BioAccord with UNIFI for Oligonucleotide Identity, Assay, Purity and Impurities Quantification

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INTRODUCTION

The therapeutic oligonucleotide field has been rapidly growing during recent years (Figure 1), meaning robust, compliant analytical methods are required in support of regulatory filings and for GMP batch release as new therapies enter the clinic and gain approval. Liquid chromatography (LC) and mass spectrometry (MS) are essential techniques for quality control (QC) testing of critical quality attributes (CQAs) of drug substances and drug products, including identity, assay, purity and impurity quantification. Additionally, gas-phase fragmentation (MS/MS or MS^E) can provide sequence information to support identity confirmation.

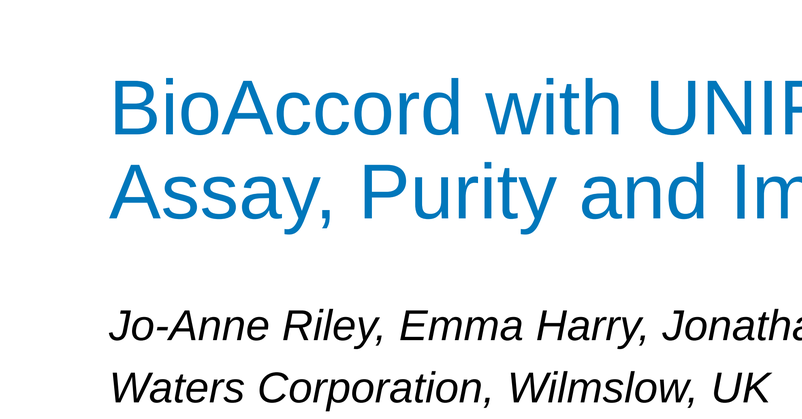

Figure 1: Approved oligonucleotide-based therapeutics over time

The Waters BioAccord™ LC-MS System is a SmartMS™ enabled bench-top UPLC-UV-ESI-TOF MS designed for biopharmaceutical analysis (Figure 2). SmartMS™ encompasses a built-in health check and automated self optimising set-up and calibration capabilities for ease-of-use and to ensure the system is always generating data of the required high quality. The waters_connect™ informatics platform with UNIFI™ is used for instrument control, data acquisition, review, and reporting. The compliance tools can be configured based upon the GxP or quality standards operating in a laboratory.

Figure 2: Waters BioAccord™ System with UNIFI™

Here, the Waters BioAccord™ System with UNIFI™ software was demonstrated to be an easy-to-use robust platform for achieving comprehensive oligonucleotide analysis.

METHODS

Gem91; a fully phosphorothioated 25-mer was used as a representative therapeutic oligonucleotide. Samples were prepared from a lyophilised powder by reconstituting in water to a concentration of 1 mg/mL (128 µM) for analysis.

UPLC CONDITIONS

Parameter	Value			
System	BioAccord™ System with ACQUITY Premier			
Column	ACQUITY Premier Oligonucleotide C18 Column, 130 Å, 1.7 µm, 2.1 mm × 50 mm (p/n: 186009484)			
Mobile phase A	8.6 mM TEA, 100 mM HFIP, pH ~8.25			
Mobile phase B	4.3 mM TEA, 50 mM HFIP in 50 % MeOH			
Wash solvent	H_2O/MeOH 1:1 (v/v)			
Column temperature	60 °C			
Autosampler temperature	6 °C			
Flow rate	0.200 mL/min			
Nominal injection volume	1 µL			
UV detection	260 nm, 20 points/sec			
Gradient	**Time (min)**	**%A**	**%B**	**Curve**
	0.00	70	30	6
	10.00	60	40	6
	11.00	0.0	100	6
	12.00	0.0	100	6
	12.10	70.0	30	6
	16.00	70.0	30	6

MS CONDITIONS

Parameter	Value
Mode	Full scan with fragmentation
Mass range	Low (50 – 2000 *m/z*)
Polarity	Negative
Scan rate	10 Hz
Capillary Voltage	Default (0.8 kV)
Cone Voltage	Default (40 V)
Fragmentation cone voltage	Custom: 50 V to 90 V
Desolvation Temperature	300 °C

DATA PROCESSING

To allow quantification based on extracted ion chromatogram (EIC) data, Analysis Method type “Quantify Assay Tof 2D Chromatographic” was used.

A mass list of known impurities was created for Gem91 and these were added as Components to the Purpose tab of the processing method, using the 7− charge state for expected masses. Additional unknown impurities can be added to the method if observed during initial data assessment. Additionally, Amount values of calibration standards were added to enable assay quantification (Figure 3).

