

Advanced Utilization of the Custom Calculator Tool for OpenLab CDS: An Oligonucleotide Example

Introduction

The Custom Calculator function in OpenLab CDS allows you to embed calculations for/on your basic result information. You can use these calculations to generate application-oriented results or quality-control statistics. Custom calculations allow you to produce new values based on mathematical and non-mathematical calculations from existing system-generated results (Figure 1). Calculations can be generated for each peak in a sample or across an entire injection set, and complex, multi-variable calculations are also possible. You can incorporate custom calculations into the processing method with a single mouse click.

Custom Calculator and custom calculations can be a powerful tool for your lab. However, it may be daunting to create a method for an advanced workflow using Custom Calculator. This overview demonstrates use of Custom Calculator for OpenLab CDS for an oligonucleotide final product peak area calculation (which requires significant calculations) and reporting for an oligonucleotide acquisition and analysis method set.

Oligomer analysis is complicated by the variety of molecular modifications that occur during the production and storage phase, which must be accounted for in the purity analysis via LCMS and software workflows. Starting with just one target compound, the species that arise can occur as different adducts during analysis (sodium, potassium, etc.). Process Impurities and degradants are also present and can be shorter or longer than the FLP (full length product) by one or more nucleobases leading to even more species to consider during data analysis. The implemented Custom Calculator workflow helps to meaningfully group the gathered data into a %Area report by groups such as FLP, Shortmer, Longmer, Depurinated, Oxidized FLP, etc. while reducing manual steps and thus saving massive amounts of time.

We compare the time savings achieved by using Custom Calculator for over 100 channels of mass spectral data instead of a manual workflow in the original software as well as the roles your lab and employees will have in running this Custom Calculator, thus showing you how you can set your lab up for success with an automated purity workflow using Custom Calculator for OpenLab CDS and reports generated from this process.

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Method creation and utilization

Synthetic oligonucleotide samples contain a high number of coeluting chromatographic impurities. Specific chromatographic methods for quantifying these impurities should be available for release and stability purposes. As a standard, these methods are based on UV and MS detection and should be stability indicating. The combination of the chromatographic selectivity and the MS selectivity will result in an accurate purity data set. This can be done by extraction of all specific m/z channels in combination with processing within predefined time windows. Combining all the resulting areas will end up with a %Area report for the list of all MS impurities, up to 120 impurities. In addition, this analytical procedure should be accurate, QC friendly, and the workload should be kept as low as possible for the related processing step.

An automation tool was developed using Custom Calculator for the extraction of the MS Full Scan channel on an Agilent MSD XT single quadrupole instrument, processing within the predefined time windows and reporting by combining all the data to one %Area report.

Figure 2 shows the workflow for creating a method from both manual and automated perspectives. Figure 3 illustrates how users can save hours each day using Custom Calculator. On a workstation PC that has the minimum Agilent recommended requirements, filling in the manual processing method at maximum demand takes at least 30–45 minutes of hands-on time, but can take up to several hours for complex sample sets. This time must be spent for every unique processing method you wish to use in the sequence.

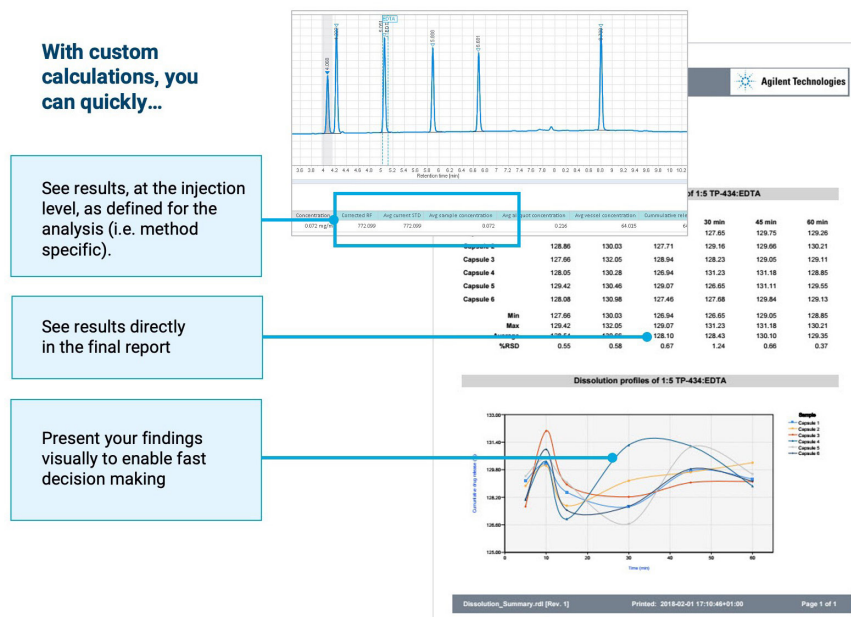


Figure 1. Overview of Custom Calculator for OpenLab CDS.

