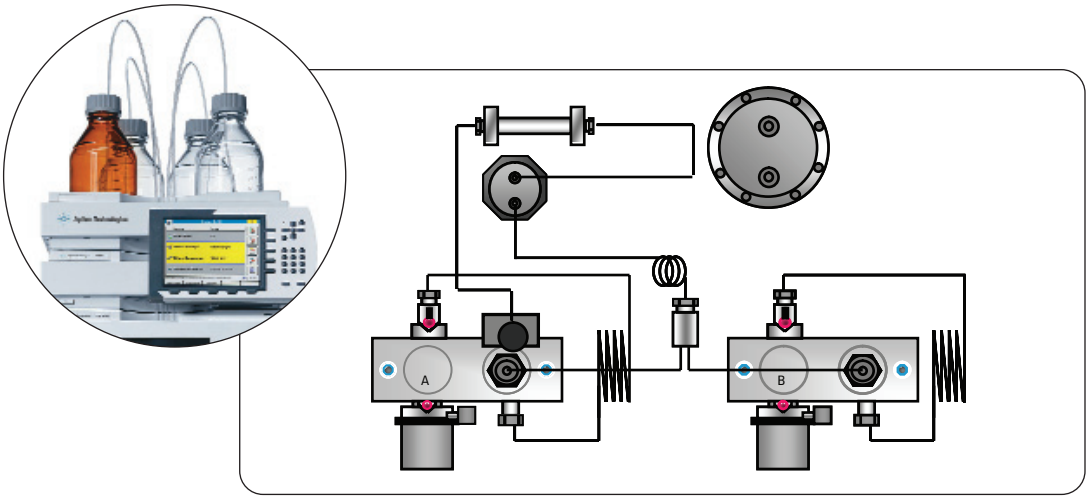
Agilent 1200 Series Rapid Resolution LC system

Switching between standard and low delay volume configurations using a software-controlled, high-pressure valve for optimized separation performance

Technical Overview



Agilent Equipment

- 1200 Series RRLC system
- 2-position / 6-port valve

Introduction

The Agilent 1200 Series RRLC system is designed for conventional LC with long columns of large internal diameter as well as for ultra-fast or rapid resolution LC with narrow bore column of about 2.1 mm id. Using a 600 bar, 2-position/6-port valve provides for switching between optimized conditions for both types of analysis. This technique offers:

- Convenient switching between standard and low delay volume configurations of the 1200 Series binary pump
- Inclusion of delay volume configuration in the method
- Changing of pump configuration within a sequence

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The influence of delay volume on separation performance

In most industries where LC is deployed as an analysis technique the flexibility of the LC equipment has recently become an important requirement. Flexibility enables the equipment to be used for both conventional LC as well as for fast, ultra-fast and rapid resolution applications. To fulfill this requirement, the Agilent 1200 Series RRLC system is designed to cater for columns of different lengths, different particle sizes from 1.8 to 5 µm and different internal diameters, and to deliver flow rates over a range from 50 µL up to 5 mL.

To ensure that optimum analysis conditions are always used, the Agilent 1200 Series RRLC system provides for adjustment of the pump delay volume through exchange of capillaries and flow cells. The influence of the pump delay volume is illustrated in figure 1. A sample was analyzed twice, first using the standard configuration and then using the low delay volume configuration. For low flow rates and 2.1 mm id columns, it is clear that the low delay volume configuration preferable to shorten the time a change in gradient needs to reach the column. As a consequence this configuration improved peak width, peak height and shortened run and cycle times (table 1). The chromatographic conditions used in figure 1 were as follows.

