

Agilent OpenLAB CDS MatchCompare for the Comparison of Peptide Mapping Samples

Application Note

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Introduction

Peptide mapping, a key analysis used in the characterization of bio-therapeutic proteins, results in complex chromatograms that are generally difficult to study. The enzymatic treatments applied to the proteins are reproducible, but minor changes in protein structure can result in very slight changes in the quantities of protein fragments. Thus, overlaying the chromatograms in a side-by-side or A/B type of comparison is the general approach used in peptide mapping.

Agilent OpenLAB MatchCompare is an ideal tool to use in the study of peptide mapping samples. Originally developed for GC analysis of fragrances, it excels at comparing a sample chromatogram against a reference with user-settable tolerances. Thus, it excels at the fingerprint matching type of analysis used in the comparison of complex samples.

Rather than subjective interpretation, MatchCompare develops objective comparison data allowing the user to determine similarity/dissimilarity of samples. MatchCompare software was initially used for gas chromatography, however the software may also be used with liquid chromatography. This study compared complex peptide maps. This approach would allow, for example, a QC/QA lab to quickly determine how similar samples are to a standard or another sample.

The method uses a shallow gradient and takes advantage of the Agilent BlendAssist feature to allow the pump to make dilute mobile phases.

MatchCompare was used for data processing running on Agilent OpenLAB CDS 2 DA.



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Methodology

Parameter	Value
Sample	BSA Digest
Column	Agilent Advanced Biopeptide Mapping Column
HPLC System	
Sampler	(G4226A), 15 µL Injection volume
Pump	(G4204A) Flow rate: 0.250 mL/min BlendAssist: Water/water 1 % TFA (0.05 %) 97.00 A, C ACN/ACN 1 % TFA (0.04 %) Yes 3.00 B, D; 0.05 % water, 0.04 % ACN
Diode-array	(G4212A), 210 nm, 280 nm
Column oven	(G1316C), 35 °C

Results and Discussion

Figure 1 presents the main OpenLAB CDS screen showing a series of BSA tryptic digests, the first two of which are overlaid. The chromatograms show that there is a retention time shift in the early stages of the chromatogram – most likely the result of matrix effects. MatchCompare can easily handle these shifts.