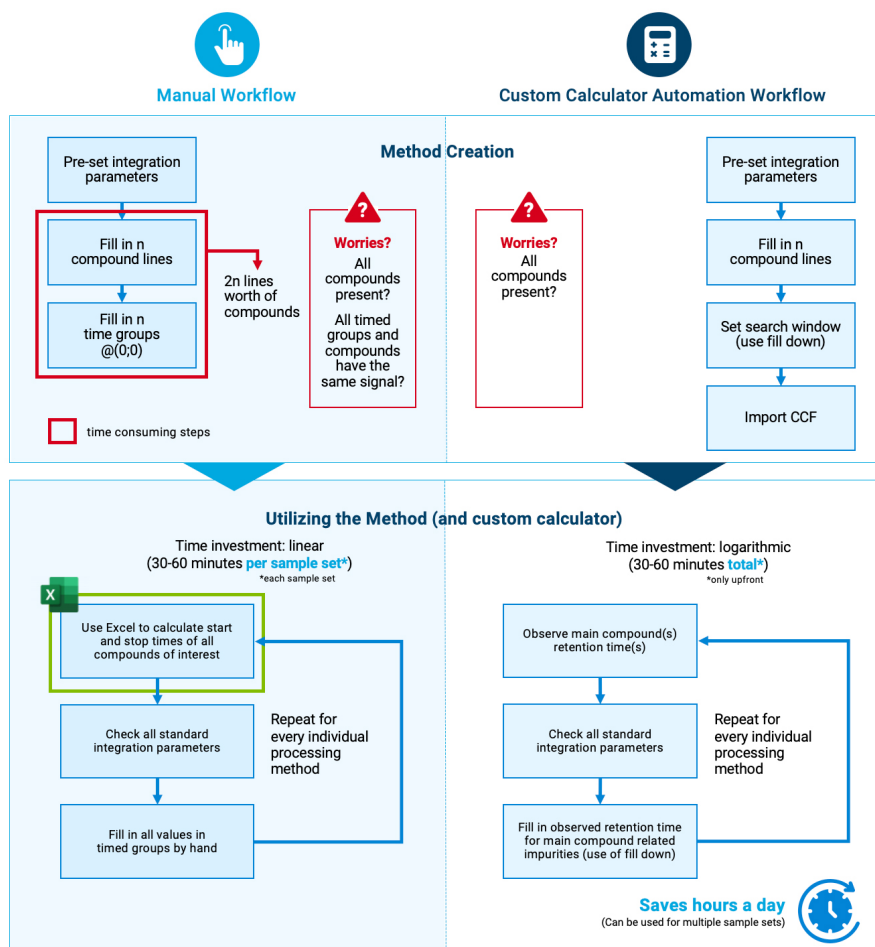


Figure 2. Comparison of manual and automated method workflow creation. The Custom Calculator automation workflow can result in substantial time savings over manual methods.

On average, manual processing takes a minimum of 30 minutes, but can often take up to 2 hours per sample set. While an automated processing method takes 15 minutes (with straightforward methods) to create and can then be used for the lifetime of the instrument method. The automated method takes two minutes for the computer to populate; the computer will do most of the work if the experimental criteria allow automatic calculations. This is time in which a user is not required to be hands-on and it is part of the automated method. A repeat may be needed if peak shift is too severe. As shown in Figure 3, the efficiency improvements of creating and utilizing a Custom Calculator automation workflow can be substantial as the number of samples that are analyzed increases.

Once the Custom Calculator tool is programmed for the specific oligo project, only the specific retention time from that analysis sequence should be added to the tool. Custom Calculator is able to quickly extract, process, and accurately report the %Area MS purity data of sequences containing even a high number of samples.

