

Automated Method Development for HPLC/MS

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Introduction

One of the most daunting experiences for any Chemist is to be given a sample and being asked to develop a method from the beginning. In this overview of automated method development we will look at some of the considerations for developing a robust method quickly. One possible way to find a good method is an internet search. Definitely a Google search for details on the compound you are looking at will certainly help. However, some of the methods may not fit your sample matrix or your equipment. The most common parameters used for method development are

mobile phase
mobile phase pH
bonded phase
Temperature

By using the Agilent Method Development Scouting Wizard we are able to create a Campaign to optimize our separation using these parameters. We can look at all these parameters automatically with no Chemist intervention. The system flushes between experiments and stores the unused columns in appropriate solvents. By adding the specificity of Mass Spectrometry we remove the need to run standards for all the compounds in a mix. With single quad MS, can track the movement of peaks based on the M/Z which give an easy identification of the compound.

For pH adjustments, you can use a quaternary pump to do on line changes in ionic strength. With the injector programming feature of the autosampler, you can do on line dilution and derivitization during the method development process.

The entire sequence with all the methods and flushes are built by the Wizard. With Chemstation Data analysis, you can do quick data review. By using extracted ion information from the single quad MSD we can see trends in parameter changes with one sample injection per set of sample parameters instead of 11 injections for a 10 compound Mix.

Experimental

In this experiment we will develop a method to look to speed up an analysis of Biodiesel production samples. We are able to develop a method that will be implemented UV only HPLC method by using LC MS for the method development

Example: initial method scouting experiments for a new sample

Bonus.RP	2.0	MeOH	5 min
SB C18	8.0	ACN	15 min
SB CN			
Eclipse C18			

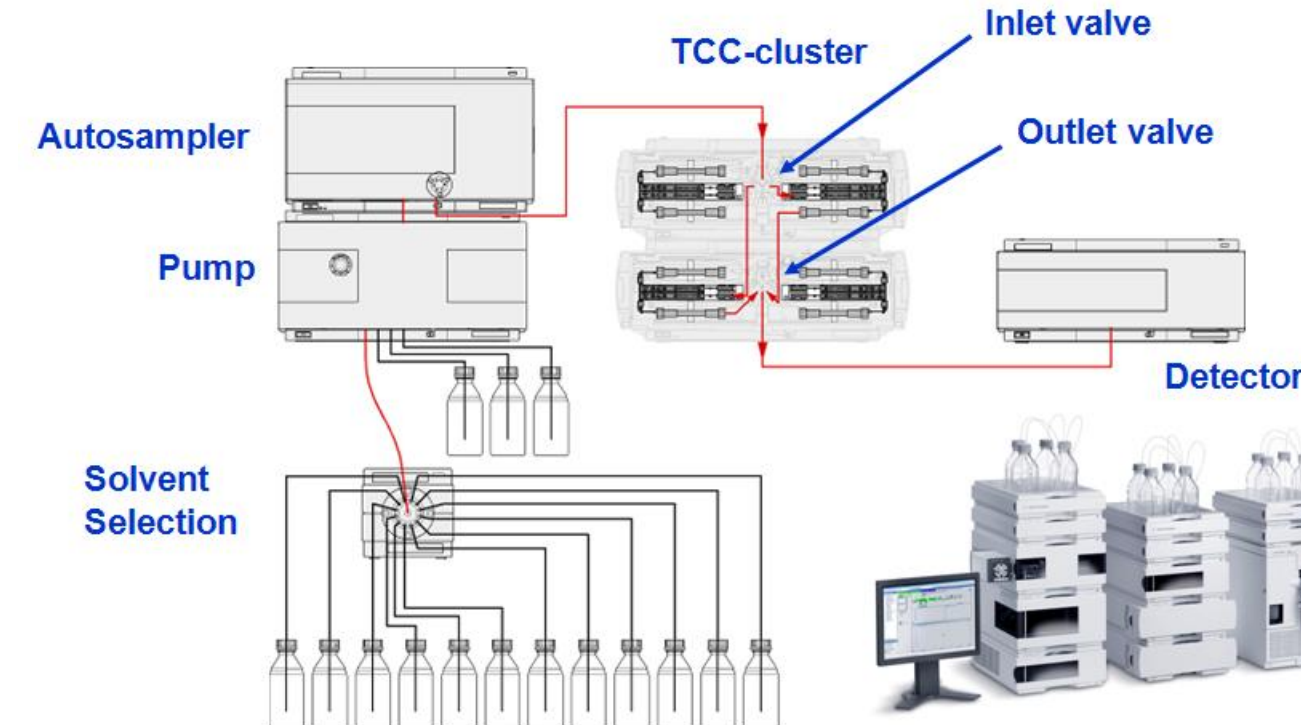
32 Experiments in total - this is already 4 h method run-time!

