Fast Method Development Using the Agilent 1290 Infinity Quaternary LC System with Column Selection Valve

Technical Overview

Authors

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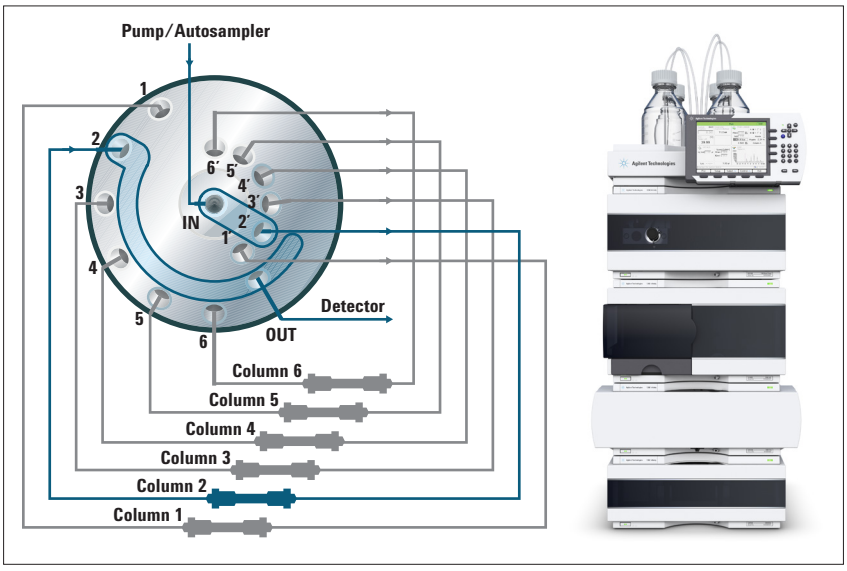
Abstract

The Agilent 1290 Infinity Quaternary LC System in combination with a column selection valve built in the Agilent 1290 Infinity Thermostatted Column Compartment enables stationary and mobile phase scouting by switching columns and solvents automatically for more convenient and fast method development. This Technical Overview demonstrates how method scouting with different columns and mobile phases can be setup easily. In this Technical Overview, a method development system was installed with four different columns and three mobile phases using the 1290 Infinity Quaternary LC System and the Agilent 1200 Infinity Series 6-Position/14-Port Column Selection Valve.



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Introduction

For method development procedures, it is best to vary the stationary and mobile phase. The aim is to optimize the method and find the perfect stationary and mobile phase to separate the analytes. With the 1200 Infinity Series 6-Position/14-Port Column Selection Valve in the 1290 Infinity Thermostatted Column Compartment, it is possible to reach that aim quickly and easily.

Using a column selection valve facilitates the determination of the optimum stationary phase without the need to change columns manually after one stationary phase has been tested. Using a quaternary pump assists in finding the optimum mobile phase without needing to frequently change mobile phases manually.

The Agilent 1200 Infinity Quaternary LC systems offer the possibility to integrate a 6-position/14-port column selection valve in the thermostatted column compartment. Up to six columns can be installed and used for instant method development.

This Technical Overview gives an example using four columns and three different mobile phase compositions to optimize the separation of nine compounds.

Experimental

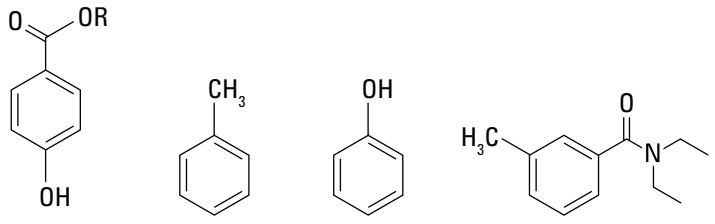
Instrumentation

Description	Model number
Agilent 1290 Infinity Quaternary Pump	G4204A
Agilent 1290 Infinity Autosampler	G4226A
Agilent 1290 Infinity Thermostat	G1330B
Agilent 1290 Infinity Thermostatted Column Compartment with 6-position/14-port column selection valve	G1316C G4234B
Agilent 1290 Infinity Diode Array Detector	G4212A

