Oligonucleotide Samples are Amenable to Automated LCMS Analysis

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Summary:

Aim: Manual analysis of data from Oligonucleotide analysis by mass spectrometry was tedious and required high expertise.

This poster shows the extent to which this can be automated and simplified by software.

Samples were from a low resolution (single quad) instrument with an established workflow.

Workflows were made more productive with automation available in the software and the MS instrument return on investment was extended.

Introduction

In the real world, LCMS analysis of synthetic Oligonucleotide samples has previously been tedious, laborious, and required expert overview. A trained analyst might take half a day to complete a sample. Negative consequences are:

- Low efficiency
- Poor use of valuable capital (human and instrument)
- Production bottlenecks
- Inability to match increased demand for throughput

Large parts of existing workflows remain manual and time consuming, such as analysis of impurities.

It makes economic and scientific sense to adopt better tools. This poster illustrates how an organization's efficiency and capability can be improved with automation and software.

Methods and Toolkit

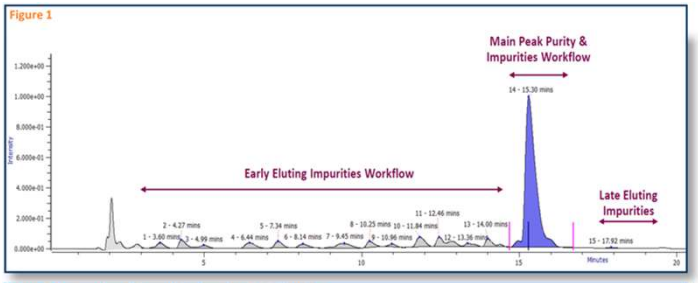
Analytical scientists with a desire to improve efficiency and methods whilst reducing tedium and waste in workflows

Agilent LC-UV-MS systems acquiring data, or other mass spectrometer.

Templated Workflows from Protein Metrics' Byos software optimized for Oligonucleotides.

Impurities Can Be Rapidly Identified and Quantified

Figure 1 shows 20 minutes of the standard 40-minute LC run in use for the typical Oligonucleotide samples displayed in the chromatogram trace in Byos software. The blue highlighted peak shows the main peak and the response (by UV or by MS) can therefore be quantified relative to other peaks, or the sum of all other peaks that are automatically assigned. The user can highlight the various peaks in the inspection pane, with purity calculations performed through an automatically generated report (example given in Figure 4) to perform the workflow demonstrated in Figure 2 (inserts). Reports can also be templated, Figure 3 shows an example.



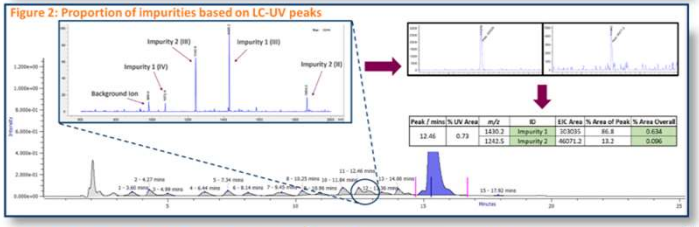
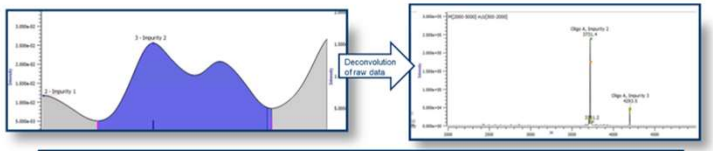


Figure 3: Templated report to calculate impurities based based on several measures. The highlighted tab shows shows the setup exposed for the LC-UV peaks

Agilent Chemstation Data (low res) provided raw data and was imported into Byos software for processing. The visualization pane for the LC-UV peak also links to the MS data which is automatically deconvoluted to the mass scale. The reporting can then compare both techniques for an orthogonal analysis of the relative levels of impurity. The software can process low-res MS data and automate peak identification.
Detection of unknown impurities is possible simultaneously and a user can subsequently classify them if identifiable.

Figure 4: Integrated LC-UV trace containing Impurities 2 and 3. Raw data is automatically deconvoluted to the mass scale and the relative intensities of intensities of the peaks can be used to provide a relative measure of the impurities.



Peak # ↑	Apex time ↑	RenormedArea ↑	Delta name ↑	Mono mass	*MS Ratio%	*UV-MS
1	16.1204	1.95272	Impurity 2	[redacted]	86.09	1.68
			Impurity 3	[redacted]	13.91	0.27

Time Savings Demonstrated

TIME: Cuts processing time by >60%

AUTOMATION: >65% of workflow can be automated / semi-automated

FUNCTIONALITY: Software provides >70% of functionality to process samples

REPORTING: >95% of required data can be reported, and <10% manual transcription

ACCURACY: Full-length product is within 4% and impurities within 50% relative of previous data

Data and analysis in this poster is the work of Edward Wilkinson and colleagues at AstraZeneca Macclesfield with support from Protein Metrics. An extremely detailed and thorough study has been presented the 11th Annual Oligo Networking Event in the United Kingdom with methodology and assessments by the team members. Please contact us for further details if desired.

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