

Tune Your Mixing Volume for Gradient Generation to Achieve Better Detection Limits

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Keywords

Baseline Ripples, Gradient Formation, High-pressure Gradient Proportioning, HPG, Low-pressure Gradient Proportioning, LPG, Solvent Mixing, LOD, LOQ, SpinFlow Mixer

Summary

This Technical Note discusses two technical approaches for gradient formation and examines the reasons for fluctuations in solvent composition. In case of different extinction coefficients of the used solvents these fluctuations become visible in UV detection resulting in characteristic baseline ripples. At the end we will provide assistance in choosing the right mixing system for best measurement results.

Background

Gradient elution is the most popular technique in HPLC. There are two different technical solutions for gradient formation: high-pressure gradient (HPG) proportioning and low-pressure gradient (LPG) proportioning.

In the HPG configuration, two independent pumps deliver the solvents and combine them using a Tee-piece directly following the pump head outlets (on the high-pressure side). Once the streams are combined, they move directly into the mixer. The distinctive flow rates of both pumps result in a well-defined solvent composition. Figure 1 illustrates the typical flow path of HPG pumps. The Tee-piece is in this figure part of the purge unit.

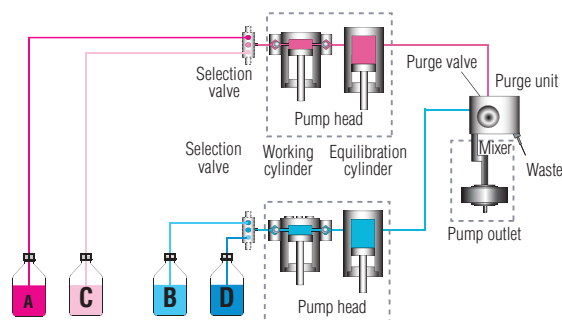


Figure 1. Typical flow path of an HPG pump.



In the LPG configuration, the proportioning of up to four solvents is performed using solenoid valves in front of the pump head inlet (on the low-pressure side). The timed opening and closing of the individual solvent channels in the proportioning valve ensures to achieve the correct mobile phase composition. The mobile phase passes the pump head before it reaches the mixer. The dwell volume of the pump head therefore adds to the gradient delay volume (GDV) of the pump. Figure 2 illustrates the typical flow path of LPG pumps.

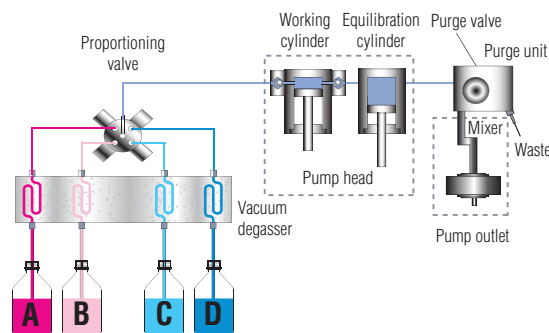


Figure 2. Typical flow path of a LPG pump with built-in degasser unit.

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With both pump variants, fluctuations in solvent composition occur during gradient formation although in different ways. In case of different extinction coefficients of the used solvents these fluctuations become visible in UV detection resulting in characteristic baseline ripples.

In the LPG configuration, the proportioning is performed by opening and closing of valves for each solvent during the aspiration phase of the pump. This forms discrete solvent plugs, as illustrated in Figure 3A. For a perfect homogenization of the resulting solvent mixture, highly efficient longitudinal mixing is essential. In the HPG configuration, the composition is formed by two solvent streams. In this case, the solvent composition can fluctuate due to slight flow instabilities (also seen as pressure pulsation) in each of the two solvent delivery lines, as illustrated in Figure 3B. This is a known phenomenon which occurs with all piston pumps. The lower the pressure pulsation, the more constant the solvent composition. For a perfect homogenization of the solvent mixture, radial and longitudinal mixing is required.