Chromatographic Conditions

Parameter	Condition
Sample:	Uracil, phenol, methyl-, ethyl-, propyl-, butyl-, and heptylparabene, N,N-diethyl-m-toluamide, toluene
Columns:	Agilent ZORBAX RRHT SB C18, 4.6 × 100 mm, 1.8 µm (p/n 728975-902) Agilent ZORBAX RRHT Eclipse Plus C18, 4.6 × 100 mm, 1.8 µm (p/n 95996-902) Agilent ZORBAX RRHT Extend C18, 4.6 × 100 mm, 1.8 µm (p/n 728975-902) Agilent ZORBAX RRHT Eclipse Plus Phenyl-Hexyl, 4.6 × 100 mm, 1.8 µm (p/n 959964-912)
Mobil phase:	A: Water, B: Acetonitrile, C: Methanol
Gradient 1:	20% B at 0 minutes, 95% B at 8 minutes
Gradient 2:	20% C at 0 minutes, 95% C at 8 minutes
Gradient 3:	10% B and 19% C at 0 minutes 45% B and 45% C at 8 minutes
Flow rate:	1 mL/min
Stop time:	15 minutes
Post time:	5 minutes
Injection volume:	3 µL
Temperature:	40 °C
Detection:	254 nm/10 nm; Ref. 360 nm/100 nm 10 mm flow cell Peak width > 0.013 minutes (20 Hz)

Analyzed Compounds



Parabenes* Toluene Phenol N,N-diethyl-m-toluamide

*R = methyl-, ethyl-, propyl-, butyl- and heptyl-

Software

Agilent OpenLAB CDS ChemStation Edition version C.01.04

Parameter Setting and Functionality of the Column Selection Valve

This Technical Overview used a column selection valve in the 1290 Infinity Thermostatted Column Compartment. For easy installation, the valve drive enables the exchange of valve heads from the front of the instrument without any tools. After instrument configuration, the column selection valve appears in the user interface (Figure 1).

In the column selection valve parameter screen, the valve parameters can be changed and different positions (one to six, use current position, next position after run) can be chosen. Figure 2 shows there are six positions available, which can hold up to six columns.

This Technical Overview used four columns in valve position 1 to 4, one bypass in position 5, and one waste in position 6. Bypass means flushing the system including the detector. Waste means flushing the system up to the valve, excluding the detector. If mobile phase scouting is necessary, it is recommended to have a bypass position to flush the whole instrument during a sequence.

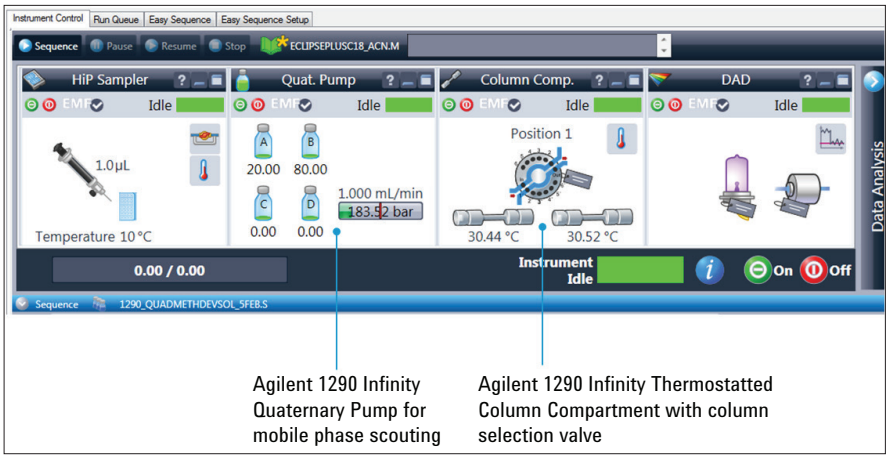


Figure 1. User Interface showing column selection valve mounted in column compartment.

