Optimal Method Development Utilizing Modern UHPLC Columns via a Quality–By-Design (QbD) Methodology

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Introduction

As the trend continues towards smaller particles and more recently superficially particles to achieve shorter analysis times and increased sample throughput, there have been concerns about limited bonded phase availability for ease of method development. Current products in the UHPLC columns space have expanded the bonded phase availability considerably, allowing for easy method development. When combined with a Quality-by-Design (QbD) method development approach, one can utilize a larger number of different selectivities to rapidly develop an optimized method, and predict the appropriate method parameters to produce the desired optimum run conditions. Here, a QbD approach was utilized to demonstrate method development with newer UHPLC column technologies in the same manner as traditional 3.5 and 5µm HPLC columns to rapidly achieve ideal method conditions.

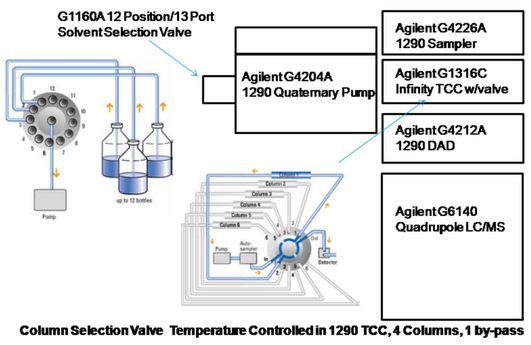
Introduction

Generally, chromatographic method development is carried out using a trial-and-error approach and requires a large amount of manual data interpretation. This one variable at a time approach is widely practiced. To work within limitations, chromatographers have to choose a certain subset of parameters and begin varying one at a time – single variable optimization. The result is that localized optima are established before moving on to the next parameter. The true optimum can be missed using this approach as it fails to take into account the interactions between individual parameters. The result of the trial-and-error approach commonly used today is a compromise between the best use of available resources and experimental rigor.

QbD principles can be directly applied to this process to achieve better, more robust separations. The variety of parameters involved in chromatographic separation means that the combinations and permutations of possible parameters for investigation quickly become unfeasible to consider by manual experimentation. To cover a sufficiently large experimental space, the matrix of possible column types, temperatures, gradients and buffer concentrations would require thousands of experiments, requiring far more time and resources than are typically available to chromatographers in an industrial R&D lab. Using available automated valves and computer aided analysis it becomes possible to carry out far more complex experiments, using data gained in early experiments to help track components and model chromatographic method performance.

Experimental

System Configuration



Column Selection Valve Temperature Controlled in 1290 TCC, 4 Columns, 1 by-pass

An Agilent 1290 Infinity Series LC Method Development Solution was also used in this work.

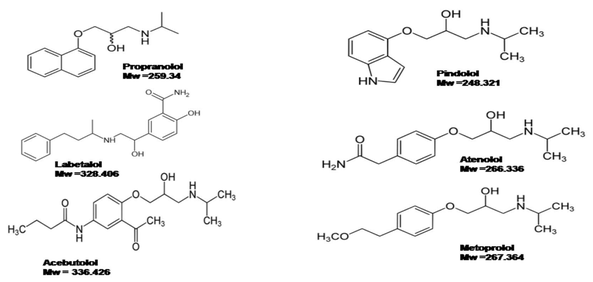
- G4204A Infinity Quaternary 1200 bar pump
- G1316C 1290 Thermostatted Column Compartment (TCC) with G4234B 6 position 14 port 1200 bar column valve
- G1160A 12 position, 13 port solvent selection valve
- G4212A Diode Array Detector
- G6140 Single Quadrapole Mass Spectrometer
- G4226A High Pressure Auto-sampler

- In addition to control by Open Lab version C 1.04 this system was used with ACD/Labs Autochrom 2012 for LC/MS