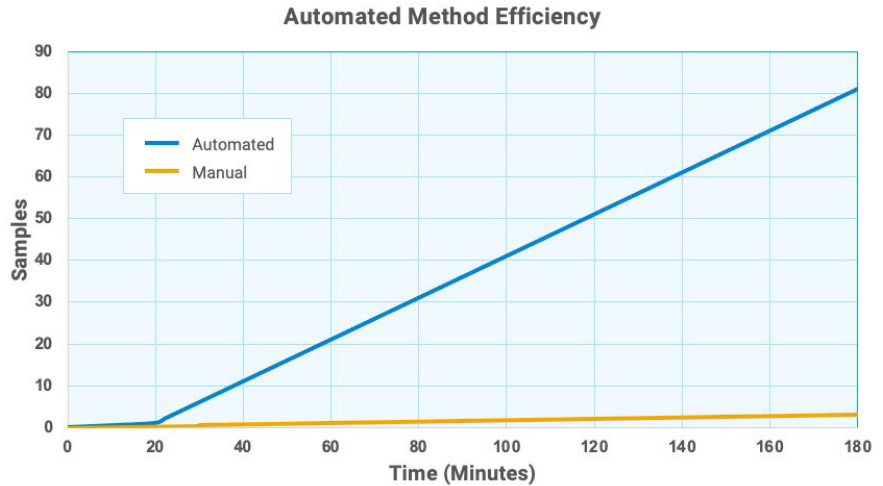


Figure 3. Significant time savings and increased sample results processing/reporting throughput can be achieved by making and utilizing a Custom Calculator automation method.

Workflow and roles

Figure 4 shows the technical details of how the Custom Calculator operates with the OpenLab CDS method and how it interacts with compound details. Also shown are the simple steps lab members would take in order to utilize the power of this Custom Calculator, and what would be expected from the lab analyst and admin in order to keep the program running.

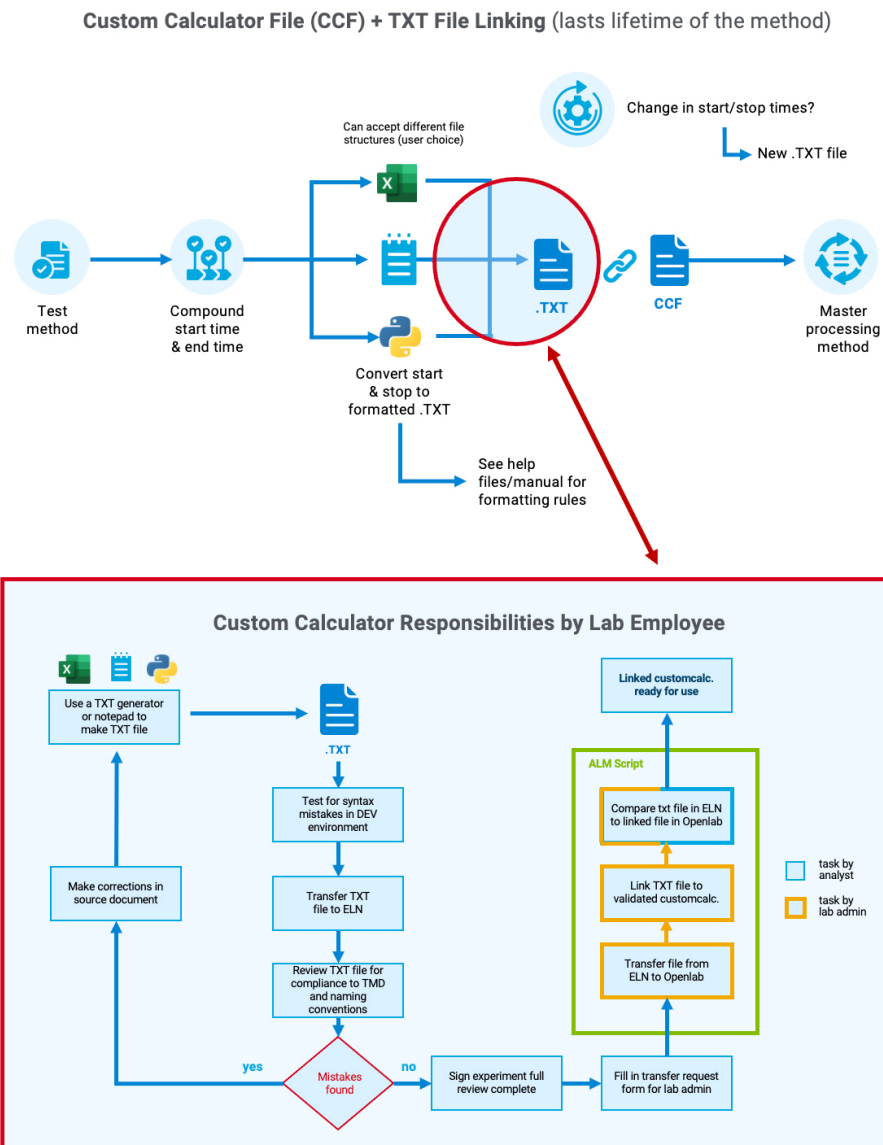


Figure 4. This figure shows the technical details of how the Custom Calculator operates with the OpenLab CDS method and how it interacts with compound details. This figure then focuses on the simple steps clearly defined roles for lab members in order to utilize the power of Customer Calculator and what would be expected from the lab analyst and admin in order to keep the program running.

