Single Quadrupole Mass Spectrometry Quick Workflows Specified to Oligonucleotides Screening

Kosuke Uchiyama[1], Noriko Kato[1], Qiuyi Wang[1], Simon Ashton[2], Neil Loftus[2], Risa Suzuki[1, 3], Yuka Fujito[1]

[1]Shimadzu Corporation, Kyoto, Japan; [2]Shimadzu Corporation, Manchester, United Kingdom; [3]Shimadzu Scientific Instruments, Inc., Columbia, Maryland

Overview

- A Quick Picking algorithm has been developed to automate deconvolution of multiply charged components for charactering chemically synthesized oligonucleotides without the input of a defined full-length product (FLP) sequence.
- The algorithm has been applied to the analysis of a 20-mer and 80-mer antisense DNA using a nominal mass single quadrupole LC-MS instrument. Each detected peak is automatically deconvoluted and molecular mass information reported.

Introduction

A workflow has been developed to deliver molecular mass information on the full-length product and related impurities for chemically synthesized oligonucleotides by applying a deconvolution algorithm to all detected components within a targeted data stream (UV/PDA, TIC or BPC). It has been designed to simplify the data processing experience without the need for a sequence input delivering results quickly by automating mass assignment of all detected components in a data stream.

2. Materials and Methods

2.1 LC-MS/MS method

20-mer sample. A phosphorothioate-modified oligonucleotide purified was used as a sample (20-mer anti-sense oligo; C229 H316 N69 O121 P19 S19; average mass 7168.05, Te-smCe-sTe-sTe-sGe-sGd-sTd-sTd-sAd-sCd-sAd-sTd-sGd-sAd-sAd-sAe-sTe-smCe-smCe-smCe) spiked with 10% impurities (N-1(5') and N-3(5') shortmers) to the full-length product.

80-mer antisense DNA sample. 80-mer PolyT oligonucleotide sample (C800 H1041 N160 O558 P79; average mass 24273.62) was also analyzed was analyzed by ion-pair reversed-phase chromatography mode using HFIP (hexafluoroisopropanol) and 10 mM TEA (triethylamine)..

LC Separation. Components were separated using the Shimadzu Nexera XS™ inert LC and Shim-pack Scepter™ Claris column using HFIP (hexafluoroisopropanol) and 10 mM TEA (triethylamine) as ion pair additives.

Mass Spectrometry Detection. Shimadzu Single Quadrupole LCMS™-2050. Data acquired in profile mode. MS mass scan *m/z* 600-2000; 1 secs; negative ion mode.

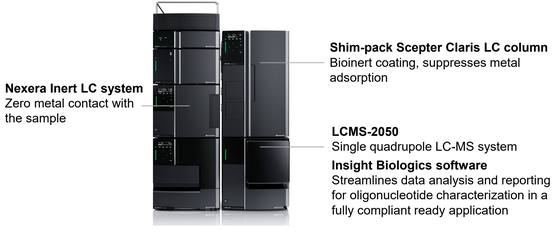


Figure 1. LC-MS configuration for characterising chemically synthesized oligonucleotides

3.Results

3.1 Workflows and Methods

Insight Biologics software application supports two workflows to help accelerate oligonucleotide characterization. The default data processing behavior was driven by a defined FLP sequence to confirm sequence composition, identify product related impurities and assess impurity profiles. Data processing has been further augmented by enabling automated deconvolution routines to be applied without a defined FLP sequence input to help workflows that need to quickly check on molecular mass information in a synthesis (Figure 2).

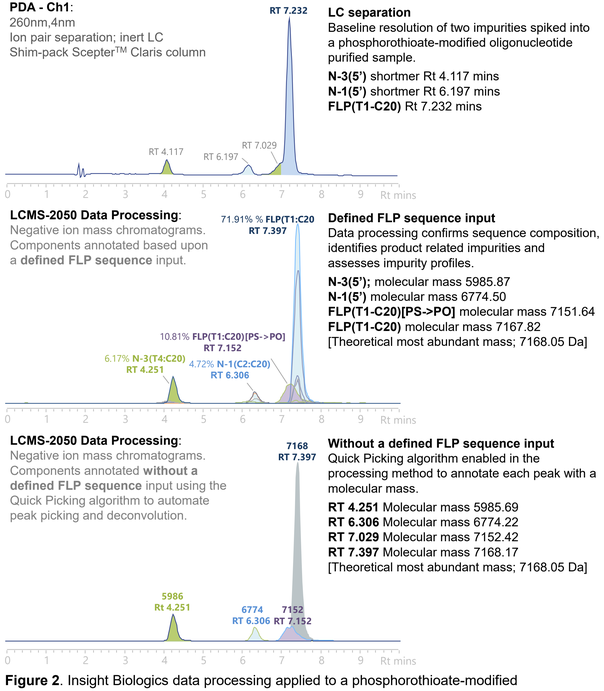


Figure 2. Insight Biologics data processing applied to a phosphorothioate-modified oligonucleotide purified sample spiked with N-1(5') and N-3(5') shortmer impurities. The Quick Picking algorithm generated equivalent molecular mass information compared to a conventional data processing pathway using a defined FLP sequence.