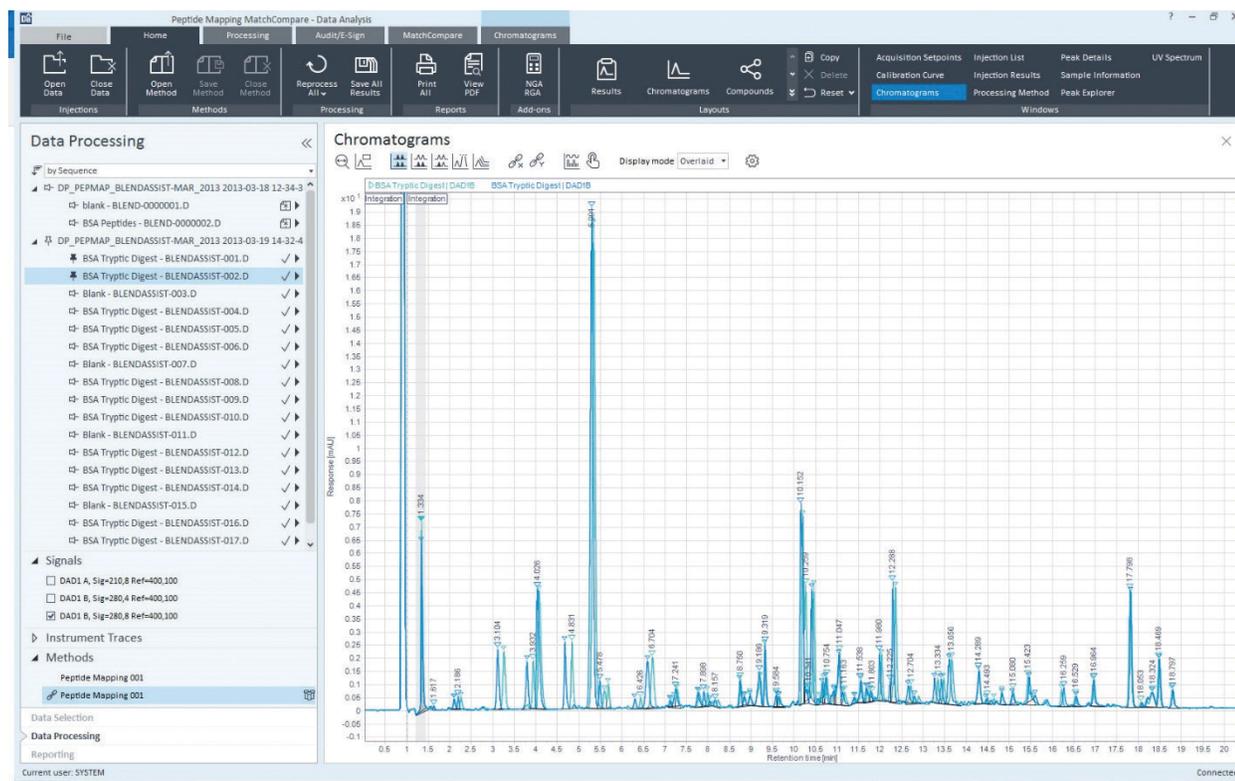


Figure 1. Overlay of two BSA tryptic digest samples shown in Agilent OpenLAB CDS 2 DA.

Given the complexity of these samples, one might be tempted to use the Peak Explorer in OpenLAB CDS 2. Figure 2 shows an examination of the first few sets of the BSA digests using Peak Explorer. The samples are related, but the chromatograms are too complex for Peak Explorer alone.