Report generation

The overall goal of this specific Custom Calculator method is to generate an MS Area % report for the Oligonucleotide and all impurities of the sample. Below is a real and highly customizable (yet redacted) report out from this entire Custom Calculator process for use by lab employees, administration, and the laboratory notebook system.

Conclusions

With a one-time setup of a Custom Calculator workflow such as this, your lab can save hours of hands-on time per sample set of oligonucleotide injections, eliminate manual errors on all your oligo analysis steps, and automatically report out purity details without the need for any manual calculations. Utilization of this software can save your lab hours per day and eliminate painful and time-consuming manual steps previously required of your lab analysts and admins.

Agilent provides custom solutions and consultancy approach/services to embed OpenLab into customer workflows. Please reach out for more information or for assistance applying calculations specific to your work.

MS Area % REPORT							Analytical Development
Data file: [REDACTED]							
Sample name: 0.25mg/ml [REDACTED]							
Description:							
Result Set Version: 2022-0718-0911-42644							
Sequence Name: 20220715 172840 compar with quad pump							
Notebook Code:							
Elims Nr:							
Sample Info:							
Identifier: 1							
Name	m/z	RT Start	RT Stop	Area Blanc	Corrected Area	Area %	
compound 1	1438.9	13.45	13.95		0.00	0.00	
compound 2	1451.0	13.40	13.70		0.00	0.00	
compound 3	1463.0	13.90	14.20		1170.26	0.01	
compound 4	1637.2	13.90	14.20		1628.04	0.01	
compound 5	1640.5	13.80	14.10		1036.57	0.01	
compound 6	1641.9	13.85	14.15		0.00	0.00	
compound 7	1645.1	13.90	14.20		21347.39	0.11	
compound 8	1647.6	13.90	14.20		1666.11	0.01	
compound 9	1664.7	14.52	14.82		4632.45	0.02	
compound 10	1668.5	14.20	14.50		6806.11	0.03	
compound 11	1669.9	13.90	14.20		1704.25	0.01	
compound 12	1682.5	13.90	14.20		12149.27	0.06	
compound 13	1685.7	13.85	14.15		0.00	0.00	
compound 14	1690.2	13.90	14.20		28749.61	0.15	
compound 15	1698.5	13.78	14.08		17658.89	0.09	
compound 16	1700.7	13.95	14.25		88707.30	0.45	
compound 17	1702.7	13.85	14.15		0.00	0.00	
compound 18	1705.1	13.45	13.85		193043.26	0.99	
compound 19	1705.9	13.85	14.15		346351.05	1.77	
compound 20	1708.7	11.15	14.00		239922.34	1.22	
compound 21	1709.1	14.00	14.30		18416621.08	94.01	
compound 22	1712.3	14.25	14.55		25261.92	0.13	
compound 23	1712.7	13.75	14.05		136058.06	0.69	
compound 24	1717.8	14.35	14.65		17227.70	0.09	
compound 25	1719.7	14.70	15.00		21026.15	0.11	
compound 26	1738.7	14.55	15.65		0.00	0.00	
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						Page 1 of 2	

MS Area % REPORT							Analytical Development
Name	m/z	RT Start	RT Stop	Area Blanc	Corrected Area	Area %	
compound 27	1748.4	14.35	14.65		0.00	0.00	
compound 28	1770.8	14.20	14.50		0.00	0.00	
compound 29	1773.2	14.15	14.45		0.00	0.00	
compound 30	1776.4	14.20	14.50		0.00	0.00	
compound 31	1777.8	14.30	14.60		2509.73	0.01	
compound 32	1780.9	14.15	14.45		4705.98	0.02	
Summary							
Sum of compounds group 1	1.77	Sum of compounds group 3		0.14			
Sum of compounds group 2	0.21	Sum of compounds group 4		0.04			
Total Area	19589983.53						
E-Signatures:							
No E-Signature Data Available.							
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						Page 2 of 2	

Figure 5. Example of a final report generated by this automated process.

To learn more about what you can do with Custom
Calculator, visit:
www.agilent.com/chem/openlab-custom-calculator

To learn more about OpenLab CDS, visit:
www.agilent.com/chem/openlab-cds

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