- Sample: isocratic standard sample, containing dimethylphthalate, diethylphthalate, biphenyl and o-terphenyl
- Column: Agilent Eclipse Plus, 100 x 2.1mm, 1.8 µm
- Solvents: A: Water, B: Methanol 50/50
- Flow: 0.5 mL/min
- Gradient: 5 min, 95 %B
- Injection volume: 1 µL
- Column temperature: 60 °C
- Detection wavelength: 245/10 nm
  Reference wavelength: 450/100 nm
  Peak width: 0.01min
  Slit width: 8 nm

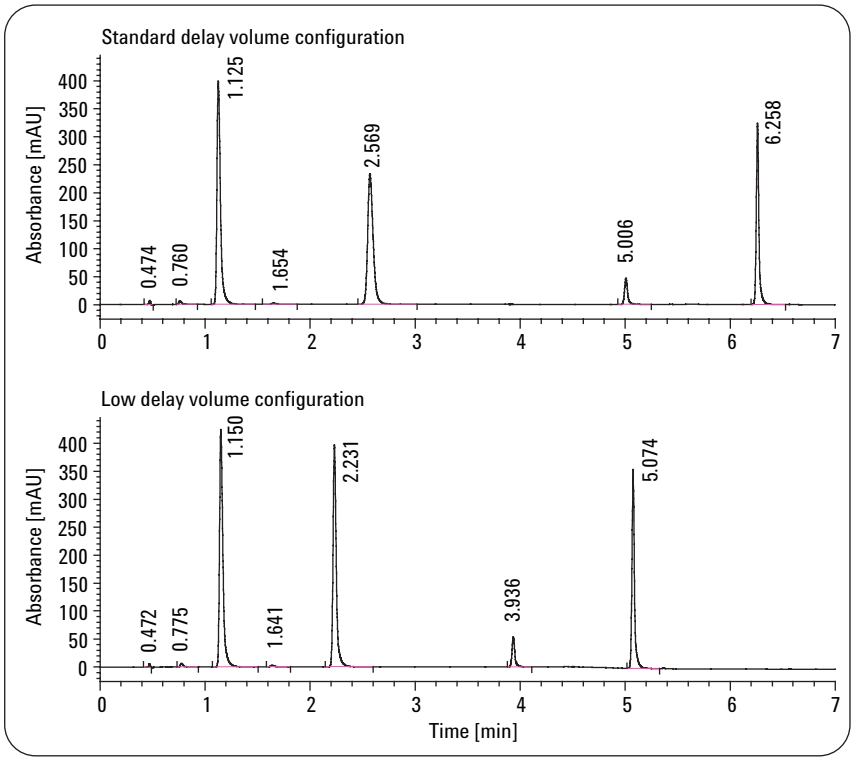

Figure 1
Influence of standard and low delay volume configuration for low flow rates on 2.1 mm id columns.

Parameter measured	Standard delay volume configuration	Low delay volume configuration
Retention time peak 2	2.569 min	2.231 min
Retention time peak 4	6.258 min	5.074 min
Height peak 2	233.27 mAU	395.80 mAU
Peak width peak 2	0.0525 min	0.0300 min

Table 1
Performance comparison of standard and low delay volume configurations.