3.2 Data Processing Pathways

Given the need to process data acquired on a complex synthetic oligonucleotide sample with tools to support ion additions, sequence deletions [shortmers], sequence additions [longmers] and a dictionary of predictable impurities to annotate FLP and impurity components with high reporting confidence there is also a need to provide flexibility in generating molecular weight information quickly.

- Quick Picking is designed to generate molecular mass information for all multiply charged components in a sample without a user defined FLP sequence input. It is simple to set up and once enabled in the processing method it can be automatically applied requiring a few clicks to fully process the sample (Figure 3).
- Component detection and data processing routines are driven by peak integration settings selected from either TIC, BPC or LC (PDA) data streams.
- For non-complex samples, there is high correspondence of molecular mass information between processing a sample with a defined FLP sequence input and using the automated Quick Picking algorithm. It is noted that for complex samples with closely eluting components the algorithm has been designed to report a single component with the highest signal intensity.

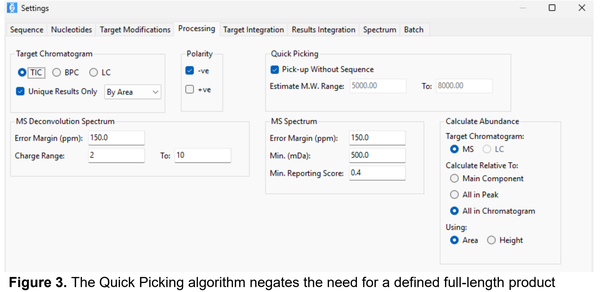
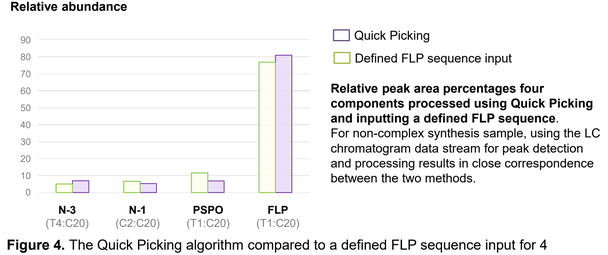


Figure 3. The Quick Picking algorithm negates the need for a defined full-length product (FLP) sequence and allows a target data stream to be selected (TIC, BPC or LC). In this example, the LC data stream was used in component detection to automate charge state deconvolution of 4 components.



Relative peak area percentages four components processed using Quick Picking and inputting a defined FLP sequence. For non-complex synthesis sample, using the LC chromatogram data stream for peak detection and processing results in close correspondence between the two methods.

Figure 4. The Quick Picking algorithm compared to a defined FLP sequence input for 4 components detected in the LC chromatogram data stream.

3.3 Quick Picking Algorithm Applied toPolyT80

As one example of applying the algorithm to an 80-mer PolyT oligonucleotide sample (average mass 24273.62). For molecules with a higher molecular mass the initial estimate parameters are changed to a charge state range between 10-31 and a mass range between 10,000-30,000.

- Using a nominal mass single quadrupole LC-MS system, the Quick Picking algorithm calculated the molecular mass of an 80-mer PolyT oligonucleotide Quick Picking to be 24272.68 Da (or 24272.68 u). [Mass error less than 1Da].

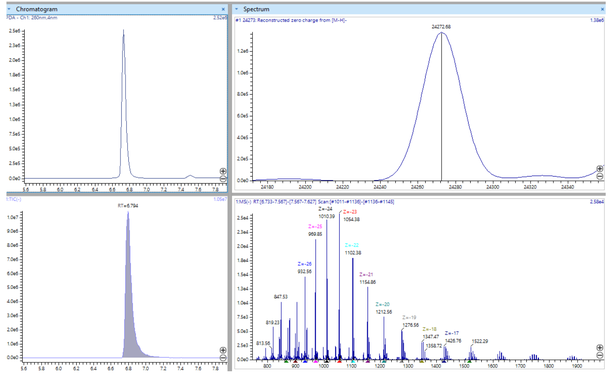


Figure 5. The Quick Picking algorithm applied to the automated analysis of an 80-mer PolyT oligonucleotide sample without a targeted user defined sequence input resulting in a mass error less than 1 Da.

4. Conclusions

- LabSolutions Insight™ Biologics offers flexibility by supporting both user-defined and automated deconvolution workflows for comprehensive oligonucleotide analysis.
- A Quick Picking algorithm has been developed and applied to the analysis of 20-mer and 80-mer antisense DNA. In the case of a 20-mer sample, the FLP and associated impurities were also automatically detected and molecular weight information reported. The analysis showed high correspondence with data processing results using a defined FLP sequence input.
- The Quick Picking algorithm enables a molecular weight confirmation of any detected peak and can be a powerful tool for synthesis confirmation in cases where the product is not as intended.
- This LC-MS analysis platform provides a rapid and cost-effective solution for purity analysis and molecular weight confirmation, making it ideal for quality control environments.