Performance PerSPECtives

Waters® Breeze™ Systems

Starting Out Right

Mobile Phase Preparation Considerations: Regardless of the grade of solvent purchased, bottled reagents contain particulate matter and dissolved gasses that can compromise chromatographic performance or damage HPLC instrument components (e.g.pump seals) or columns. Waters Performance PerSPECtive WPP 217 entitled: "Factors Contributing to Superior Alliance® System Performance: Importance of Proper Solvent Conditioning" addresses how solvent preparation affects Alliance HPLC system performance. This Performance PerSPECtive provides information to help users obtain optimal results from their automated Breeze™ HPLC System.

Step 1: Solvent Filtration Vacuum filtration of all HPLC eluents through a sub-micron (0.20 – 0.45 µm) pore size membrane filter removes potentially damaging particles that can adversely affect column life and Breeze HPLC System performance. Solvent filters are available in a variety of membrane types (Nylon, PVDF, PTFE, etc.) in order to accommodate virtually all solvent compatibility concerns.

Step 2: Degassing: While most chromatographers filter their eluents prior to use, many fail to consider the importance of proper solvent degassing, especially when using an absorbance detector at wavelengths below 220 nm (See "Ghost Peaks and Aerated Sample Solvent" by Y. Egi and A. Ueyanagi in LC-GC Feb. 1998. Vol. 16, No. 2. Pages 112 – 118). **Adequately degassed solvent can improve retention time reproducibility for better peak identification, improve injection volume reproducibility for improved peak quantitation, and improve baseline stability for enhanced detection sensitivity.**

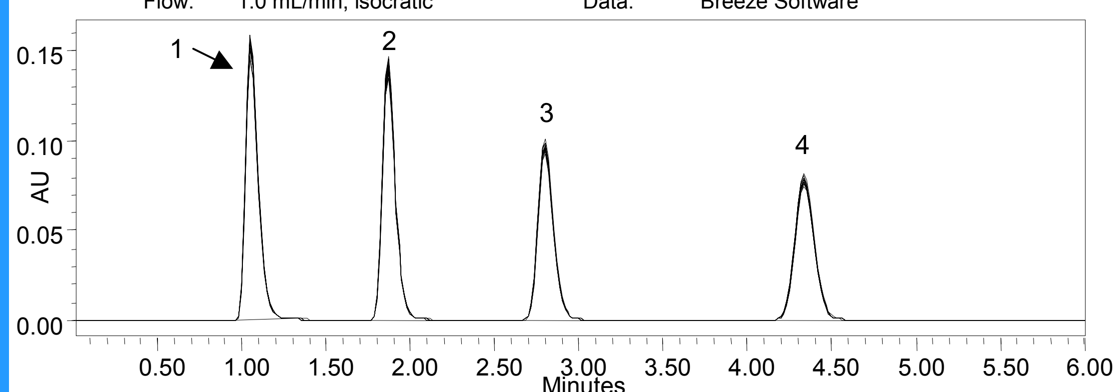
Breeze HPLC System users can effectively degas their HPLC solvents using one of two methods:

A) Off-Line sonication under vacuum
B) Waters In-Line Vacuum Degasser (WAT079700) that removes dissolved gasses from the eluents by diffusion through a membrane exposed to vacuum

The Choice of Solvent Degassing Method is Yours:
A Breeze HPLC System with autosampler was configured to test the effect of solvent degassing on the isocratic separation of the Waters PQ test mix (i.e., 1 = uracil, 2 = methyl paraben, 3 = propyl paraben, and 4 = butyl paraben). The separation conditions are detailed below:

Breeze HPLC System Configuration and Separation

Pump:	1525 Gradient HPLC Pump	Injector:	717 Plus Autosampler
Detector:	2487 at 254 nm or 210 nm	Column:	Symmetry® C_{18} (4.6 x 75 mm) at 35° C
Sample:	20 µl PQ Mix	Eleunts:	A= Water (40%) B= Methanol (60%)
Flow:	1.0 mL/min, isocratic	Data:	Breeze Software



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Retention Time Reproducibility: The Waters 1525 Gradient HPLC Pump uses two high performance pumps to deliver and blend solvents after passing through the pumping system (i.e., “high pressure mixing”). Table 1 confirms that either eluent degassing by Off-Line sonication under vacuum or use of the Waters In-Line Vacuum Degasser results in excellent retention time reproducibility.

