

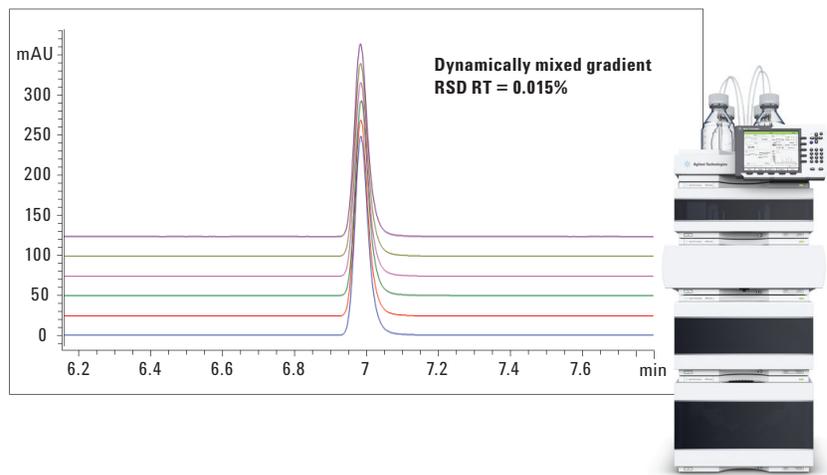
Retention time precision of Agilent 1290 Infinity Quaternary Pump

Analysis of clopidol using dynamically and premixed gradients

Technical Overview

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Abstract

The most important performance parameter for an ultrahigh performance liquid chromatography (UHPLC) pump is precision of retention times. Highest precision is required to identify compounds by retention times and subsequently accurately quantify the analyzed sample compounds. Gradient analysis can be done using either premixed mobile phases applying gradients from 0 to 100% B, as described in some USP methods, or using dynamically-mixed mobile phases applying gradients, for example, from 10 to 80% B.

This Technical Overview shows that premixing mobile phases for gradient applications provides retention time precisions below specifications, even under demanding chromatographic conditions. It also shows that dynamically-mixed gradients with the Agilent 1290 Infinity Quaternary Pump improve retention time precision significantly. The results of the 1290 Infinity Quaternary Pump are compared to results obtained using a quaternary pump from a non-Agilent manufacturer.



Agilent Technologies

Introduction

Pharmaceutical industry quality control analyses of drug preparations is done using premixed mobile phases not only for isocratic HPLC analysis but also for HPLC gradient analysis to fulfill USP method requirements^{1,2}. In this case, the complete gradient range of the pump from 0 to 100% is used. Quaternary pumps may have difficulty operating in the 0–100% organic range, therefore providing retention time precision below specification. As a result of these difficulties, some quaternary pump manufacturers specify an operating range from 5 to 95% or from 5 to 90% organic range.

This Technical Overview shows that using gradients based on premixed mobile phases produces excellent results for the 1290 Infinity Quaternary Pump. Further precision of retention times based on premixed gradients were compared to results based on dynamically mixed gradients. All tests performed on the 1290 Infinity Quaternary Pump were repeated with a non-Agilent quaternary LC system. The results of both LC systems were compared.

Experimental

Instruments

Module	Agilent Part Number	Non-Agilent LC modules
1290 Infinity Quaternary Pump	G4204A no additional mixer	Quaternary pump
1290 Infinity Autosampler	G4226A	Autosampler
1290 Infinity Thermostat for autosampler	G1330B	Thermostat for autosampler
1290 Infinity Column Compartment	G1316C	Column compartment Too small to hold 250 mm length column*
1290 Infinity Diode Array Detector	G4212A	Variable wavelength detector

*Column was kept at room temperature wrapped with insulation material.

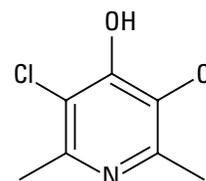
Table 1
Instruments used.

Software

Agilent OpenLAB CDS ChemStation
C.01.04 as acquisition and evaluation
software

Compound

Clopidol was selected as the analyte. Clopidol is used as prophylactics or therapeutics in the poultry and rabbit industries. It is added to feed in a concentration range of 100 to 125 mg/kg.³



Chromatographic conditions

Column:	Agilent ZORBAX SB C18, 250 × 4.6 mm, 3.5 μm
Mobile phase:	Water + 0.1% TFA, Acetonitrile + 0.09% TFA for dynamic mixing Water/ACN: 95/5 + 0.1% TFA and Water/ACN:40/60 + 0.1% TFA for quaternary premixing
Flow rate:	1 mL/min
Stop time:	15 min
Post time:	5 min
Injection volume:	1 μL with needle wash for 6 s
Column temperature:	30 °C
Diode array detection:	267/10 nm, Ref 400/ 80, 10 Hz, slit 4 nm

Gradients

Dynamic mixing of mobile phases

- At 0 min 95% Water + 0.1%TFA and 5% Acetonitrile + 0.09%TFA
- At 15 min 40% Water + 0.1%TFA and 60% Acetonitrile + 0.09%TFA

Premixing of mobile phases

- At 0 min 100% of Water/Acetonitrile = 95/5% + 0.1%TFA
- At 15 min 100% of Water/Acetonitrile = 40/60% + 0.1%TFA

Results and discussion

Two LC systems with quaternary pumps were tested using dynamically and premixed mobile phases to form a gradient. One system was the 1290 Infinity Quaternary LC, the other was a quaternary LC system from a different LC instrument supplier.