Experimental

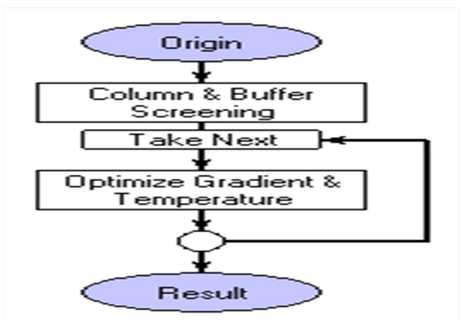
Beta blockers are used to treat hypertension and to manage cardiac arrhythmia. They diminish the effects of stress hormones. Beta blockers can be abused in sports such as archery, to reduce cardiac contraction, heart rate, and coronary blood flow and have been included in the list of forbidden substances by the International Olympic Committee.

Compounds and Structures

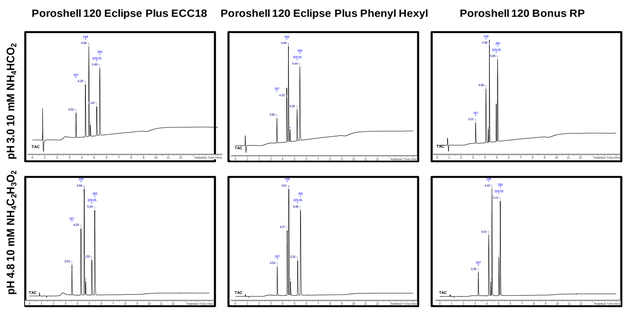


Results and Discussion

In the first step below a strategy is conceived in which the experimental direction is set up to separate a 6 component beta blocker mixture, symbolized by the flow chart below. A generic gradient is carried using the defined variables (column & buffer). The experiments are carried out, the resulting chromatograms are shown below. Using MS and UV data the peaks are identified and tracked. The best results are identified and summarized in the table on the right. Further experiments are planned and carried out.

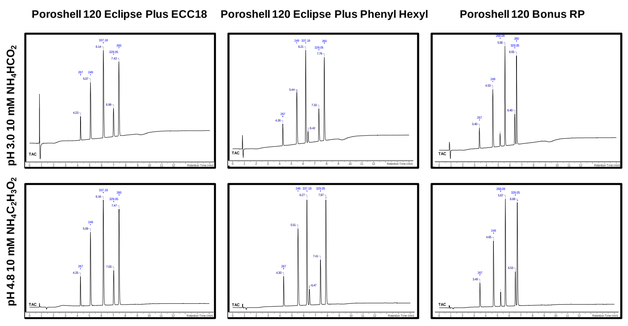


Optimization using Acetonitrile



0.3 ml/min 10% 100mM Buffer constant, 0 to 90% organic/7.33 minutes hold at 90% till 10 minutes

Optimization using Methanol



0.3 ml/min 10% 100mM Buffer constant, 0 to 90% organic/7.33 minutes hold at 90% till 10 minutes

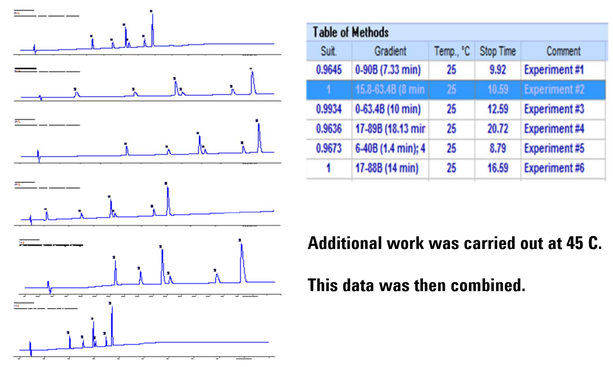
After the initial screening run, which takes only moments to set up, data is collected overnight. The first screening is processed, peaks are identified using UV and MS data. After this initial screening the best conditions (or others) are chosen for additional investigation. The suitability for further development is given a resolution score. Resolution score (Rs. Sc. on chart below) is used to identify good candidates for further work as is minimum resolution (Min Rs.) Although the Min Rs of pH 4.8 Phenyl Hexyl is higher it was decided to develop pH 3 as lower pH is generally less problematic. Individual peaks are identified using MS Data (M+1) retention times.