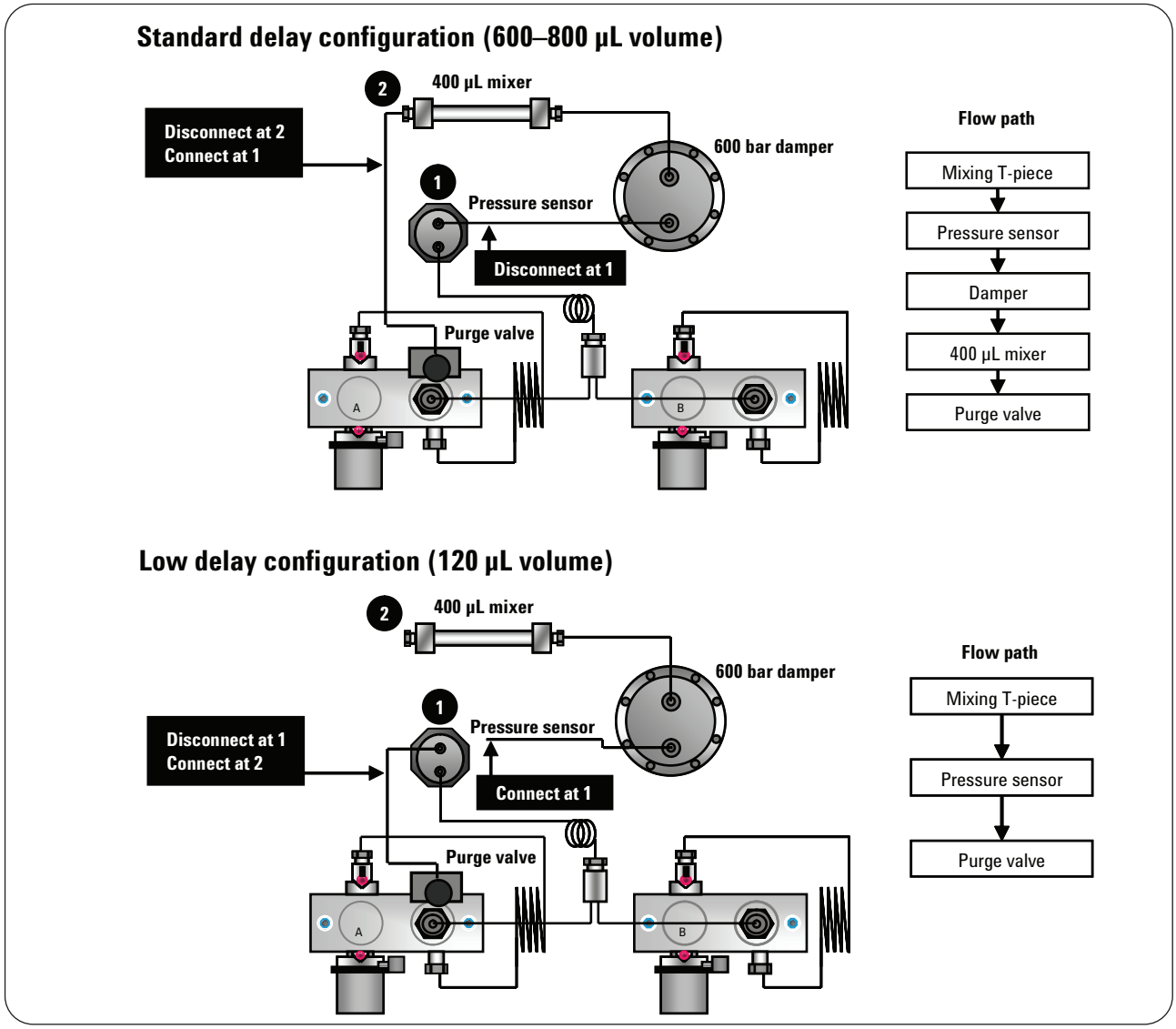

Figure 2
General CYP 3A4/5 enzymatic reaction converting midazolam substrate to 1'OH-midazolam.

Changing the delay volume configuration of the pump from standard to low

There are two ways to change the delay volume configuration of the pump from standard to low:

1. Connecting the capillaries by hand, or
2. Automated switching using a 600 bar, 6-port/2-position valve

Figure 2 shows how the capillaries must be connected when the configuration is done manually. One capillary must be disconnected form the pressure transducer (position 1). Another capillary must be disconnected from the mixer (position 2) and then connected to the pressure transducer (position 1).