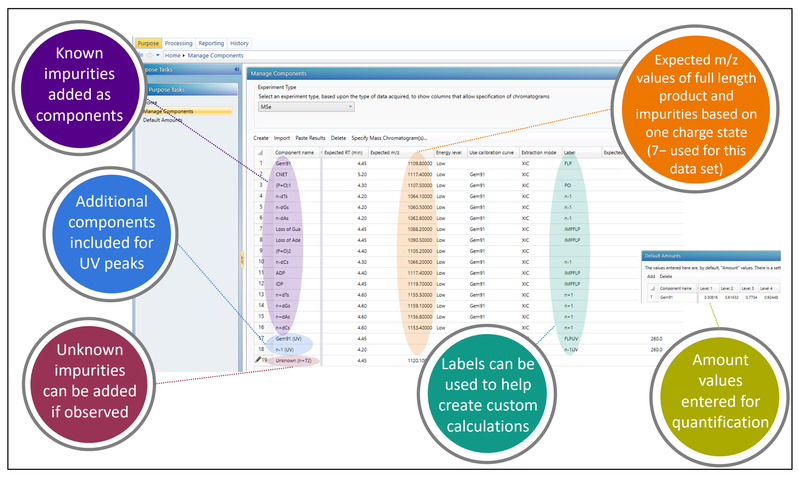

Figure 3: Component details in the Purpose tab of the processing method

Integration parameters can be optimised and set to allow consistent automated integration of UV and MS data. Calibration curves can be constructed within the software from specified UV or MS data for quantitation. Visualisation of processed data using customisable workflow steps enables easy review of integration, which can be manually adjusted where required, in a compliant way with audit trails for traceability (Figure 4).

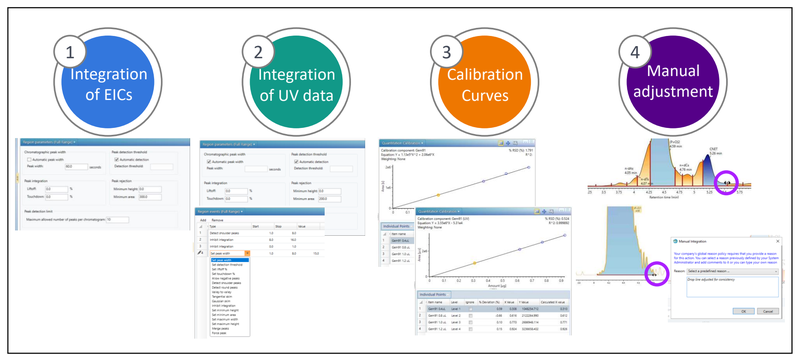

Figure 4: Examples of processing steps

Summary Calculations can be used to provide statistical results such as mean and %RSD values. These features can eliminate the need to transfer data into other programmes for calculations. To help assess system suitability results and sample acceptance criteria, Limit Checks can be included within the method to automatically highlight any values, found or calculated, which are close to or outside of the limits set (Figure 5). In addition, Custom Fields can be created to perform the calculations required to reach the final results (Figure 6).

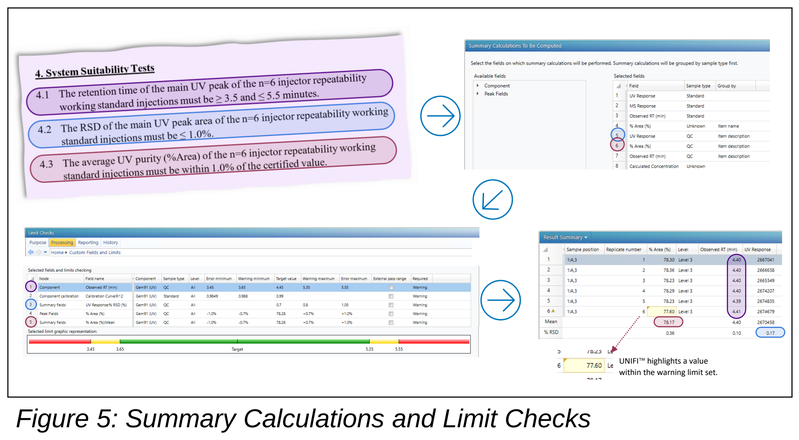

Figure 5: Summary Calculations and Limit Checks

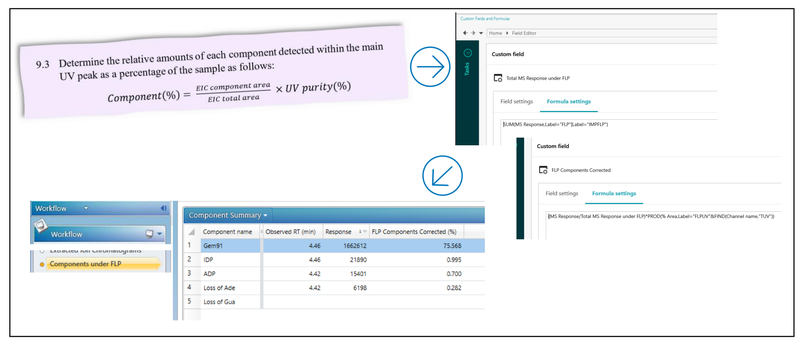

Figure 6: Custom calculations

RESULTS

Once processing method parameters are optimised and data processed, results can be viewed using customisable workflow steps. This allows efficient assessment of results against SST criteria and sample specifications (Figure 7).

