COMPLIANT-READY WORKFLOW FOR MASS CONFIRMATION OF OLIGONUCLEOTIDES AND RELATED IMPURITIES

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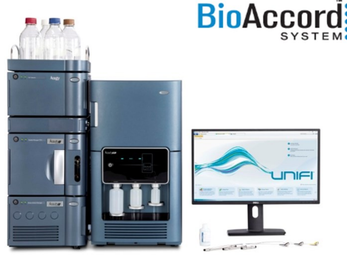
OVERVIEW

Waters UNIFI™ Scientific Information System is the first compliance-ready software platform to merge LC and high performance MS data into a single solution that encompasses data acquisition, processing, visualization and reporting. It supports a wide range of biotherapeutic workflows, including mass confirmation of synthetic oligonucleotides and their related impurities. Here we present on the automated data processing and reporting workflow for oligonucleotides using an oligonucleotide standard mixture.

INTRODUCTION

Advances in the biology, stability and delivery of oligonucleotides have led to a resurgence in the development of oligonucleotide-based therapies. With these advances comes an increasing desire to process oligonucleotide mass spectrometry data and a need for informatics development to enable compliance-ready data capture, processing and reporting, in support of regulatory filings for current and future drug candidates.

Recent improvements to our UNIFI software allow for the mass confirmation of oligonucleotides and their related impurities in a compliance-ready manner, thus supporting GMP development, manufacturing, QC release testing and associated regulatory filings.



The results of these improvements allow the user to specify 'Mass Only' target masses and to define and specify custom modifications. It also allows selecting regions of interest using retention time windows. A choice of deconvolution algorithms have been made available so oligos can be processed more effectively based on the mass range.

We explored the capabilities of this new UNIFI™ workflow by running our MassPREP Oligonucleotide Standard, containing a mixture of oligonucleotides and synthesis related impurities, on our recently introduced BioAccord™ LC-MS System.

METHODS

Materials

Oligonucleotide Separations Technology (OST) Standard supplied by Waters is a lyophilized equimolar mixture of 15, 20, 25, 30 and 35 nucleotide long oligodeoxythymidines. The OST standard is reconstituted in RNA-free water to 2µM final concentration for LCMS analysis.

LC Conditions

As part of the BioAccord™ LC-MS system, an ACQUITY™ UPLC™ I-Class PLUS fitted with an Oligonucleotide BEH (Bridged Ethylene Hybrid) C18 column (2.1 x 50 mm, 1.7 µm particles) was used to separate the five oligos in the standard at a flow rate of 200 µL/min and a column temperature of 60°C. The mobile phase composition was: Solvent A: 10mM triethylamine (TEA) and 50mM hexafluoro-2-propanol (HFIP) in Milli-Q water and Solvent B: 5mM TEA, 25mM HFIP in 50% methanol.

TIME (minutes)	FLOW (ml/min)	%A	%B
0	0.2	78	22
10.0	0.2	60	40
11.0	0.2	40	60
12.0	0.2	40	60
12.1	0.2	78	22
15.0	0.2	78	22

LC Gradient

MS conditions

Mass data was acquired by the ACQUITY™ RDa™ Mass Detector in negative ion mode over the m/z range of 400-5,000 with a full scan rate of 2Hz, cone voltage of 30V and desolvation temperature of 450°C.

The BioAccord™ LC-MS System has built-in self-diagnostic capabilities, or what we call an intelligent health system, that maximizes ease-of-use and uptime by identifying and resolving issues quickly. Intuitive software and SmartMS features automatically optimize performance and detect error conditions when they arise, proving step-by-step guidance on how to resolve.

Acquity RDa™ Self-Diagnostic Panel

Informatics

UNIFI™ Intact Protein Analysis within the Biopharma workflow has been enhanced to allow it to process 'Mass Only' oligonucleotide targets. This has enabled the workflow to target any mass without the need for the user to specify a sequence. Deconvolution includes two algorithms, MaxEnt1 to process masses larger than 5 KDa and also BayesSpray to for masses ranging from 0 to 500 KDa (Figure 2).

RESULTS

Component Name	Expected RT (min)	Time window (min)	Expected mass (Da)
Oligo_15T	3.93	0.2	4500.9331
Oligo_20T	6.67	0.2	6021.8990
Oligo_25T	8.98	0.2	7542.8649
Oligo_30T	10.67	0.2	9063.8308
Oligo_35T	11.87	0.2	10584.7967

Figure 1. The five oligodeoxythymidines that are part of the MassPREP OST Standard are added to the component table. Inputs include the expected retention time and average mass for each oligo.