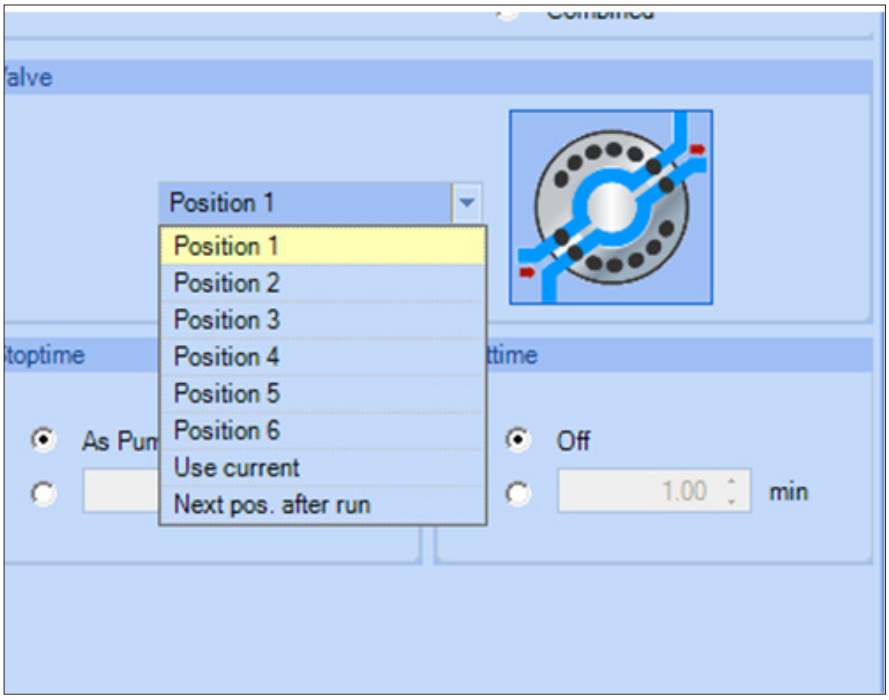


Figure 2. Column selection valve parameter screen.

Results and Discussion

The optimization of the separation conditions was done in 2 steps.

1. **Column scouting** using the four columns with different stationary phases and water and acetonitrile as mobile phases:

- **Valve position 1:** ZORBAX RRHT SB C18, 4.6 × 100 mm 1.8 µm (p/n 728975-902)
- **Valve position 2:** ZORBAX RRHT Eclipse Plus C18, 4.6 × 100 mm 1.8 µm (p/n 95996-902)
- **Valve position 3:** ZORBAX RRHT Extend C18, 4.6 × 100 mm 1.8 µm (p/n 728975-902)
- **Valve position 4:** ZORBAX RRHT Eclipse Plus Phenyl-Hexyl, 4.6 × 100 mm 1.8 µm (p/n 959964-912)

2. **Mobile phase scouting** using three mobile phase compositions:

- Water/acetonitrile with gradient 1
- Water/methanol with gradient 2
- Water/methanol/acetonitrile with gradient 3

Three channels of the 1290 Infinity Quaternary Pump were used with A=water, B=acetonitrile and C=methanol.

Column Scouting

The analysis was done on four stationary phases with the same chromatographic conditions using gradient 1. Three replicates for each column were done. Figure 3 shows overlaid chromatograms obtained on the four stationary phases.

Excellent separation was achieved on the ZORBAX RRHT Eclipse Plus C18 column and the ZORBAX RRHT SB C8 column. Resolution data were calculated for all four columns (Figure 4).