Experimental

An instrument capable of automatically switching between columns and solvents with an appropriate software to set up experiments.

The Agilent 1200 Series Method Development Solution - Hardware

Method Development System - Concept

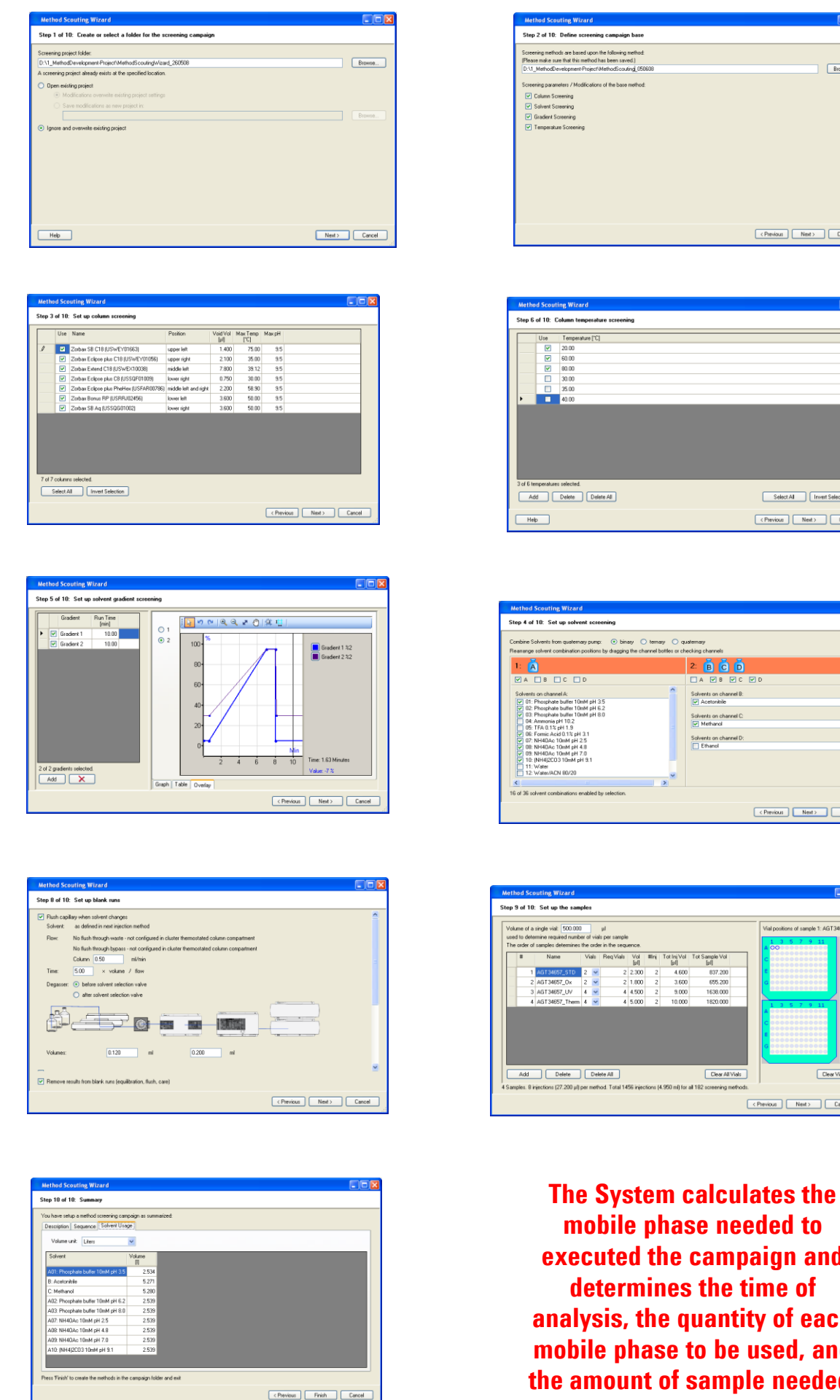


Agilent 1200 Series Method Development Solution - Method Scouting

A tool to automatically generate scouting experiments by varying columns, solvents, gradients and temperatures

- A Wizard guides the user through the setup of the scouting campaign
- Automatic generation of method variations based on a user-defined standard method
- Automatic generation of flush and column equilibration methods
- Automatic generation of a ChemStation sequence using above methods
- Scouting experiment can be setup for multiple samples
- User settings and resulting methods / sequence are stored in a Scouting project and can be re-used

