A SOFTWARE PACKAGE FOR SEQUENCE CONFIRMATION AND IMPURITY CHARACTERISATION OF SYNTHETIC OLIGONUCLEOTIDES

Chris Knowles[3], Jo-Anne Riley[2], Emma Harry[2], Jonathan Fox[2], Catalin Doneanu[1], Ying Qing Yu[1]

[1]Waters Corporation, Milford, MA, USA [2]Waters Corporation, Wilmslow, UK [3]Waters Corporation, Newcastle upon Tyne, UK.

INTRODUCTION

Synthetic oligonucleotides have emerged in recent years as a powerful alternative to small molecule and protein therapeutics. Manufacturing and quality control of oligonucleotide therapeutics requires highly selective and sensitive LC/MS methods for impurity sequencing and quantification. The most often used mass spectrometry-based method for oligonucleotide analysis has been reversed-phase chromatography employing a variety of ion-pairing reagents and modifiers in negative ESI-MS mode (IP-RP LC-MS). One critical step for identification of oligonucleotide impurities is mass spectrometry-based sequencing and data interpretation. Here we introduce a software workflow that can identify impurities across batches of Gem91; a well characterised fully synthetic phosphorothioated antisense oligonucleotide therapeutic with inidcations for AIDS. Analysis was performed via LC-MS for intact mass assignment and sequence confirmation for both the target sequence and a coeluting PS>PO impurity via targeted MS/MS and untargeted (MS^E) fragmentation.

METHODS

Gem91 was used as a representative therapeutic oligonucleotide with the sequence:

dC dT* dC* dT* dC* dG* dC* dA* dC* dC* dC* dA* dT* dC* dT* dC* dT* dC* dC* dT* dC* dT* dC* dT**

(d = deoxyribose sugar, * = phosphorothioated backbone)

Two batches of Gem91 were analysed by IP-RP-UPLC-ESI-MS with an ACQUITY™ Premier coupled to a Xevo™ G3 QTof high resolution accurate mass MS

•ACQUITY Premier Oligonucleotide C18 Column, 130Å, 1.7 µm, 2.1 × 50 mm

•TEA and HFIP in water and methanol based mobile phase system with 16-minute run time

Data were processed using the INTACT Mass and CONFIRM Sequence apps within the compliant ready waters_connect™ informatics platform.

RESULTS

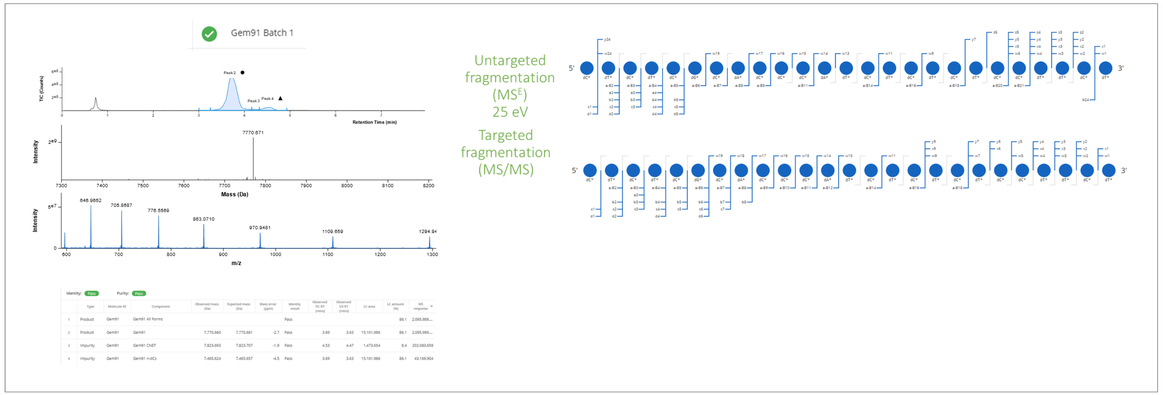

Figure 1

An overview of the INTACT Mass deconvoluted results for batch 1 (left) shows the correct assignment of the target mass within 10ppm, which is confirmed via 100% sequence coverage using MS/MS and MSE fragmentation data in CONFIRM Sequence (right). Other impurities assigned to different peaks in the TIC include CNET and n-dCs, both of which are chromato-graphically separated from the main peak.