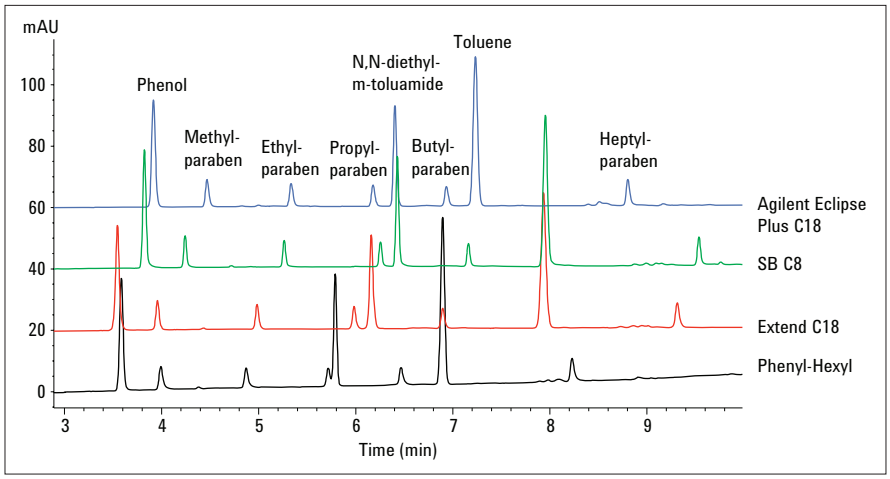


Figure 3. Overlay of chromatograms obtained from column scouting.

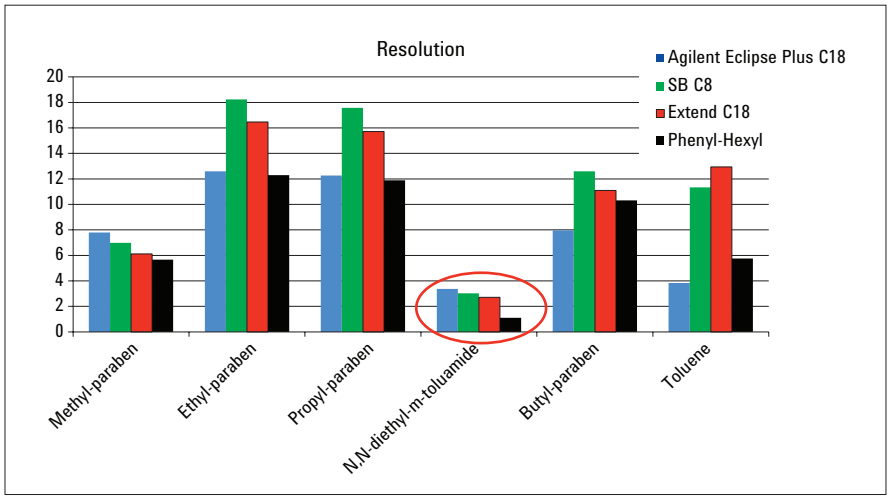


Figure 4. Resolution data achieved based on the column scouting experiments.

The separation between propylparaben and N,N-diethyl-m-toluamide was the most critical part of the chromatogram. The best resolution was achieved using the ZORBAX RRHT Eclipse Plus C18 column. This column was selected for all further experiments.

Mobile Phase Scouting

The ZORBAX RRHT Eclipse Plus C18 column was used for the mobile phase scouting experiments. Acetonitrile, methanol, and a 50/50% mixture of methanol and acetonitrile was used as organic phase (Figure 5). As a result, the best separation was obtained for acetonitrile as organic mobile phase. Methanol also provided a separation for all peaks, but with less resolution for butylparaben (Figure 6). The separation of heptylparaben, the peak with blue asterisk (Figure 5), from some impurities before this peak was better using acetonitrile. Further, the elution of N,N-diethyl-m-toluamide, the peak with red asterisk (Figure 5), changed to higher retention times and eluted after the toluene peak. The mixture of methanol and acetonitrile resulted in coelution of N,N-diethyl-m-toluamide, the peak with green asterisk (Figure 5), and toluene. Figure 6 combines the resolution data.