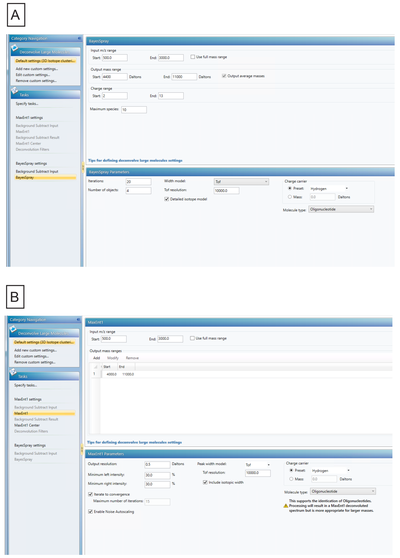


Figure 2. Two deconvolution algorithms are available for processing oligonucleotide mass data, both resulting in average mass output. BayesSpray (A) is more suitable for smaller oligonucleotides (less than 5,000 MW), and MaxEnt1 (B) is better for larger oligonucleotides (greater than 5,000 MW).

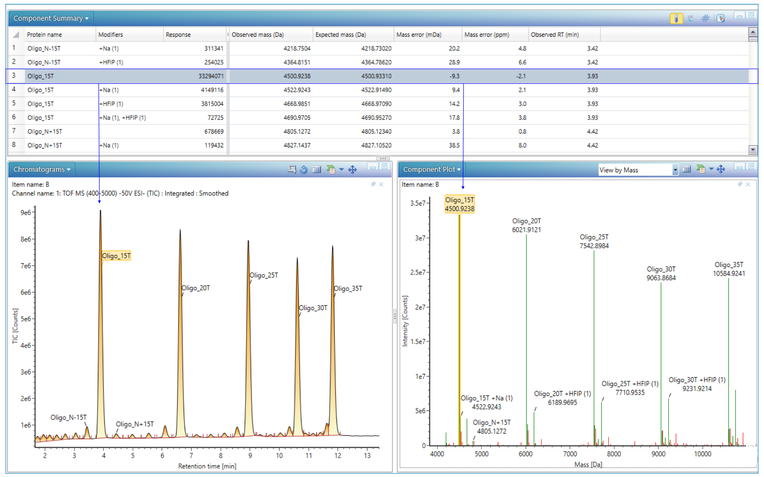


Figure 3. The five standard oligonucleotides part of OST Standard were successfully detected . Adducts as well as impurities are also highlighted . UNIFI™ visualization is synchronized across all panels under the Review tab; 15T oligo is highlighted in the component summary and i is automatically displayed in the chromatogram and component plot.

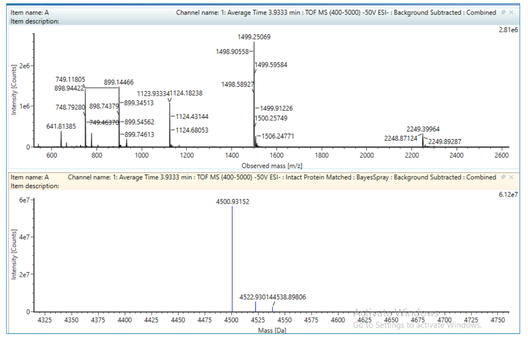


Figure 4. Example spectra of 15 nucleotide standard (top) and its subsequence mass deconvoluted results using BayesSpray.

Visualization Tools

Summary plots are visual representation of attributes across samples including retention time, observed mass, mass error and MS response among others.

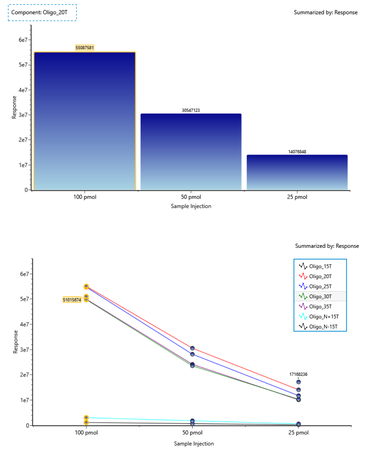


Figure 5. Summary plot representing MS response of a single component , Oligo_20T, across 100pmol, 50pmol and 25pmol samples (Top) . Summary plot illustrating all identified oligonucleotide components across the samples (Bottom).

CONCLUSIONS

- Herein we demonstrate a compliance-ready workflow for the mass confirmation and impurity profiling of oligonucleotide samples.
- Oligonucleotide workflow offers a choice of two different deconvolution algorithms, providing flexibility to work with small and large oligos, with each delivering average mass output.
- Oligo-related impurities can be customized and stored in library for automatic confirmation.
- Reports are fully customizable and can be automatically produced as part of the processing workflow.

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