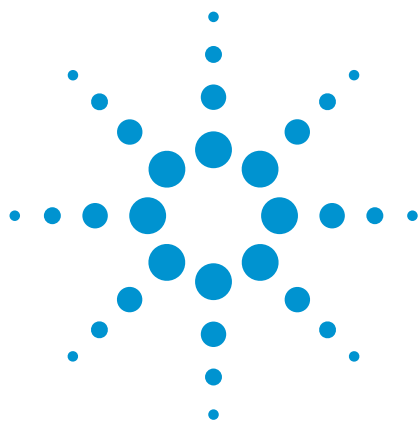




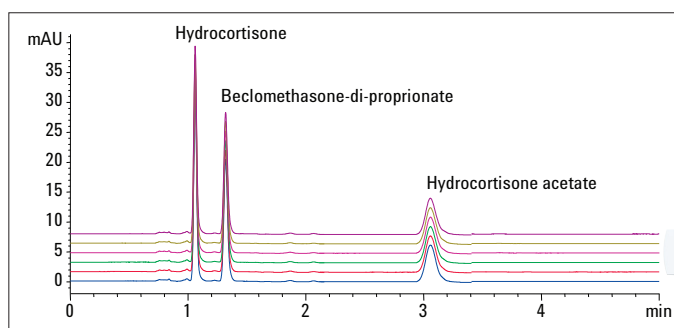
Agilent 1290 Infinity Quaternary Pump: Comparing premixed isocratic conditions with dynamically-mixed conditions

Analysis of glucocorticoid drugs

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 enable identification of compounds by retention times and subsequently to be able to accurately quantify the analyzed sample compounds. To achieve highest precision of retention times, many applications are performed using isocratic conditions by premixing the mobile phase and then pumping the mixture through a single pump channel. This Technical Overview demonstrates that the Agilent 1290 Infinity Quaternary Pump eliminates the need to premix the mobile phase.



Agilent Technologies

Introduction

Recently in the pharmaceutical industry, quality control analyses of drug preparations are frequently done using isocratic UHPLC conditions. To ensure highest precision for the retention times, premixed phases are often preferred. To ensure that the premixed phases always have the same compositions, the mixing procedures have to be very reproducible. Slight composition changes could occur between preparations and also between different users. For compounds that react to even small composition changes, non-reproducible mixing will result in lower precision of retention times.

This Technical Overview demonstrates that the Agilent 1290 Infinity Quaternary Pump is able to provide highest precision for retention times using dynamically-mixed mobile phases over prolonged periods, and that the retention time precision is comparable to results obtained from different charges of premixed mobile phases. Three glucocorticoids were chosen as analytes, which show significant retention time shifts even for slight composition changes, see Figure 1.

Experimental

Instrumentation and software

The Agilent 1290 Infinity LC used for the experiments consisted of the following modules:

- Agilent 1290 Infinity Quaternary Pump (G4204A)
- Agilent 1290 Infinity Autosampler with Thermostat (G4226A, G1330B)
- Agilent 1290 Infinity Thermostatted Column Compartment (G1316C)
- Agilent 1260 Infinity Diode Array Detector (G4212A)