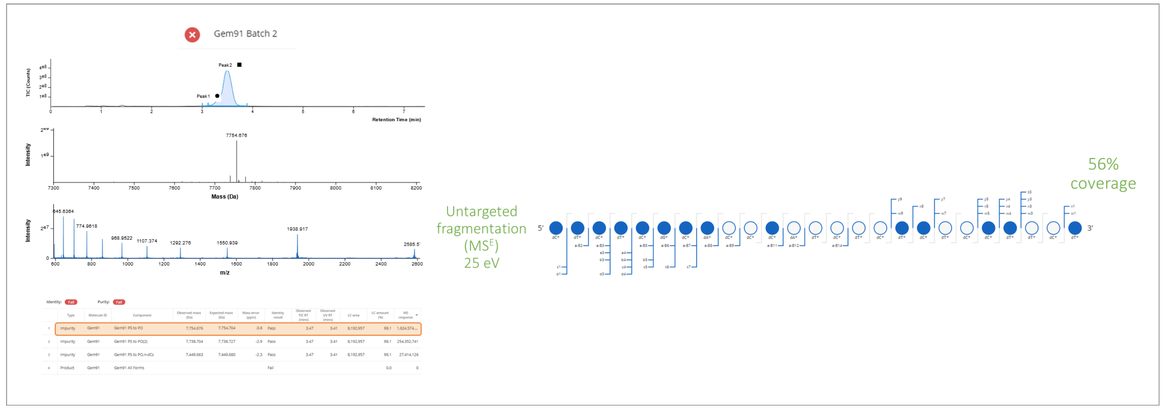

Figure 2

An overview of the INTACT Mass deconvoluted results for batch 2 (left) shows the target mass has failed identity testing, with an impurity assignment suggesting that there has been a PS>PO conversion event during synthesis (orange) that has coeluted with the main peak (peak 2). CONFIRM Sequence analysis of MSE fragmentation data (right) shows 56% coverage when the target sequence is used, with a loss of coverage in the center of the sequence, indicating that the impurity is located towards the center of the sequence.

Figure 3

CONFIRM Sequence analysis each PS>PO sequence variant using the MSE fragmentation data (top) shows 100% coverage for impurity sequences where a PS>PO conversion has occurred at position 14 or 15, with coverage results lower than 90% for all other Positions. An example fragmentation dot map (bottom) is shown for the sequence with a PS>PO conversion at position 14 (dC) highlighted orange.

CONCLUSION

- **The compliant ready waters_connect™ informatics platform contains dedicated software for oligonucleotide analysis**
- **waters_connect™ INTACT Mass software was able to identify the target mass and assign Impurities in a highly customizable method. Here we show that a failed batch has an assigned PO?PS conversion as a suggested impurity**
- **waters_connect™ CONFIRM Sequence software is able to localize the tentatively assigned PS>PO conversion with 100% sequence coverage for 2 out of 25 possible positions (position 14 and 15)**

References

1. Agrawal S, Tang JY. GEM 91--an antisense oligonucleotide phosphorothioate as a therapeutic agent for AIDS. Antisense Res Dev. 1992 Winter;2 (4):261-6. doi: 10.1089/ard.1992.2.261. PMID: 1363378.
2. DeLano M, Walter TH, Lauber MA, Gilar M, Jung MC, Nguyen JM, Boissel C, Patel AV, Bates-Harrison A, Wyndham KD. Using Hybrid Organic-Inorganic Surface Technology to Mitigate Analyte Interactions with Metal Surfaces in UHPLC. Anal Chem. 2021 Apr 13;93(14):5773-5781. doi: 10.1021/acs.analchem.0c05203. Epub 2021 Apr 2. PMID: 33798331.
3. McLuckey SA, Van Berkel GJ, Glish GL. Tandem mass spectrometry of small, multiply charged oligonucleotides. J Am Soc Mass Spectrom. 1992 Jan;3(1):60-70. doi: 10.1016/1044-0305(92)85019-G. PMID: 24242838.

TO DOWNLOAD A COPY OF THIS POSTER, VISIT WWW.WATERS.COM/POSTERS