuing, result viewing and report generation.

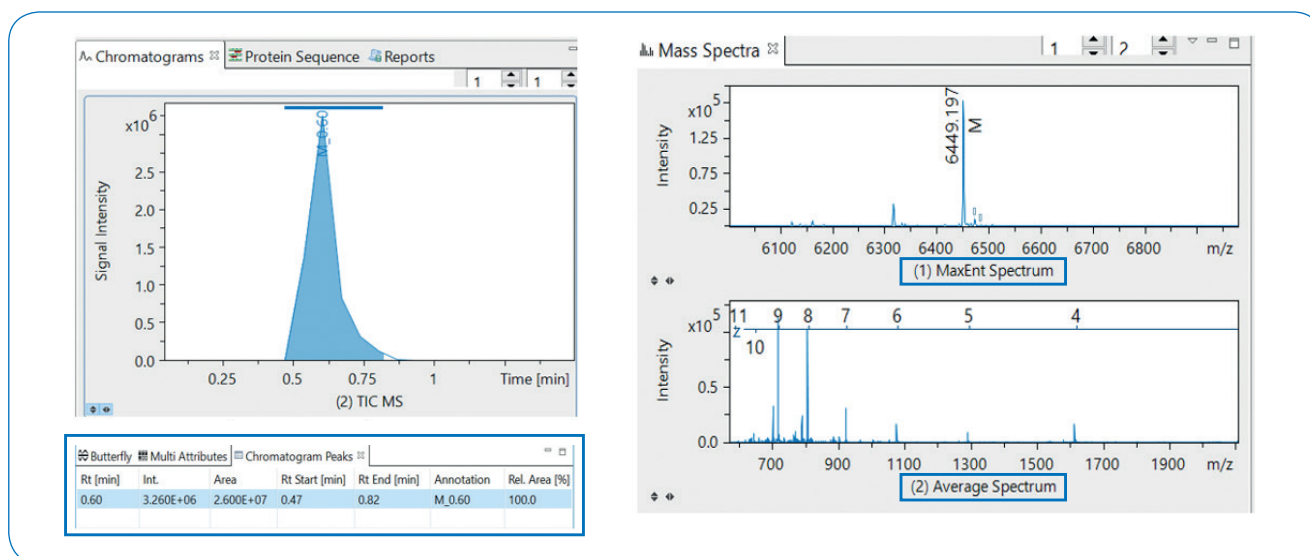- The multi-attribute screening workflow allows users to obtain critical information about synthesis process modifications for rapid QC of the final product.

Learn More

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http://www.bruker.com/massspectrometry

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