Table 1: Effect of Solvent Degassing on Retention Time Reproducibility

Retention Time Reproducibility Using Off-Line Solvent Sonication Under Vacuum (N=6)

	Peak 1	Peak 2	Peak 3	Peak 4
Mean	1.034	1.774	2.616	4.007
Std. Dev.	0.001	0.001	0.001	0.002
% RSD	0.095	0.055	0.046	0.043

Retention Time Reproducibility Using Waters In-Line Vacuum Degasser (N=6)

	Peak 1	Peak 2	Peak 3	Peak 4
Mean	1.033	1.774	2.617	4.011
Std. Dev.	0.000	0.000	0.001	0.001
% RSD	0.018	0.018	0.021	0.019

Area Count Reproducibility: Compressibility of the mobile phase (due to the presence of dissolved gas) can negatively impact the ability of an autosampler to accurately deliver precise injection volumes. Autosampler injection volume precision (and therefore consistent peak area counts) is enhanced by the use of degassed solvents. Data in Table 2 shows that, while excellent area count reproducibility is obtained using the Off-Line solvent sonication under vacuum technique, improved results are obtained when the Waters In-Line Vacuum Degasser is used for solvent degassing purposes.

Table 2: Effect of Solvent Degassing on Area Count Reproducibility

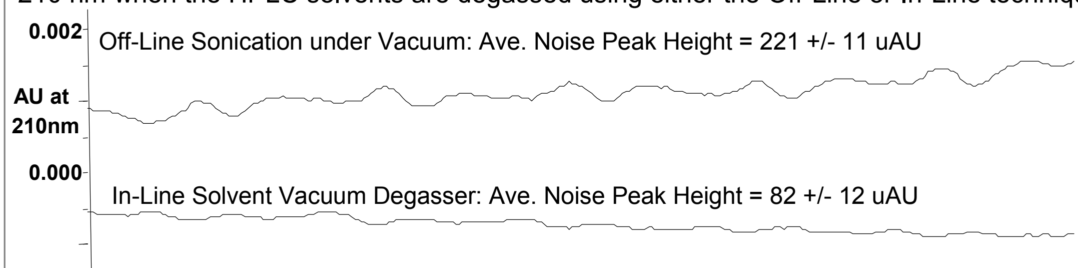
Area Count Reproducibility Using Off-Line Solvent Sonication Under Vacuum (N=6)

	Peak 1	Peak 2	Peak 3	Peak 4
Mean	819584	805535	632378	644649
Std. Dev	1370	1454	1352	1030
% RSD	0.167	0.181	0.214	0.160

Area Count Reproducibility Using Waters In-Line Solvent Vacuum Degasser (N=6)

	Peak 1	Peak 2	Peak 3	Peak 4
Mean	859820	854044	668731	681039
Std. Dev.	2700	896	604	643
% RSD	0.314	0.104	0.090	0.094

Baseline Noise at Low UV: The presence of dissolved gas in the HPLC solvent can adversely effect baseline noise at low UV wavelengths (<220 nm). Figure 2 shows excellent baseline stability at 210 nm when the HPLC solvents are degassed using either the Off-Line or In-Line technique.



Summary:

- Inadequate preparation of Breeze HPLC System solvents can compromise chromatographic performance or damage system components or columns.
- Use of filtered and degassed solvents can improve retention time reproducibility for better peak identification, improve injection volume reproducibility for superior peak quantitation, and improve baseline noise levels for enhanced detection sensitivity.

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