Agilent ChemStation Method Scouting Wizard

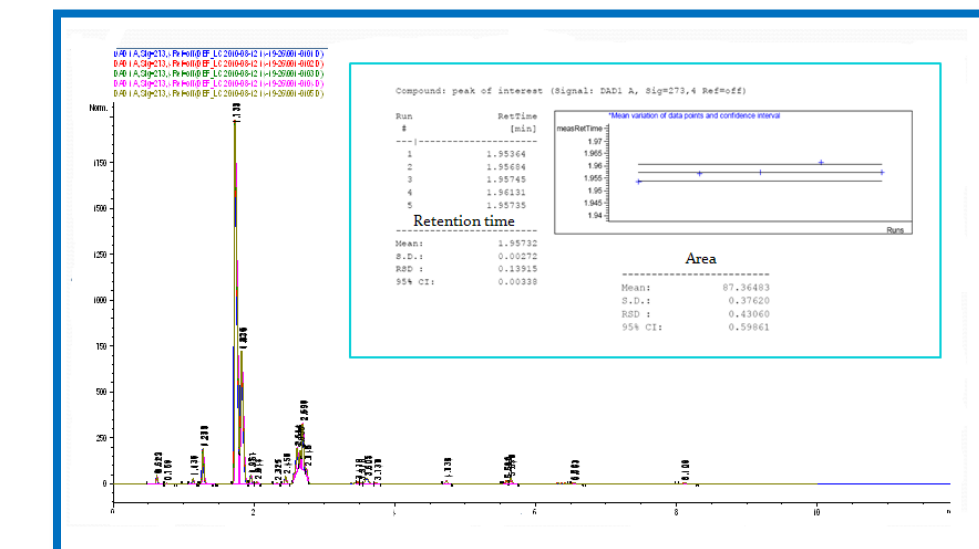
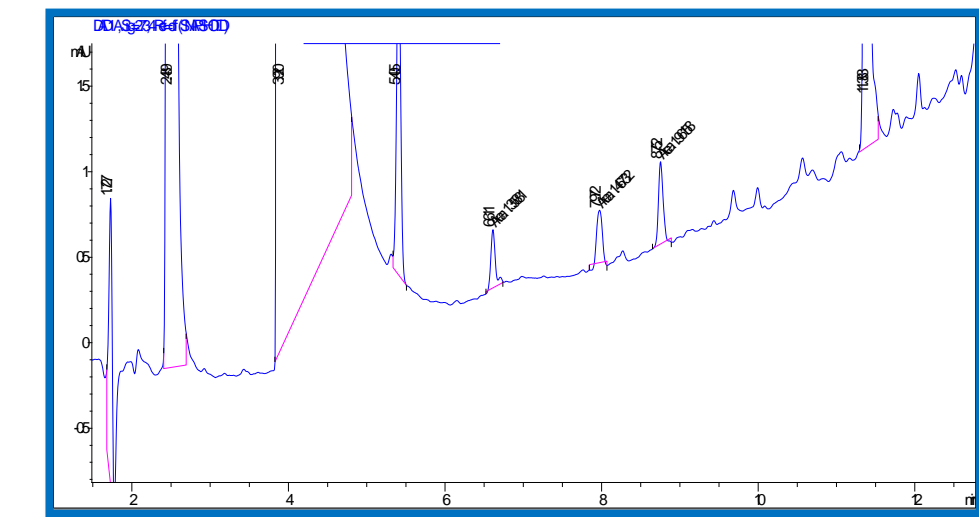
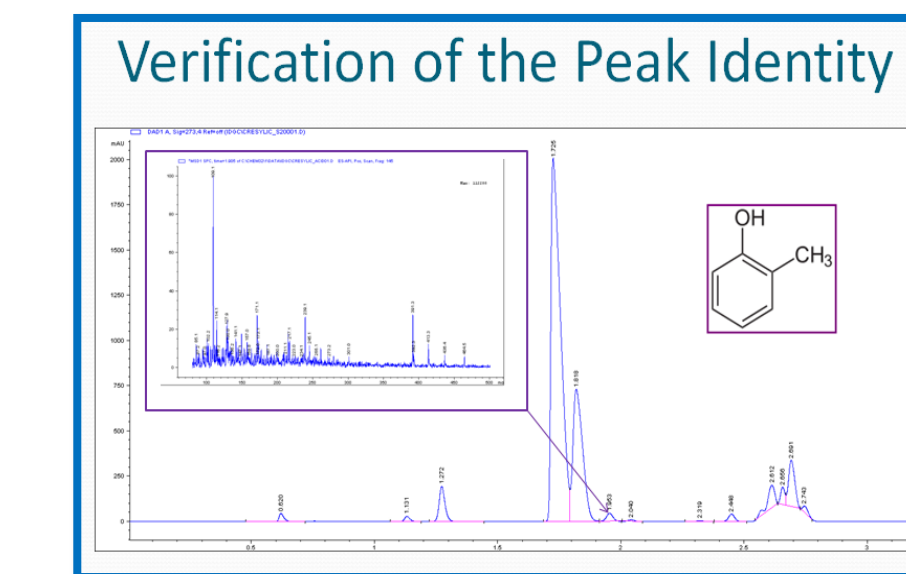
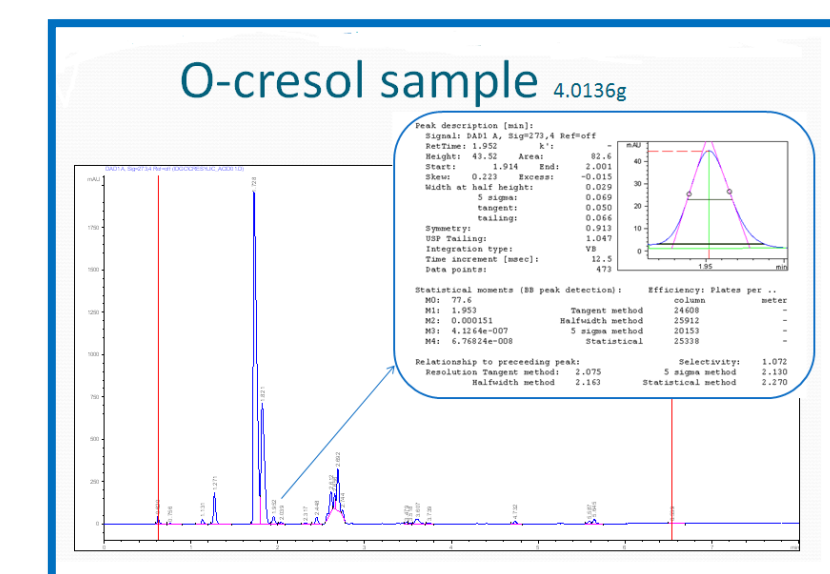
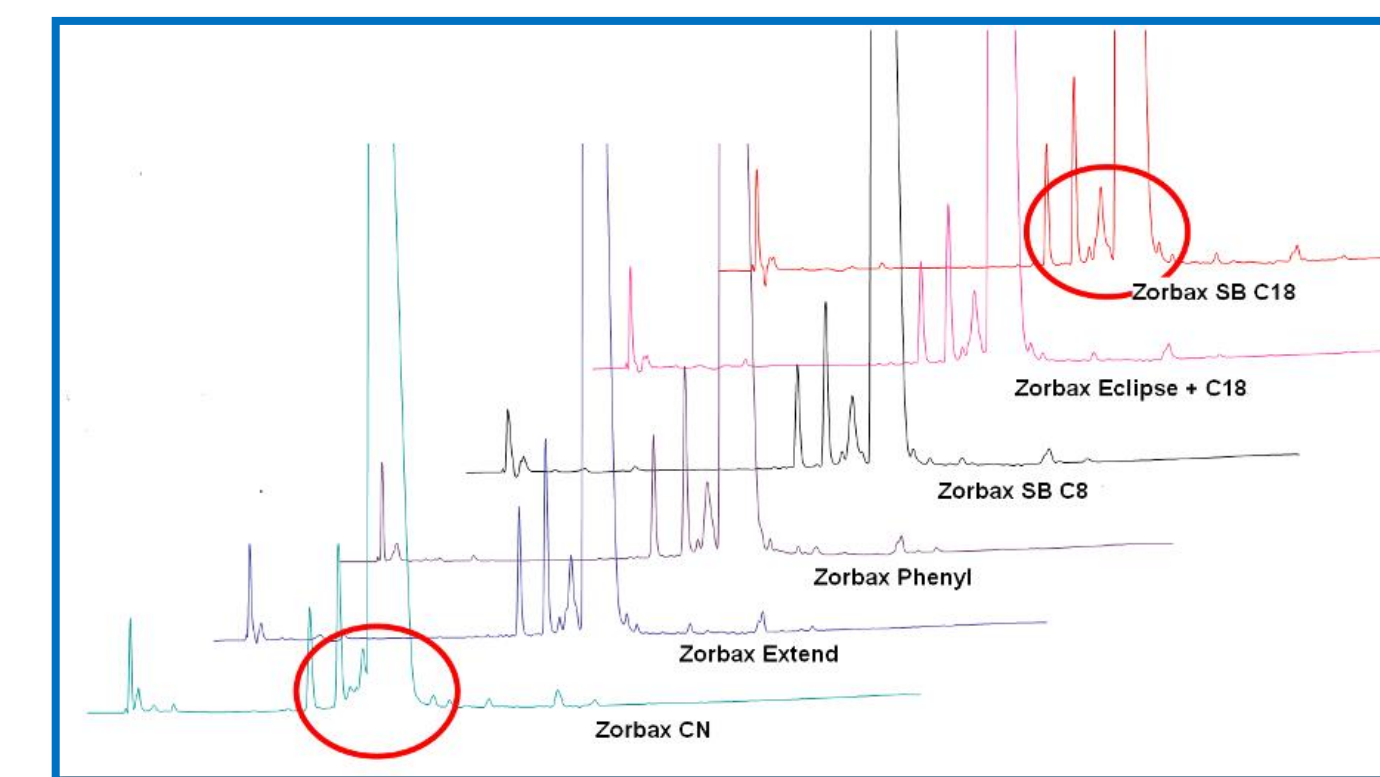
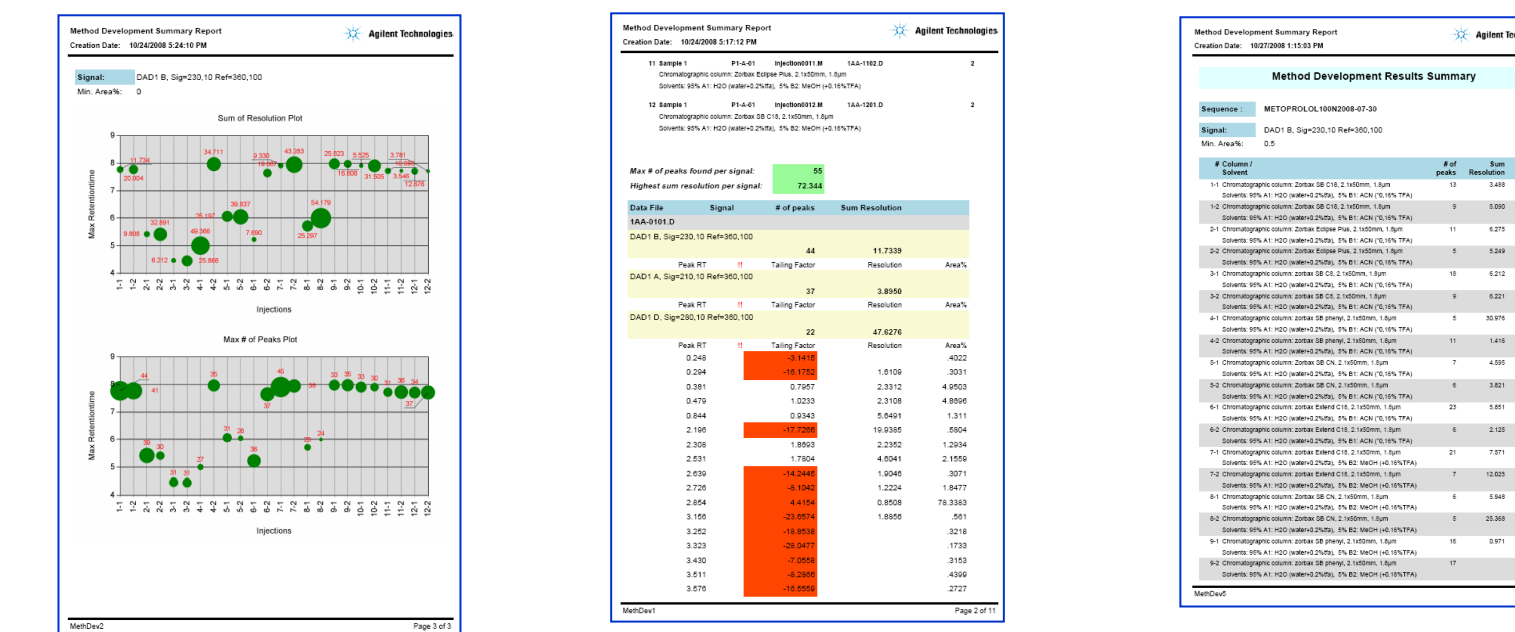


The System calculates the mobile phase needed to execute the campaign and determines the time of each mobile phase to be used, and the amount of sample needed.

Results and Discussion

Agilent 1200 Series Method Development Solution - OLIR Reporting with OpenLab Intelligent Reporter:

All available chromatographic information can be used to report - cross-sample, cross-sequence, cross-lab! Tables, Diagramms, conditional formatting



Flow 0.5ml/min

A mobile phase H2O 0.1% Acetic Acid

B mobile phase MeOH 0.1 % Acetic Acid

Gradient 40% to 100% MeOH over 8.00 minutes

Stoptime 9.0 min Posttime 1.0 min

Zorbax Eclipse Plus 2.1 x 100mm 1.8 micron

1 microliter injection volume

273 wavelength with a bandwidth of 4 slit width of 4

40 degrees C

80 Hz sampling rate on detector

Conclusions

MS detection added will have the ability to quickly develop methods and do method validation. We say MS allowed us to remove verification of standards from the experiments