Experiment	Data Type	Status	Rs Sc...	Min Rs	Total	Unlabeled	267	249.03	337.13	268.09	329.05	260.02
Poroshell 120 EC-C18, pH 3 & MeCN	-	Complete	1	1.843	6/6		3.52	4.3	4.55	4.69	5.2	5.45
Poroshell 120 Phenyl Hexyl, pH 3 & MeCN	-	Complete	1	1.587	6/6		3.5	4.34	4.47	4.61	5.2	5.44
Poroshell 120 Bonus RP, pH 3 & MeCN	-	Complete	1	1.725	6/6		3.21	4.08	4.37	4.26	4.94	5.05
Poroshell 120 EC-C18, pH 4.8 & MeCN	-	Complete	1	1.848	6/6		3.53	4.29	4.55	4.69	5.21	5.44
Poroshell 120 Phenyl Hexyl, pH 4.8 & MeCN	-	Complete	0.993	1.483	6/6		3.52	4.38	4.51	4.66	5.26	5.49
Poroshell 120 Bonus RP, pH 4.8 & MeCN	-	Complete	1	1.701	6/6		3.26	4.15	4.42	4.32	5.01	5.14
Poroshell 120 EC-C18, pH 3 & MeOH	-	Complete	0.8	0.598	6/6		4.24	5.07	5.13	5.17	5.88	5.43
Poroshell 120 Phenyl Hexyl, pH 3 & MeOH	-	Complete	1	1.861	6/6		4.26	5.45	6.21	6.41	7.33	7.78
Poroshell 120 Bonus RP, pH 3 & MeOH	-	Complete	1	1.844	6/6		3.4	4.54	5.56	5.15	6.39	6.55
Poroshell 120 EC-C18, pH 4.8 & MeOH	-	Complete	0.8	0.445	6/6		4.24	5.09	6.15	6.2	7.02	7.47
Poroshell 120 Phenyl Hexyl, pH 4.8 & MeOH	-	Complete	1	2.122	6/6		4.29	5.51	6.27	6.48	7.42	7.87
Sample	-	-	1	2.122	6/6		4.29	5.51	6.27	6.48	7.42	7.87
Trial #1	-	Complete	1	2.122	6/6		4.29	5.51	6.27	6.48	7.42	7.87
DAD1.UV	LC/UV	Complete	1	2.178	6/6	1	4.3	5.51	6.27	6.47	7.41	7.88
DAD1A.ch	Trace, 230 nm	Complete	1	2.052	6/6		4.3	5.51	6.27	6.47	7.41	7.87
MSD1.MS	LC/MS, ES+	Complete	1	1.513	6/6		4.29	5.52	6.26	6.48	7.43	7.87
Poroshell 120 Bonus RP, pH 4.8 & MeOH	-	Complete	1	1.677	6/6		3.48	4.65	5.66	5.25	6.52	6.68

Results and Discussion

From the previous data, a column and condition is selected for further development. Two or more gradients are used to develop a chromatographic model which are used to predict the subsequent conditions.