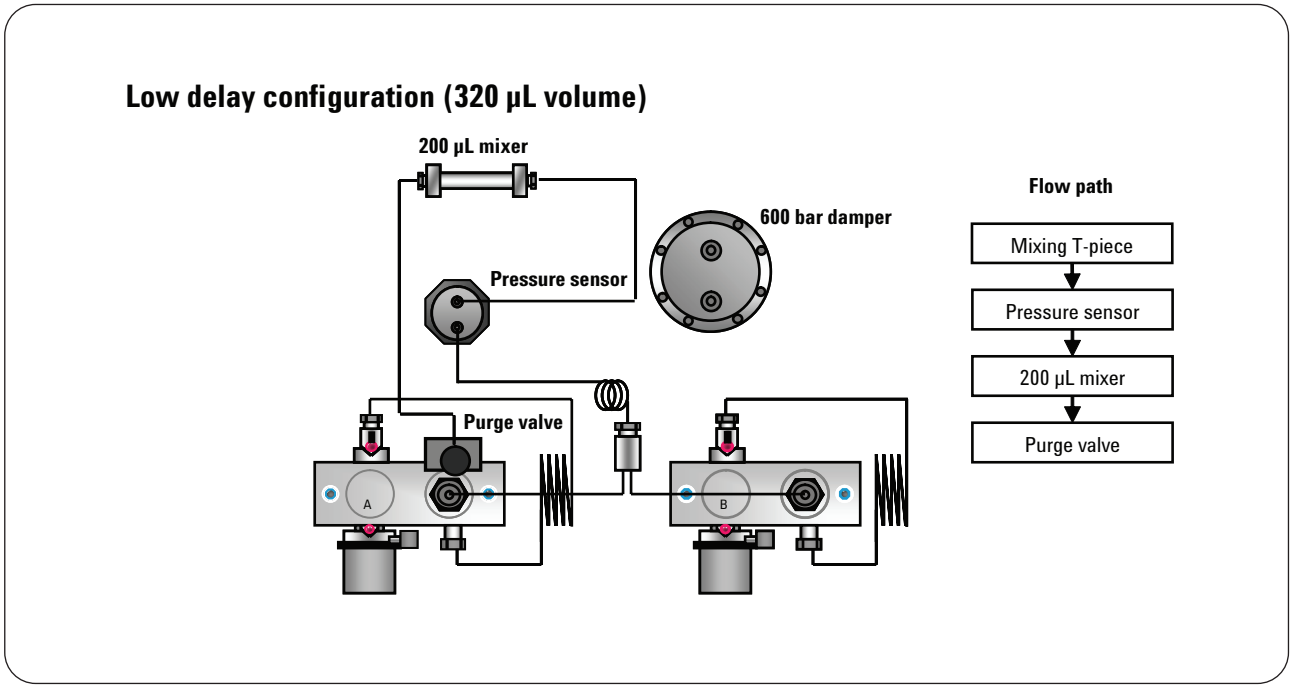

Figure 3
Addition of a low volume mixer in the pump configuration for low delay volume.

For optimum noise performance, which is especially important when performing trace analysis, an additional low volume mixer (Agilent order number 5067-1565) can be installed (figure 3).

A more convenient way to change the delay volume configuration is to use a 2-position/6-port valve (Agilent order number G1158B). This offers the advantage of switching between the two configurations without the need to disconnect and reconnect capillaries. The pump configuration can now be included in the analysis method for better traceability and it is now possible to have different pump configurations within a sequence of analyses. Figures 4, 5 and 6 show the connection schemes.