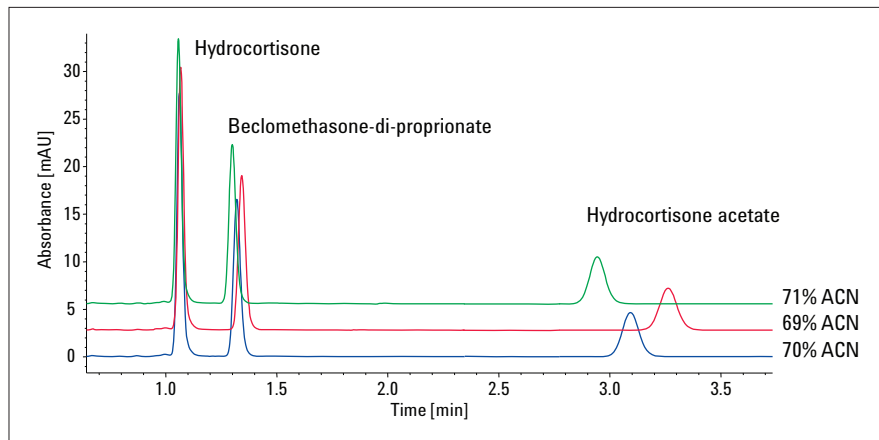


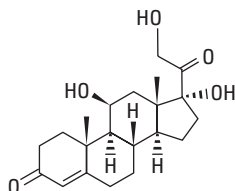
Figure 1
Influence of acetonitrile percentage on elution.

Software

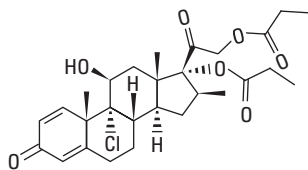
Agilent ChemStation revision C.01.03.
All Agilent LC modules had RC.Net drivers with appropriate firmware revision.

Compounds analyzed

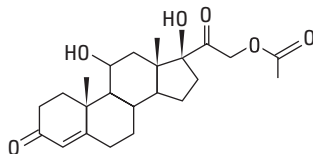
Hydrocortisone



Beclomethasone-di-propionate



Hydrocortisone acetate



Chromatographic conditions

Column:	Agilent ZORBAX Eclipse Plus C18, 100 × 4.6 mm, 5 µm
Mobile phase:	Water:Acetonitrile / 30:70
Flow rate:	1 mL/min
Stop time:	5 min
Injection volume:	3 µL with needle wash for 6 s
Column temperature:	30 °C
Detection:	254/10 nm, Ref 400/80, 20 Hz, slit 4 nm

Results and discussion

All experiments were performed using an 1290 Infinity LC equipped with the 1290 Infinity Quaternary Pump. For all experiments using premixed mobile phases, the C channel of the pump was deployed; for all experiments with dynamically-mixed mobile phases, channels A and B were used. Figure 2 shows six example chromatograms based on dynamically-mixed phases.

The following experiments were performed and the relative standard deviation (RSD) of retention times (RT) was evaluated:

1. RSD of RT for six consecutive runs using a dynamically-mixed mobile phase
2. RSD of RT for six consecutive runs using a premixed mobile phase
3. RSD of RT of the 10th run of six sequences using dynamically-mixed mobile phases over several days
4. RSD of RT of the 10th run of six sequences using six premixed mobile phases prepared by one user
5. RSD of RT for three runs using three premixed mobile phase prepared by three different users

Figure 3 shows the results for experiments 1 and 2. The precision of the retention times for the experiments with premixed mobile phases is typically slightly better than the precision for experiments with dynamically-mixed mobile phases, as expected. Nevertheless, the precision of the retention times using premixed phases is only approximately two times better, in worst case, but still very good overall.

In experiments 3 and 4, six sequences were applied each containing 10 runs with dynamically-mixed mobile phases and 10 runs with premixed mobile phases. For each sequence, the premixed phase was freshly prepared by one user. The mobile phases for the dynamic mixing were just refilled. The 10th run from each sequence was taken and the RSD of the six retention times were evaluated for the dynamically and the premixed experiments. In Figure 4, the chromatograms for the six sequences (dynamically and premixed) are overlaid. The precision over several sequences using dynamically-mixed mobile phases was significantly better than the precision for data obtained

