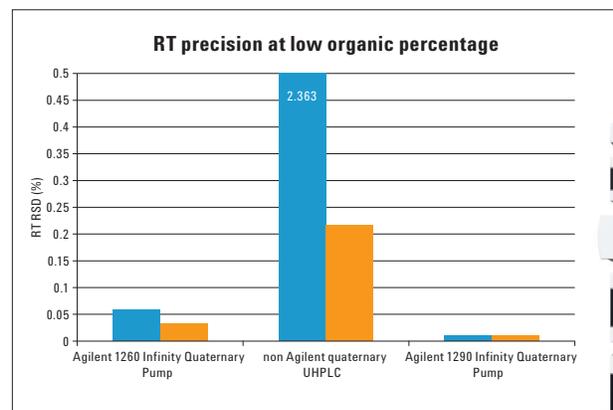


# Analysis of pharmaceutical drugs with low mobile phase organic or aqueous percentages using the Agilent 1290 Infinity Quaternary LC System

## Technical Overview

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### Abstract

Excellent precision of retention times over the complete composition range of an LC pump is a demand that is not easy to fulfill. The Agilent 1290 Infinity Quaternary LC Pump offers a composition range from 1 to 99%. Even at critical gradient segments below 5% or above 90% organic phase, the 1290 Infinity Quaternary Pump provides excellent performance due to its innovative design.

This Technical Overview provides two examples showing the performance of the 1290 Infinity Quaternary Pump below 5% and above 90% organic phase. The results are compared with results obtained on the Agilent 1260 Infinity Quaternary Pump and a non Agilent quaternary system.



**Agilent Technologies**

## Introduction

The 1290 Infinity Quaternary Pump has a wide usable composition range, from 1 to 99%. This enables the analysis of compounds at low and high organic percentages with excellent precision and accuracy. Other UHPLC quaternary pump designs have limited composition ranges, for example from 5 to 90%. This becomes problematic if hydrophilic compounds with low retention have to be analyzed starting a 0% organic mobile phase.

This Technical Overview demonstrates that the 1290 Infinity Quaternary Pump provides excellent precision data at low (2 to 12%) organic and at high (90 to 95%) organic percentages. Data are compared to data obtained on the 1260 Infinity Quaternary Pump and on a non Agilent quaternary UHPLC system.

## Experimental

### Instruments

Table 1 shows the instruments used.

### Software

OpenLAB CDS ChemStation C.01.04

## Results and discussion

To evaluate the pump performance at critical composition segments, the following experiments were performed on the three quaternary UHPLC systems:

- Analysis of theophylline and caffeine at low organic percentage, starting with 2% acetonitrile and ending with 12% acetonitrile after 5 minutes
- Analysis of testosterone acetate at high organic percentage, starting at 90% acetonitrile and ending at 95% after 5 minutes

The three UHPLC systems have different specifications for composition range, pressure range and delay volume. The system delay volume for

Module	Agilent 1290 Infinity Quaternary LC System Agilent product number	Agilent 1260 Infinity Quaternary LC System Agilent product number	Non Agilent quaternary UHPLC system n/a
Quaternary pump	G4204A	G1311B	Quaternary pump
Autosampler	G4226A	G1329B	Autosampler
Autosampler cooler	G1330B	G1330B	Autosampler cooler
Column compartment	G1316C	G1316C	Column compartment
Detector	Diode array detector (G4212A)	Diode array detector (G4212B)	Variable wavelength detector

**Table 1**  
Instrumentation used in the experiments.

Parameters	
<b>Chromatographic conditions at low organic percentage</b>	
Compounds:	Theophylline and caffeine, purchased from Sigma Aldrich
Column:	Agilent Poroshell 120 EC-C18, 4.6 × 50 mm, 2.7 μm (p/n 699975-902)
Mobile phases:	Water/Acetonitrile
Gradient:	2% to 12% ACN in 5 minutes
Flow rate:	2 mL/min
Stop time:	5 minutes
Post time:	4 minutes
Column temperature:	40 °C
Injection volume:	5 μL
DAD:	254/10 nm, Ref. 360/100 nm, 20 Hz
<b>Chromatographic conditions at high organic percentage</b>	
Compound:	Testosterone acetate, purchased from Sigma Aldrich
Column:	Agilent ZORBAX Eclipse Plus C18, 4.6 x 100 mm, 5 μm (p/n 827700-902)
Gradient:	90% to 95% ACN in 5 minutes
Flow rate:	1 mL/min
Stop time:	5 minutes
Post time:	3 minutes
Column temperature:	30 °C
Injection volume:	3 μL
DAD:	240/4 nm, Ref 400/60 nm, 20 Hz

**Table 2**  
Chromatographic parameters.

the 1290 Infinity Quaternary LC System and the non Agilent UHPLC system are comparable. Consequently, comparable retention times can be expected, (see Table 3).