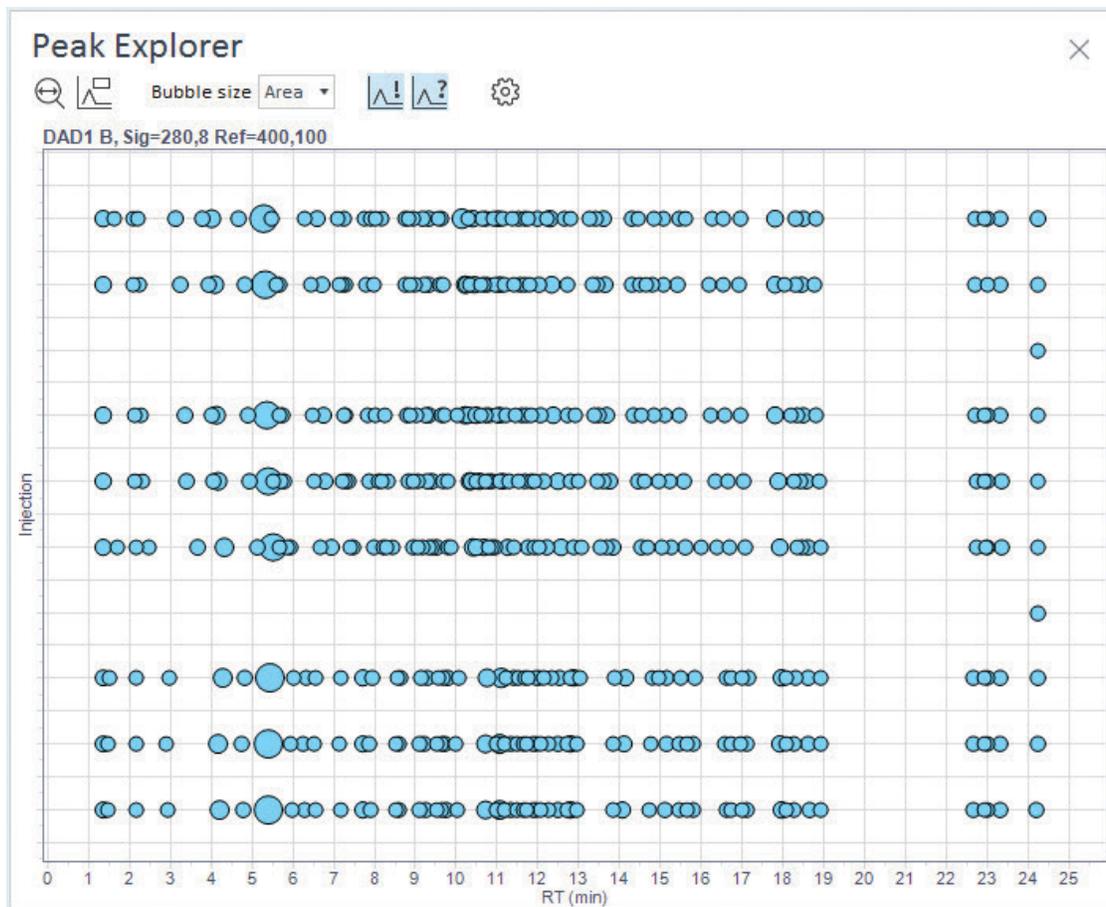


Figure 2. Peak Explorer window showing a comparison of three sets of BSA digests.

OpenLAB CDS MatchCompare can add value in this case with a detailed comparison among these samples. We set the first chromatogram as our reference, as shown in Figure 3. This action imports the peak table (Name, RT, Area %, and Height), and adds a column for Tolerance. The tolerances are initially set up with the defaults. These are changeable, as shown in the small table on the right. You could also edit tolerances for individual peaks in the main reference table.

Reference name: **BSA Tryptic Digest - BLENDASSIST - 001.ref** Nb of peaks: 63

Name	Rt [min]	% Area	Height	Tol [%]
Unknown	1.3336	3.7667	7	10.00
Unknown	1.6172	0.0886	0	30.00
Unknown	2.0937	0.3648	0	15.00
Unknown	2.1862	0.3536	0	15.00
Unknown	3.1045	2.3043	2	10.00
Unknown	3.7855	1.7716	2	10.00
Unknown	4.0259	5.6378	5	10.00
Unknown	4.6674	2.0068	3	10.00
Unknown	5.2905	22.6256	19	6.00
Unknown	5.4784	0.8912	1	15.00
Unknown	6.2944	0.3807	0	15.00
Unknown	6.5855	2.5041	2	10.00
Unknown	7.1044	0.1770	0	15.00
Unknown	7.2408	0.8181	1	15.00
Unknown	7.7551	0.6902	1	15.00
Unknown	7.8977	0.4840	1	15.00
Unknown	8.0277	0.1744	0	15.00
Unknown	8.1572	0.2523	0	15.00
Unknown	8.7348	0.8175	1	15.00
Unknown	8.8348	0.3442	0	15.00
Unknown	8.9610	0.4243	0	15.00
Unknown	9.1864	1.5911	1	10.00
Unknown	9.3191	1.6947	2	10.00
Unknown	9.5761	0.3441	0	15.00
Unknown	9.6349	0.3872	0	15.00
Unknown	10.1521	8.9962	8	10.00
Unknown	10.2743	0.4259	1	15.00
Unknown	10.3985	3.7573	4	10.00
Unknown	10.6427	0.8168	1	15.00

% Area >=	% Area <	Tolerance (%)
0.00	0.10	30.00
0.10	1.00	15.00
1.00	10.00	10.00
10.00	100.00	6.00

Figure 3. Setting the reference file for Agilent OpenLAB CDS MatchCompare.

Once the reference is saved, a second chromatogram is selected, and the comparison is carried out. Figure 4 shows the results of comparing the first two chromatograms from Figure 1. In this table, peaks are color-coded:

- Green indicates that the peak was found in both the reference and sample chromatograms, matching Area % within the chosen tolerance for that peak.
- Blue means that the peak was found in both sample and reference, but Area % was outside the tolerance.
- Grey peaks were found in the reference only.
- White peaks were found only in the sample.
- A similarity value is also given, combining all peaks using a proprietary algorithm and showing, in this case, that the samples are about 91 % similar.

Figure 5 shows the comparison presented in an area plot. The same color coding from the comparison table is used, and one can see that two white peaks found in the sample only are present, as well as some blue peaks that match retention times but do not have the same areas. Finally, there are also some grey peaks present in the reference that are absent from the sample.