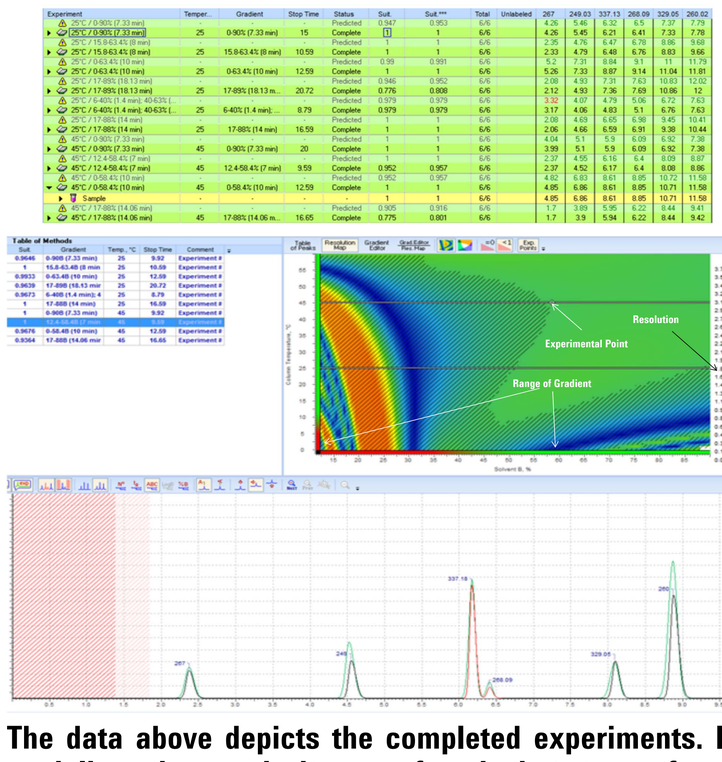
Optimization of Poroshell 120 Phenyl Hexyl Methanol pH 3, Gradient @ 25



Additional work was carried out at 45 C.

This data was then combined.

Poroshell 120 Phenyl Hexyl, Methanol, pH 3.0 Optimize Gradient and Temperature



The data above depicts the completed experiments. In modeling the work it was found that two of our methods meet our analysis criterion in terms of resolution, run time and minimum k'. A quadratic function was used to fit the retention data, which is in excellent agreement with the model. In addition a 25 C analysis was also found to yield excellent results. (Final gradient: 12.4% to 58.4 %/7 minutes @ 45 C)

Conclusions

Quality is built into the development of the method itself, resulting in better separations. The application of QbD to chromatographic method development builds quality into products by implementing several principles that include: Systematic design of method space through multivariate parameter optimization and minimizing risk by developing methods with automated control. The systematic examination of variables prior to experimentation also allows a larger experimental space to be covered, focusing experimentation on the optimal range for each variable, resulting in better quality methods.

- These columns are available in several kits:

Part Number	Kit Contents
5190-6152	RRHD pH Method Development Kit (SB-C18, Eclipse Plus C18, Extend-C18), 2.1x50mm
5190-6153	RRHD Eclipse Plus Method Development Kit (Eclipse Plus C18, Eclipse Plus C8, Eclipse Plus Phenyl-Hexyl), 2.1x50mm
5190-6153	RRHD Eclipse Plus Method Development Kit (Eclipse Plus C18, Eclipse Plus C8, Eclipse Plus Phenyl-Hexyl), 2.1x50mm
5190-6154	RRHD Aqueous Method Development Kit (SB-Aq, Bonus RP, Eclipse Plus Phenyl-Hexyl), 2.1x50mm
5190-6155	Poroshell 120 Selectivity Method Development Kit (EC-C18, Phenyl-Hexyl, Bonus RP), 2.1x50mm
5190-6156	Poroshell 120 Selectivity Method Development Kit (EC-C18, Phenyl-Hexyl, Bonus RP), 4.6x50mm
5190-6157	Poroshell 120 Aqueous Method Development Kit (SB-Aq, Phenyl-Hexyl, Bonus RP), 2.1x50mm
5190-6158	Poroshell 120 Aqueous Method Development Kit (SB-Aq, Phenyl-Hexyl, Bonus RP), 4.6x50mm
5190-6159	Poroshell 120 L1, L7, and L10 USP Method Development Kit (EC-C18, EC-C8, EC-CN), 4.6x100mm
5190-6160	Poroshell 120 L1, L7, and L10 USP Method Development Kit (EC-C18, EC-C8, EC-CN), 3.0x100mm

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