### Analysis of theophylline and caffeine at low organic percentage

For this experiment, two very hydrophilic compounds, theophylline and caffeine, were selected. The compounds were dissolved in water and analyzed on all three systems using the same column and the same chromatographic conditions. Figure 1 shows the three chromatograms overlaid.

The peaks should elute approximately at the same retention times on the 1290 Infinity Quaternary LC System and the non Agilent UHPLC system due to the comparable delay volumes. However, the peaks on the non Agilent UHPLC system eluted later than expected. The most likely reason for this is that the pump was not able to deliver the required 2% organic, but started working properly at higher percent organic only. The usable composition range for the 1260 Infinity Quaternary Pump is selectable to achieve either optimum conditions from 0 to 95% or from 5 to 100%. For the following experiment, the composition range from 0 to 95% was selected. Consequently, the precision of retention times was excellent.

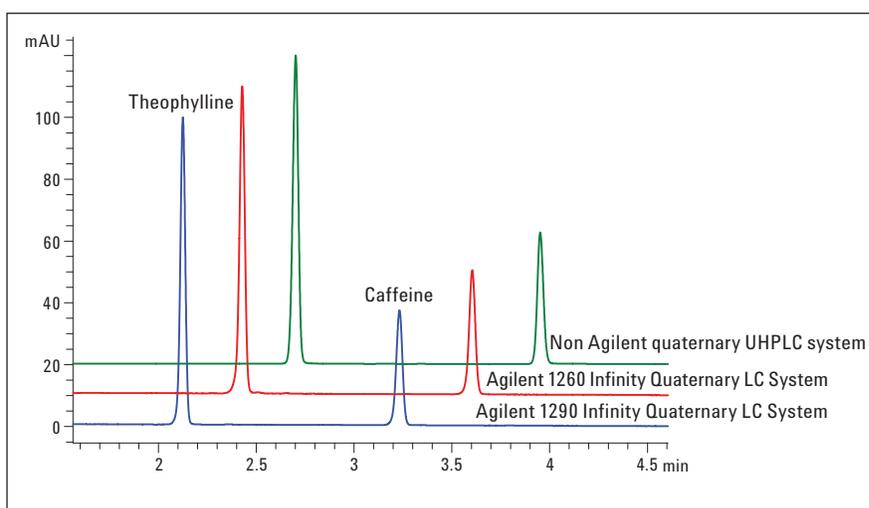
Figure 2 shows the precision data of the retention time of all three systems combined. The 1290 Infinity Quaternary LC System provided the best precision, RSD was < 0.012%.

The 1260 Infinity Quaternary LC System provided a RSD for retention times of < 0.06%. The non Agilent quaternary UHPLC system delivered very low precision data for this mobile phase composition range, resulting in RT RSD of 2.4%.

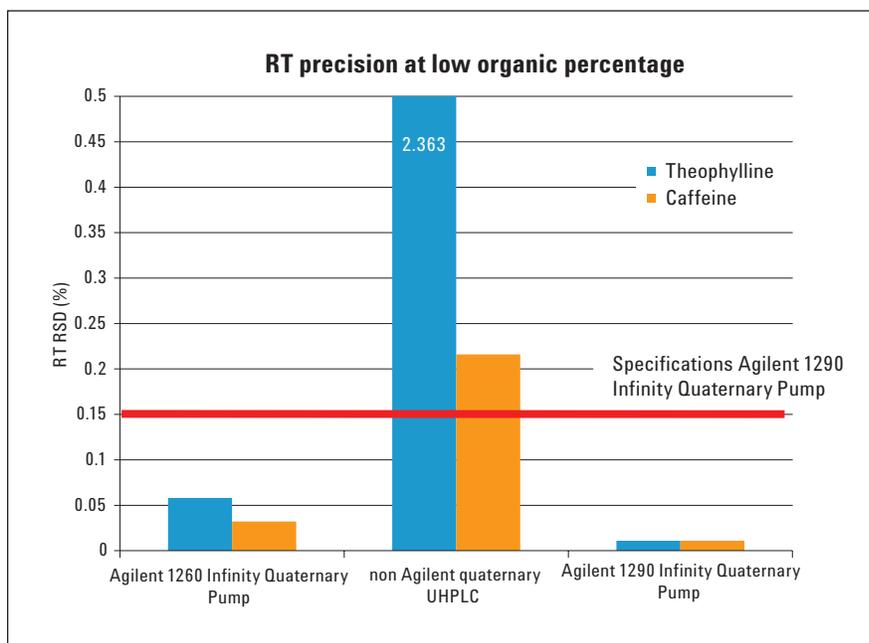
	Agilent 1290 Infinity Quaternary LC System	Agilent 1260 Infinity Quaternary LC System	Non Agilent Quaternary UHPLC system
Pressure limit	1200 bar	600 bar	1034 bar
Composition range	1 to 99%	0 to 95% or 5 to 100% selectable using primary channel	5 to 90%
System delay volume*	410 µL	980 µL	400 µL

\*measured

**Table 3**  
System specifications.



**Figure 1**  
Overlay of chromatograms at low organic percentage.



**Figure 2**  
Precision data for retention times.

## Analysis of testosterone acetate at high organic percentage

Testosterone acetate was selected for this experiment because it is a very hydrophobic compound. It was dissolved in acetonitrile and analyzed on all three LC systems under the same chromatographic conditions. As optimum composition range for the 1260 Infinity Quaternary Pump, the range from 5 to 100% was selected. All three systems showed excellent precision data. Figure 3 shows the three chromatograms overlaid.