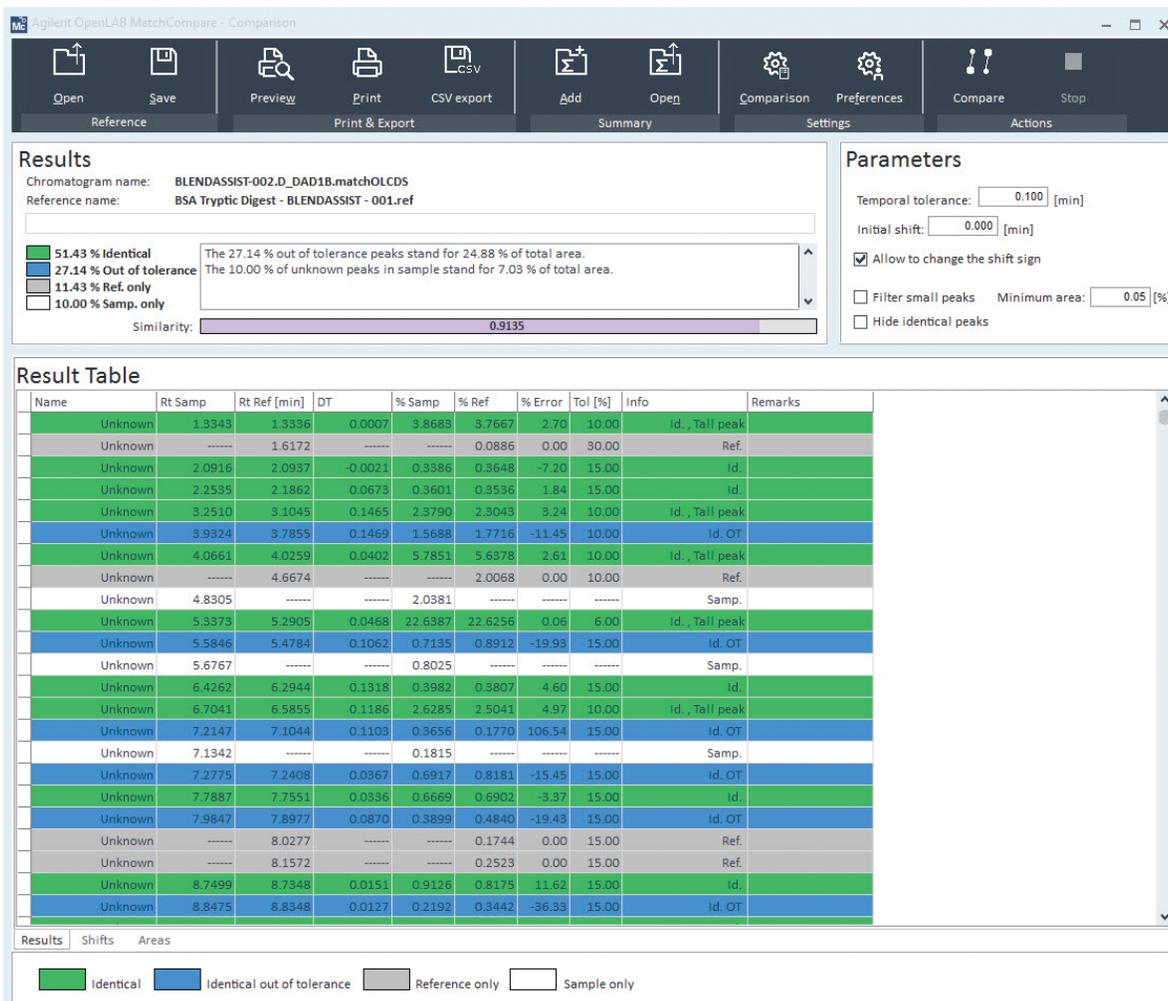


Figure 4. Agilent OpenLAB CDS MatchCompare comparison report from the two BSA digest samples in Figure 1.