Acetonitrile seems to be the best mobile phase for this application, but methanol could be used as well to separate the compounds using the ZORBAX RRHT Eclipse Plus C18, 4.6 × 100 mm, 1.8 µm column. Both mobile phase combinations provided for all peaks a resolution > 2, which is typically sufficient for reliable quantification.

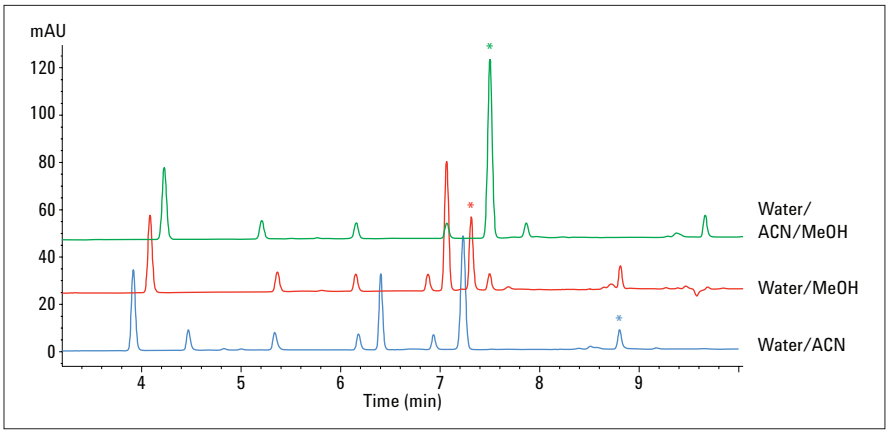


Figure 5. Overlay of chromatograms obtained from mobile phase scouting experiments.

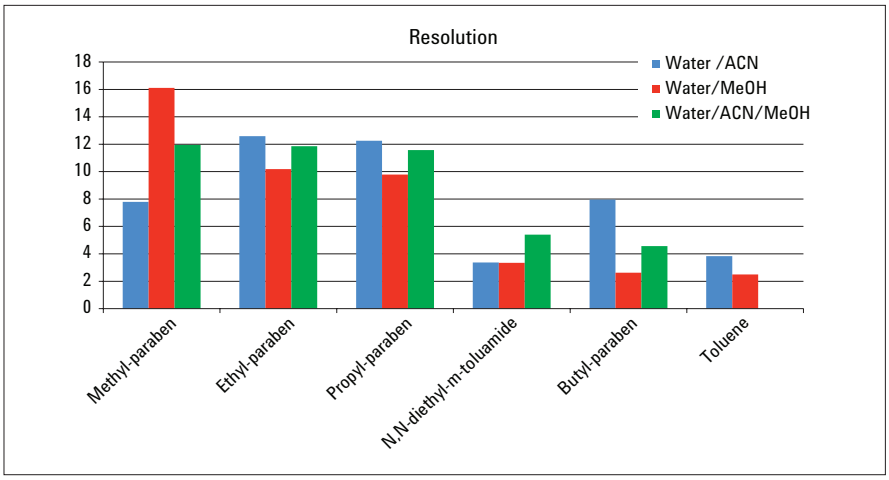


Figure 6. Resolution data obtained from mobile phase scouting experiments.

Conclusion

The Agilent 1290 Infinity Quaternary LC Systems offer the possibility to integrate a 6-Position/14-Port column selection valve in the column compartment. Up to six columns can be installed and used for method development.

This Technical Overview gives an example using four columns and three different mobile phase combinations to optimize the separation of nine compounds. Optimum separation with resolution for all peaks above two was found for the Agilent ZORBAX RRHT Eclipse Plus C18, 4.6 × 100 mm, 1.8 µm column using water/acetonitrile. Using water/methanol as a mobile phase combination, resulted in less resolution for most peaks. The optimization was done in less than 6 hours even though 20 minutes were used for each run including equilibration time.

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