The retention times obtained on the Agilent 1290 Infinity LC System and the non Agilent UHPLC system were nearly the same which was expected due to the similar delay volumes. On the Agilent 1260 Infinity LC System, the testosterone acetate peak eluted later due to the larger delay volume.

Figure 4 shows the precision data of the retention times for all three systems combined.

The precision of retention times for the Agilent 1290 Infinity Quaternary LC System and the Agilent 1260 Infinity Quaternary LC System was < 0.04% RSD. The precision for the non Agilent UHPLC system was < 0.066% RSD.

## Conclusion

The Agilent 1290 Infinity Quaternary Pump has a wide usable composition range from 1 to 99% providing excellent precision of retention times even for applications at very low or very high organic percentages. The precision for retention times was far below the specifications even at critical gradient segments. At low organic percentage, the 1290 Infinity Quaternary LC System provided a precision for retention times

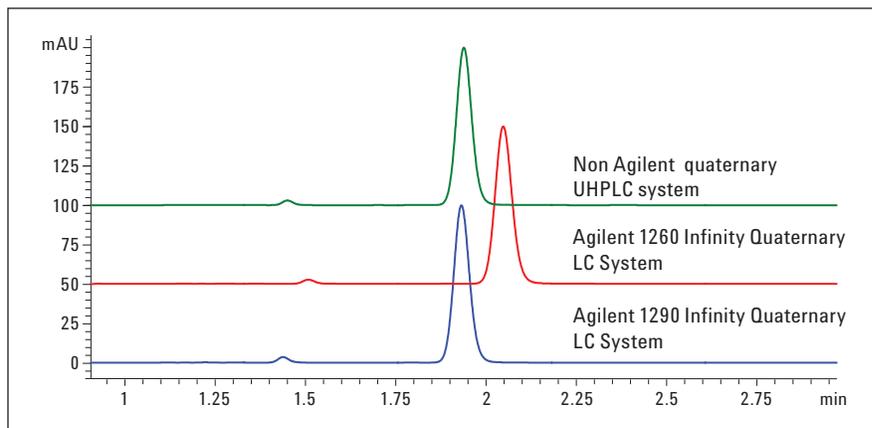


Figure 3  
Overlay of testosterone acetate chromatograms.

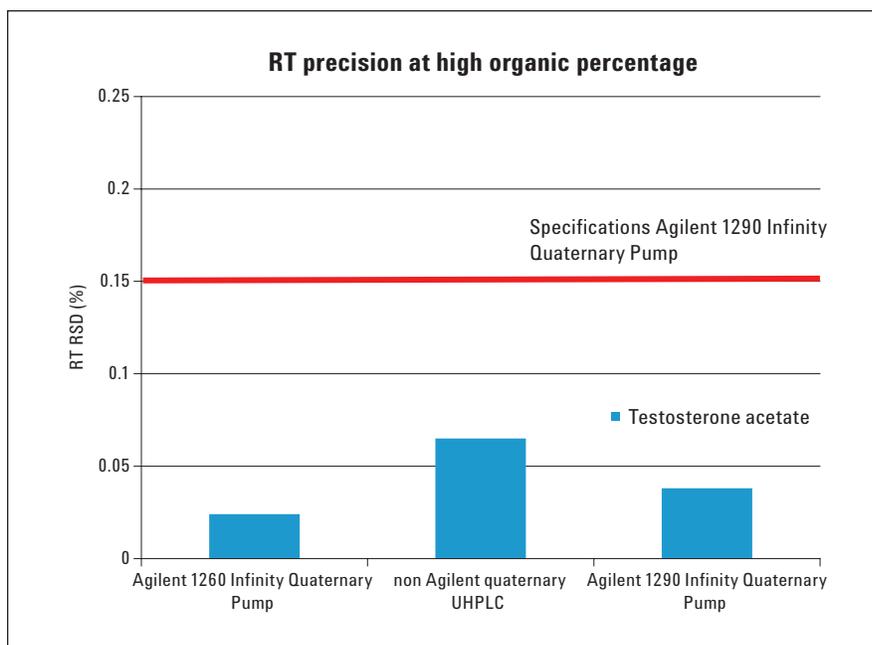


Figure 4  
Precision of retention times.

< 0.012% RSD. For high organic percentage, the precision of retention times was < 0.039%. When applying the low organic application to the non Agilent quaternary UHPLC system the precision of retention times for theophylline was as high as 2.4% RSD.

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