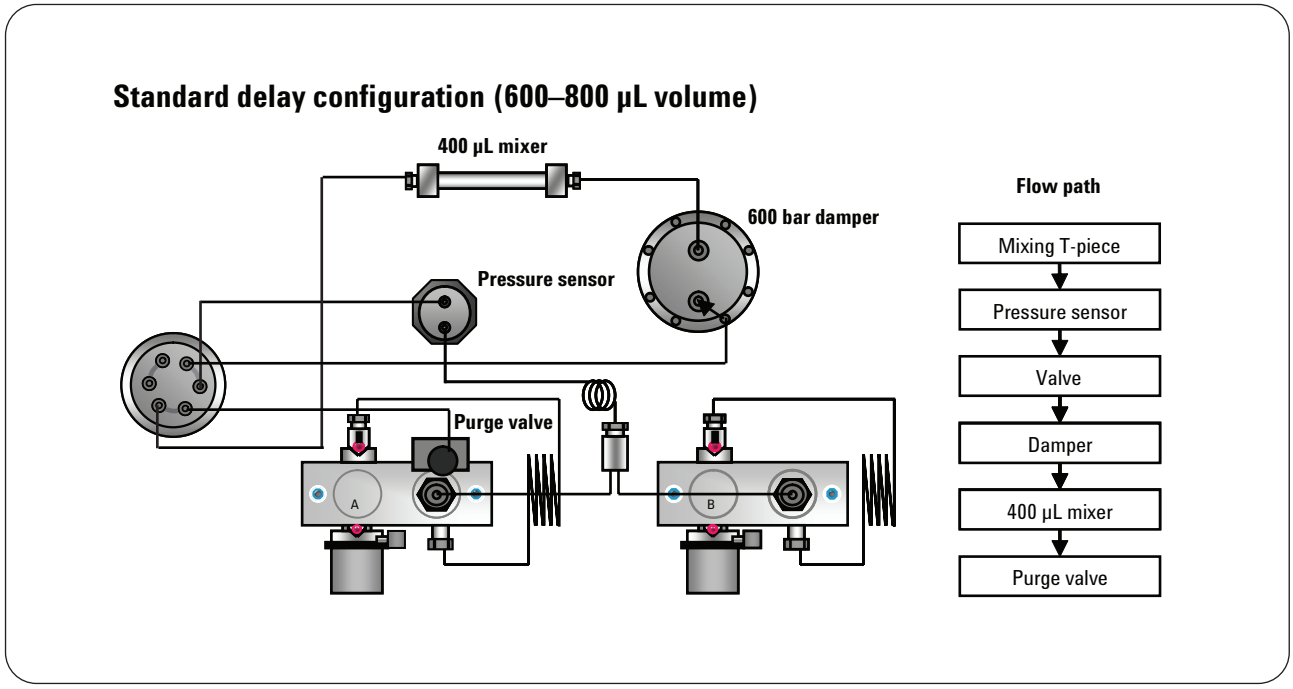

Figure 4
Pump configuration with switching valve for standard delay volume.

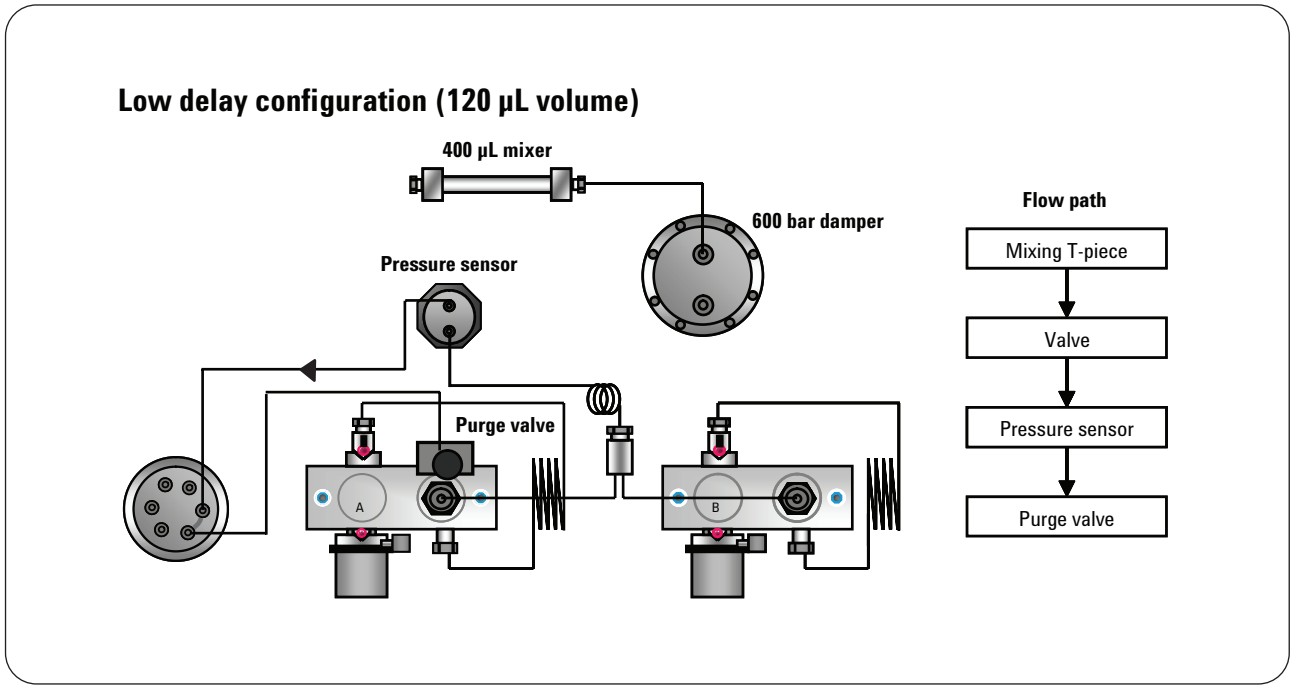

Figure 5
Pump configuration with switching valve for low delay volume.