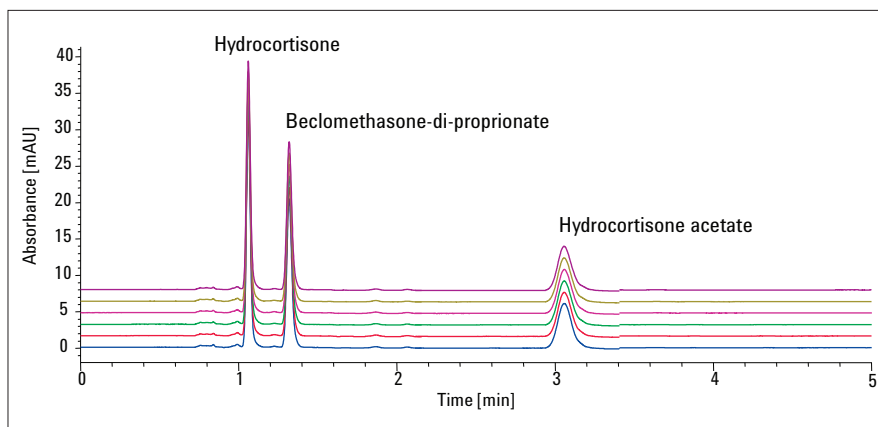


Figure 2
Analysis of glucocorticoids using a dynamically-mixed mobile phases.

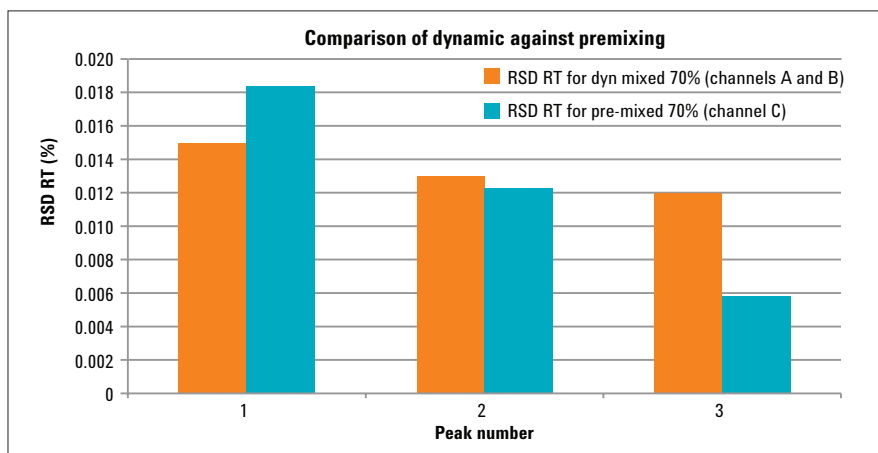


Figure 3
RSD of retention times using dynamically and premixed mobile phases.

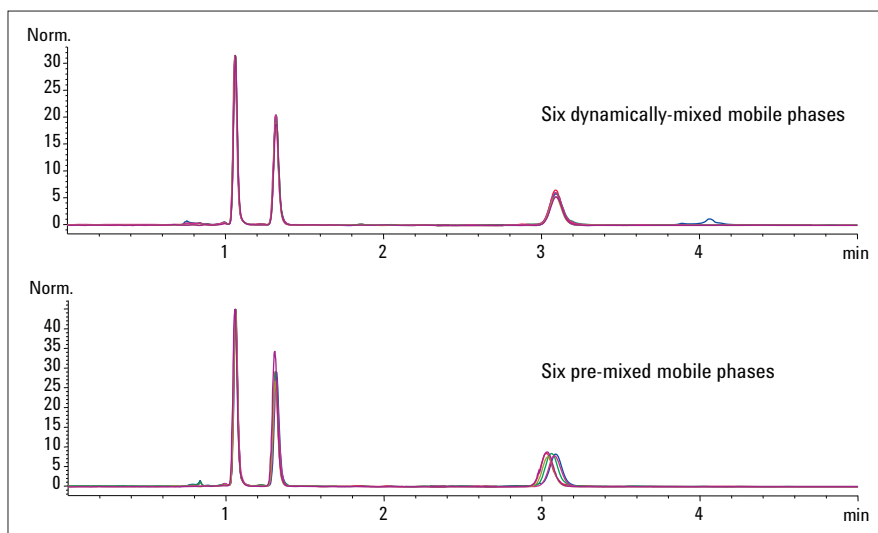


Figure 4
Comparison of chromatograms from six sequences using dynamically and six premixed mobile phases.

using premixed mobile phases, prepared by the same user. The relative standard deviation over six sequences with dynamically-mixed mobile phases was for the last peak 0.16% RSD. The relative standard deviation over six sequences with freshly prepared premixed mobile phases for the last peak was 0.83% RSD. As expected, dynamically mixed mobile phases showed better precision over several sequences even when performed on different days.