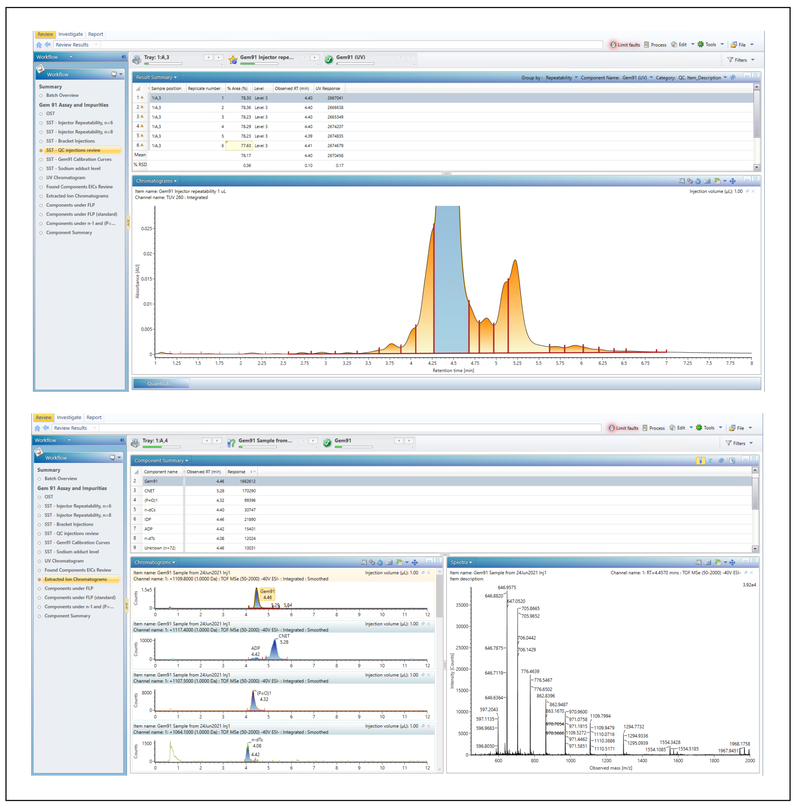

Figure 7: Data visualisation

Finally, Report Templates can be designed to display and summarise the data required for reporting of results (Figure 8). Electronic signature capability means data can be reviewed and approved by users with defined roles, for example technical reviewers, QC and QA, maintaining compliance from data acquisition to reporting.

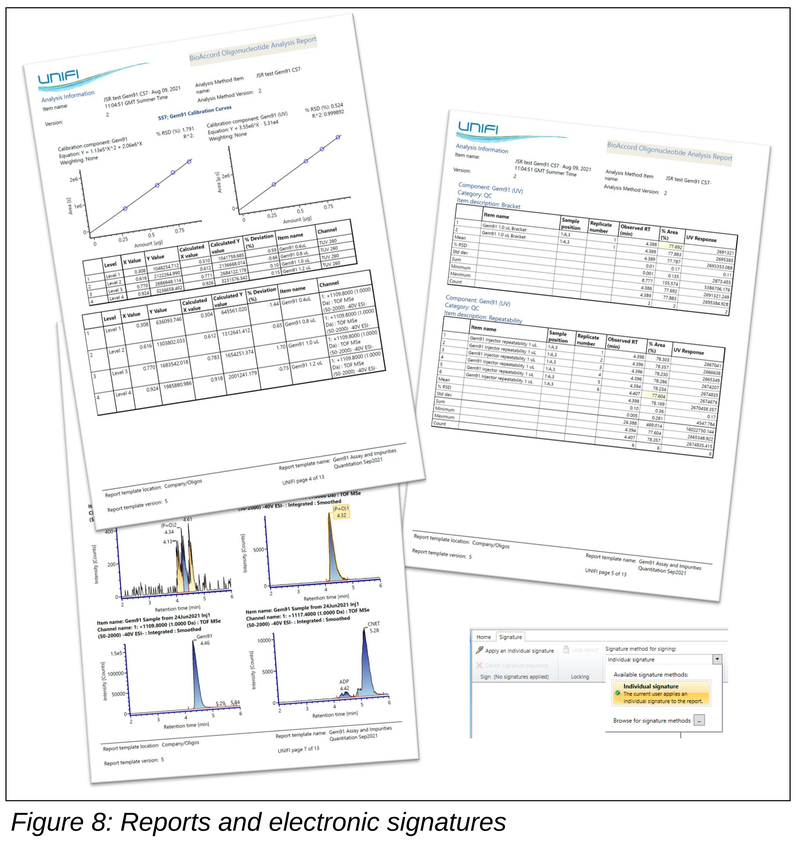

Figure 8: Reports and electronic signatures

CONCLUSION

- **QC testing of oligonucleotides can be achieved in an efficient and compliant manner using the BioAccord™ System with UNIFI™ in waters_connect™.**
- **Customisable features, including Custom Fields, Workflow Steps and Report Templates, make this a versatile and adaptable approach to suit each therapeutic.**

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