The following gradients were applied, (Figure 1):

- The premixed gradient started at 0% B and was increased to 100% B in 15 minutes. Phase A contained 95% water, 5% acetonitrile and 0.1% TFA. Phase B contained 40% water, 60% acetonitrile and 0.1% TFA.
- The dynamically mixed gradient started at 5% B and was increased to 60% B in 15 minutes. Phase A contained 100% water and 0.1% TFA. Phase B contained 100% acetonitrile and 0.09% TFA.

Clopidole eluted at approximately 30% acetonitrile. Figure 2 shows an overlay of six consecutive runs using the 1290 Infinity Quaternary Pump and an overlay of six consecutive runs obtained on the quaternary LC system from a different LC supplier using dynamically mixed gradients. Figure 3 shows an overlay of six consecutive runs using premixed gradients for the 1290 Infinity Quaternary Pump and the quaternary LC system from a different LC supplier.

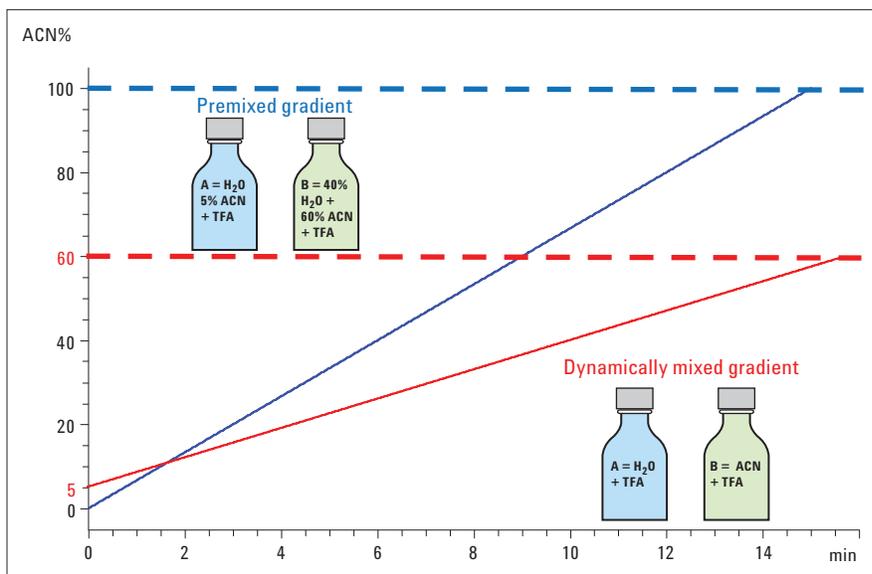


Figure 1
Gradient profiles using dynamically and premixed mobile phases

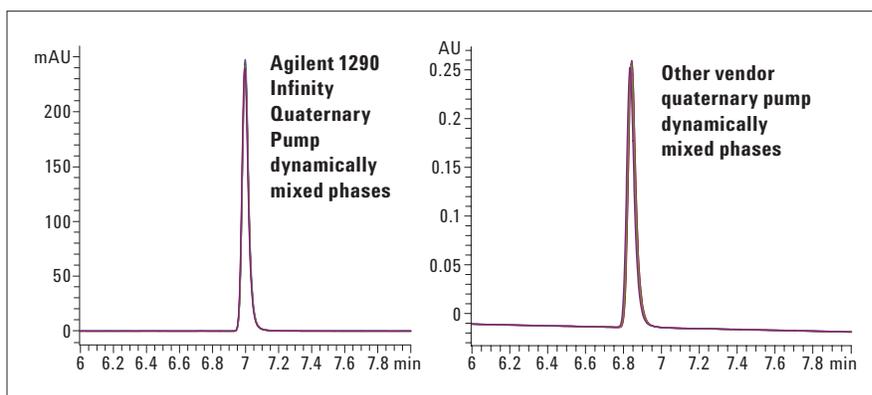


Figure 2
Overlay of six chromatograms obtained on the two LC systems using dynamically mixed gradients