Similar RSD values were obtained when different users prepared the premixed phases, see Figure 5.

The relative standard deviation for the three runs is 0.982% RSD. The results from experiments 3 to 5 are combined in Figure 6.

Conclusions

Within a sequence, a premixed phase out of one channel provided slightly better precision than a dynamically-mixed mobile phase out of two channels. In contrast, comparing several sequences over several days, the dynamically-mixed mobile phase provided better precision. This was mainly due to the individual error that occurred when one user prepared fresh mobile phase every day. The day-to-day precision of retention times for dynamically-mixed phase was approximately 0.16% RSD. The day-to-day precision for retention times using premixed mobile phases was approximately 0.89% RSD. Similar results were obtained when different users prepared the premixed mobile phases. In conclusion, using the Agilent Infinity 1290 Quaternary Pump eliminates the need to premix mobile phase. Day-to-day reproducibility of retention times is typically better than achieved with premixed mobile phases.

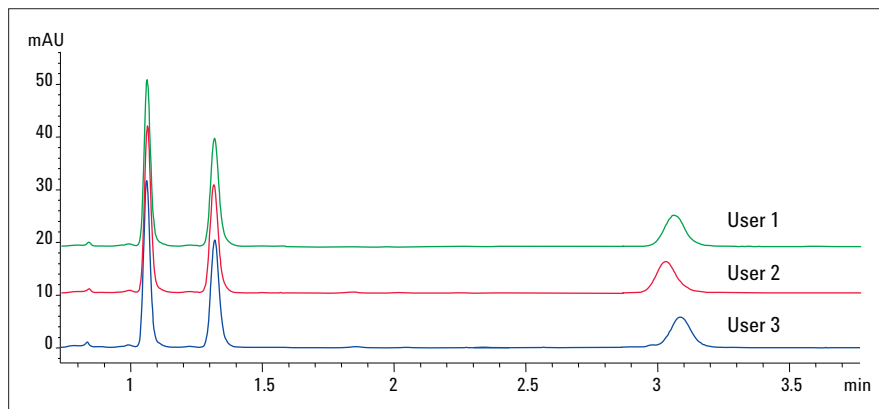


Figure 5
Comparison of chromatograms obtained by premixed phases prepared by three different users.

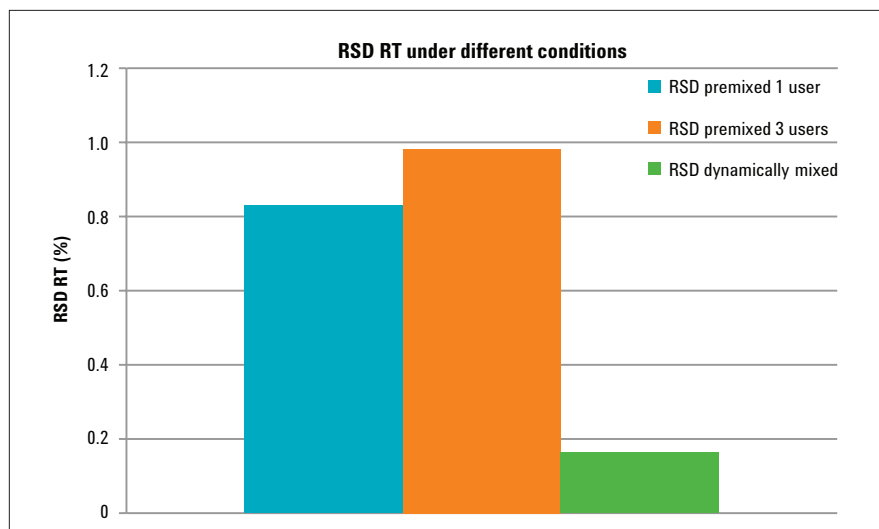


Figure 6
Precision of retention times for differently mixed mobile phases.

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

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