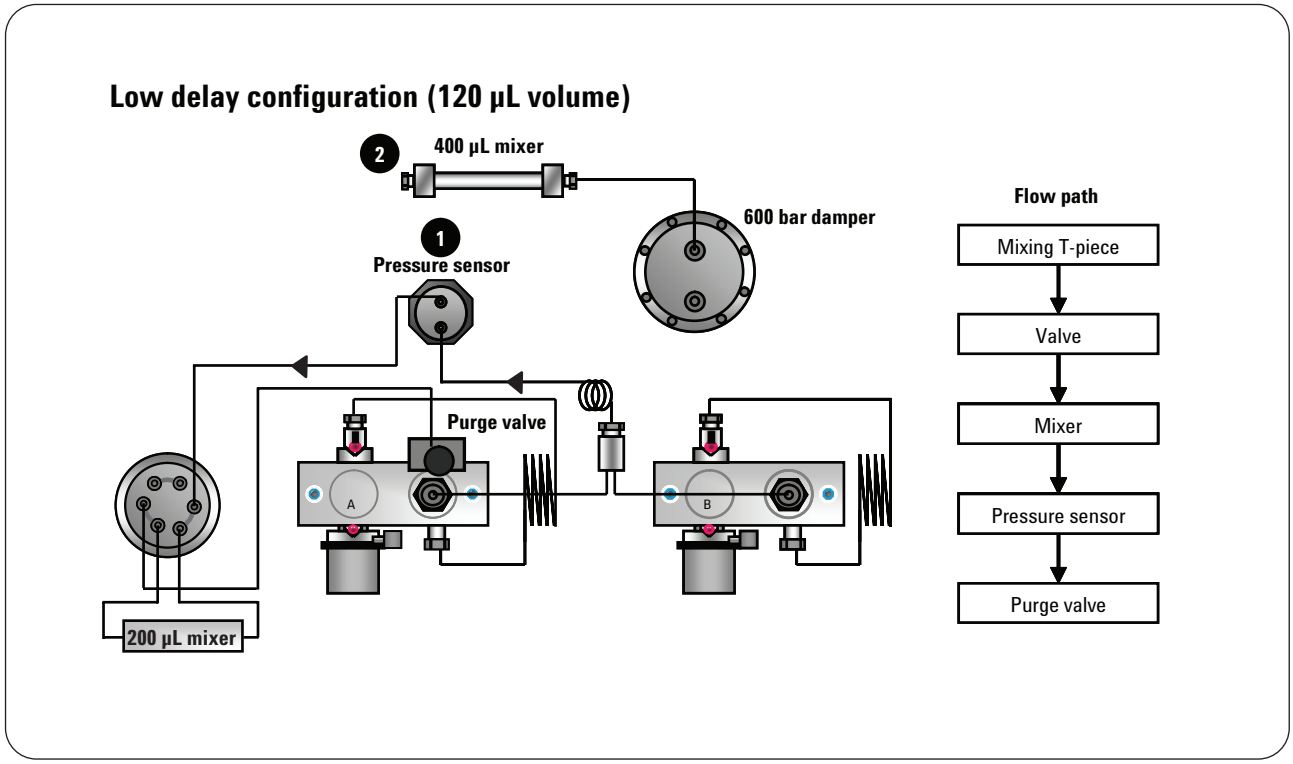

Figure 6
Pump configuration with switching valve and low volume mixer for low delay volume.

Summary

Today, a state-of-the-art LC instrument must be flexible and be able to support a wide range of LC applications. The Agilent 1200 Series RRLC system is equally suitable for conventional LC as well as for high resolution LC of complex samples using narrow-bore columns with sub-2-micron particles. Further, ultra-fast LC applications are also feasible, in which the peak widths approach those of high-resolution gas chromatography. Optimum conditions for these various applications include the adjustment of the delay volume of the pump. The Agilent 1200 Series RRLC system provides for automatic adjustment of delay volume through a switching valve, which is controlled through the Agilent ChemStation software and gives the following advantages:

- convenient switching between standard and low delay volume configurations of the 1200 Series binary pump,
- inclusion of delay volume configuration in the method, and
- changing of pump configuration within a sequence.

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Published April 1, 2008
Publication Number 5989-7732EN

Agilent Technologies