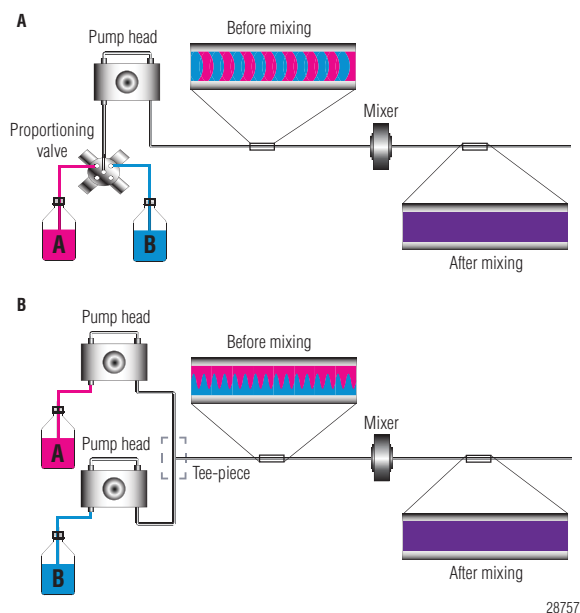


Figure 3. Schematic illustration of the compositional fluctuation in A) low-pressure mixing systems and B) high-pressure mixing systems.

In case of different extinction coefficients of the solvents incomplete mixing can cause distinctive baseline ripples, as illustrated in Figure 4. These baseline ripples can degrade the limit of detection (LOD) for analytes significantly.

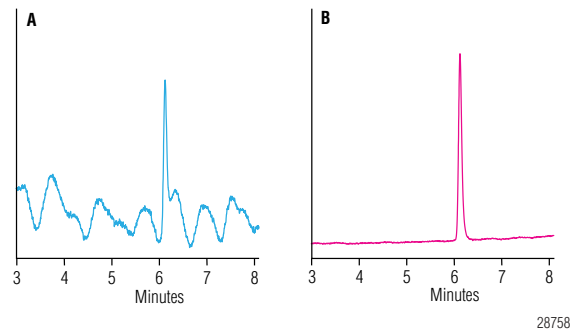


Figure 4. Sections of two chromatograms A) with baseline ripples and B) without baseline ripples.

Solutions

Choose the optimal SpinFlow mixer for your Thermo Scientific™ Dionex™ UltiMate™ 3000 RS/SD system according to the detector type (e.g. UV detection or mass spectrometry) and your application.

The following guidelines provide assistance for selecting the right SpinFlow mixer:

- For fast separations where the mixing ripple does not interfere with the detection (e.g., Thermo Scientific™ Dionex™ Corona™ Charged Aerosol Detectors or mass spectrometers), use one of the smaller SpinFlow mixer with 35 μL or 100 μL mixing volume.
- Use a medium size SpinFlow mixer (200 μL or 400 μL mixing volume) for the best balance between fast separation and low mixing ripple in UV detection.
- For highest sensitivity and when mixing ripples interfere with the detection, e.g. due to the use of UV-absorbing solvents, select a larger SpinFlow mixer with 400 μL or 800 μL mixing volume.
- For UV-absorbing solvent additives that amplify mixing ripples by interaction with the stationary phase (e.g., methods using trifluoroacetic acid as mobile phase component), use the largest SpinFlow mixers with 800 μL or 1550 μL mixing volume in order to achieve the highest sensitivity.

Find ordering information listed in Table 1 below.

Table 1. Available SpinFlow Mixer for use with Thermo Scientific Dionex UltiMate 3000 Pumps.

Description	SD pumps*	RS pumps
Mixer for 35 µL mixing volume	6040.5000	6042.5000
Mixer for 100 µL mixing volume	6040.5100	6042.5100
Mixer for 200 µL mixing volume	6040.5110	
Mixer for 400 µL mixing volume	6040.5310	
Mixer for 800 µL mixing volume	6040.5750	
Mixer for 1550 µL mixing volume	6040.5450	

*except ISO-3100SD

References

- Schmidt, C., Jendreizik, V., Niedner, W., and McLeod, F. Thermo Fisher Scientific. Dionex Poster Note, Impact of Solvent Mixing on Liquid Chromatography Performance, LPN 2880-01, Germering, Germany. <http://www.dionex.com/en-us/webdocs/110899-PO-LC-Solvent-Mixing-06Jul2011-LPN2880-01.pdf>

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