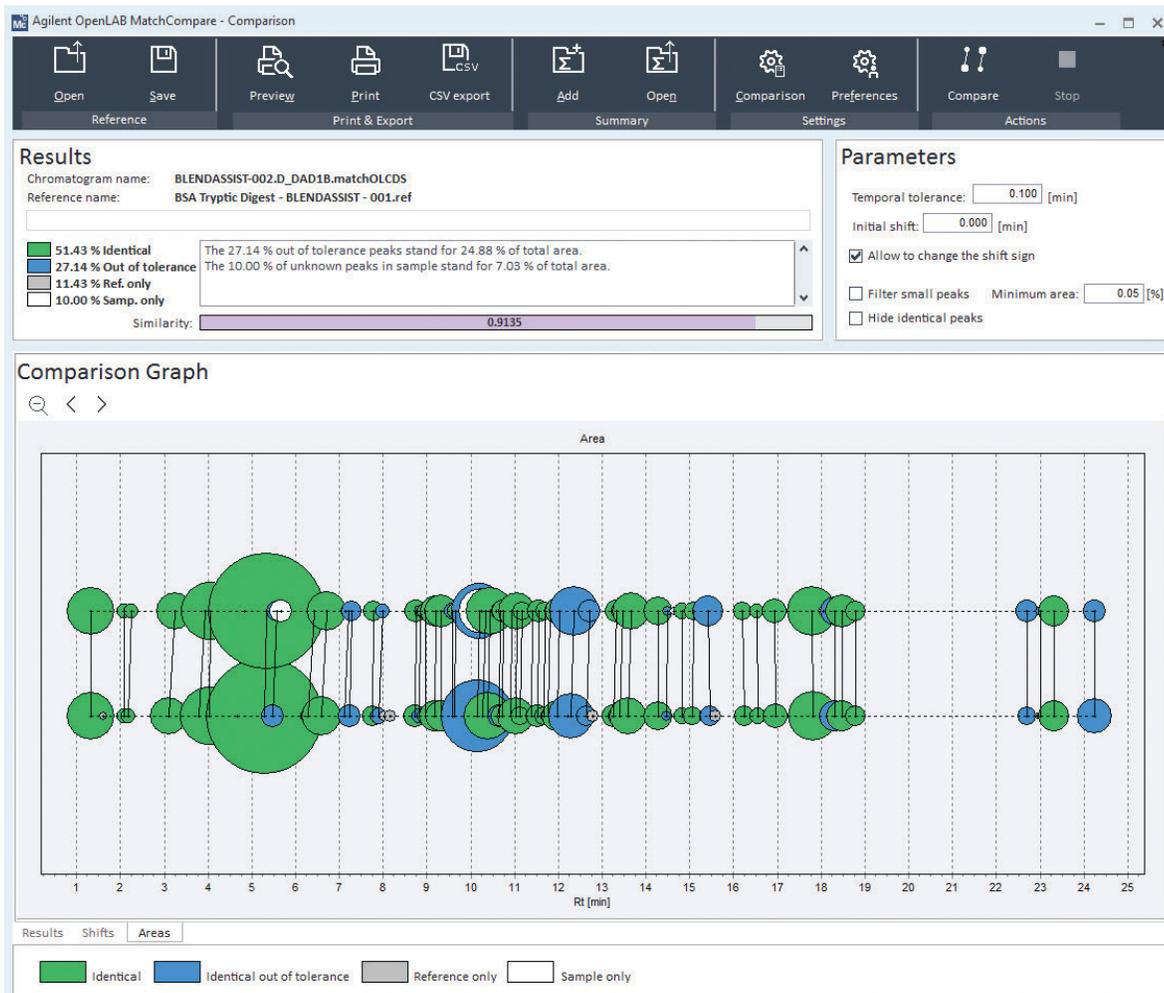


Figure 5. Area plot of the comparison from Figure 4.

Conclusion

In peptide mapping applications, Agilent OpenLAB CDS MatchCompare facilitates an initial screening of the match between healthy and diseased specimens. By running many samples on a simple LC system and using MatchCompare as a screening tool, higher-end MS systems can be reserved to study cases where significant differences have been detected.

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