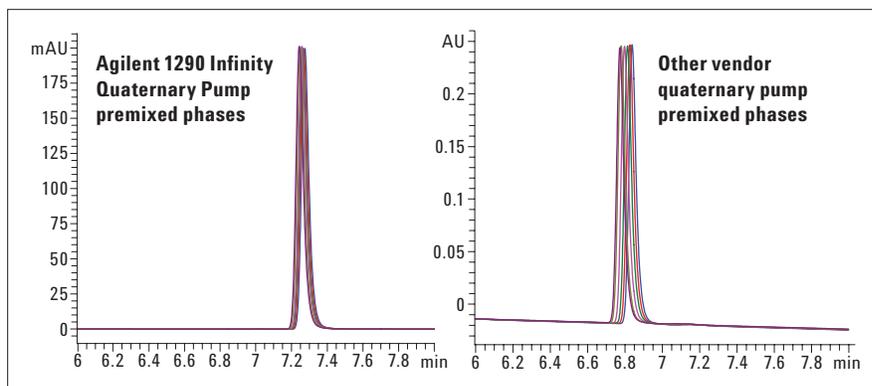


Figure 3
Overlay of six chromatograms obtained on the two LC systems using premixed gradients

The two gradients were applied to the two quaternary pumps LC systems and the precision of retention times was evaluated. The results are combined in Figure 4. The retention time precision for the premixed mobile phase with the 1290 Infinity Quaternary Pump was <0.13% RSD. The retention time precision with the non-Agilent pump was <0.39% RSD. This pump has an operating range of 5–90% B, which explains the poor precision for the premixed gradient of 0–100% B. This explains the 3 to 4 time worse precision for the premixed gradient, which starts at 0% and ends at 100% B.

Both LC systems showed a trend for the retention times to lower values using premixed mobile phases. This phenomena is well known and well documented^{3,4,5}.

Using the dynamically mixed gradient the retention time precision was significantly better for both pumps. The precision for the 1290 Infinity Quaternary Pump showed best results with a precision of retention times of 0.015% RSD over six consecutive runs. The retention time precision for the quaternary LC system from a different LC supplier showed 0.052% RSD for the retention times.

Conclusion

The 1290 Infinity Quaternary Pump had a retention time precision of <0.13% RSD for premixed mobile phases. This is below specification even under these demanding conditions. The quaternary pump from the

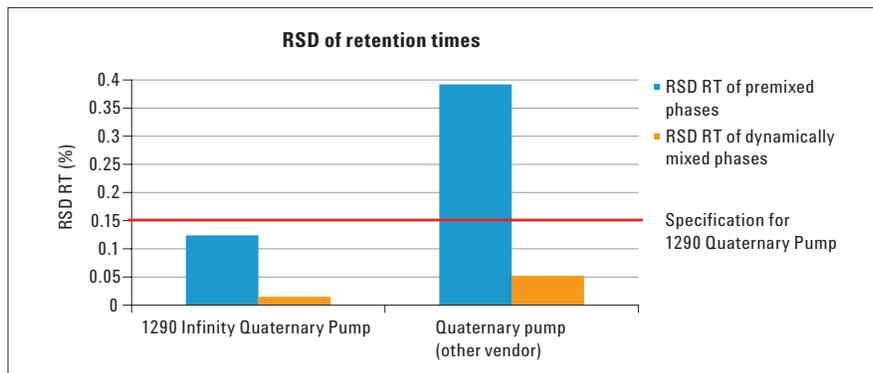


Figure 4
Precision of retention times applying premixed and dynamically mixed gradients

other vendor showed a precision for retention times of <0.39% RSD, which is a result of the limited operating range of quaternary pump.

Both quaternary pumps showed significantly better retention time precision for gradients based on dynamically mixed mobile phases. The precision for the 1290 Infinity Quaternary Pump showed the best results for dynamically mixed gradient with a precision for retention times of 0.015% RSD over six consecutive runs. The retention time precision was 0.052% RSD for the quaternary LC system from a non-Agilent manufacturer.

References

1. USP method 28 for the analysis of Atorvastatin Calcium, The United States Pharmacopoeia (2003), 26th ed., US Pharmacopoeial Convention, Rockville, MD, p. 1151

2. USP method 32-NF27 for the analysis of Olanzapine

3. Introduction to Modern Liquid Chromatography, Lloyd R. Snyder, Joseph J. Kirkland, John W. Dolan, ISBN=1118210395

4. HPLC for pharmaceutical scientists, Yuri Kazakevich, Rosario LoBrutto, ISBN=0471681628

5. Guglielmo Dusi et al "Determination of nicarbazin and clopidol in poultry feeds by liquid chromatography", Journal of chromatography A 882 (2000) 79-84

[www.agilent.com/
chem/1290QuatPump](http://www.agilent.com/chem/1290QuatPump)

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