Performance PerSPECtives

Waters® Breeze™ Software
Automated System Performance Monitoring

Question: How can I determine whether my Waters Breeze HPLC system is functioning up to my performance specification expectations?

Answer: Breeze Performance Monitoring Report!

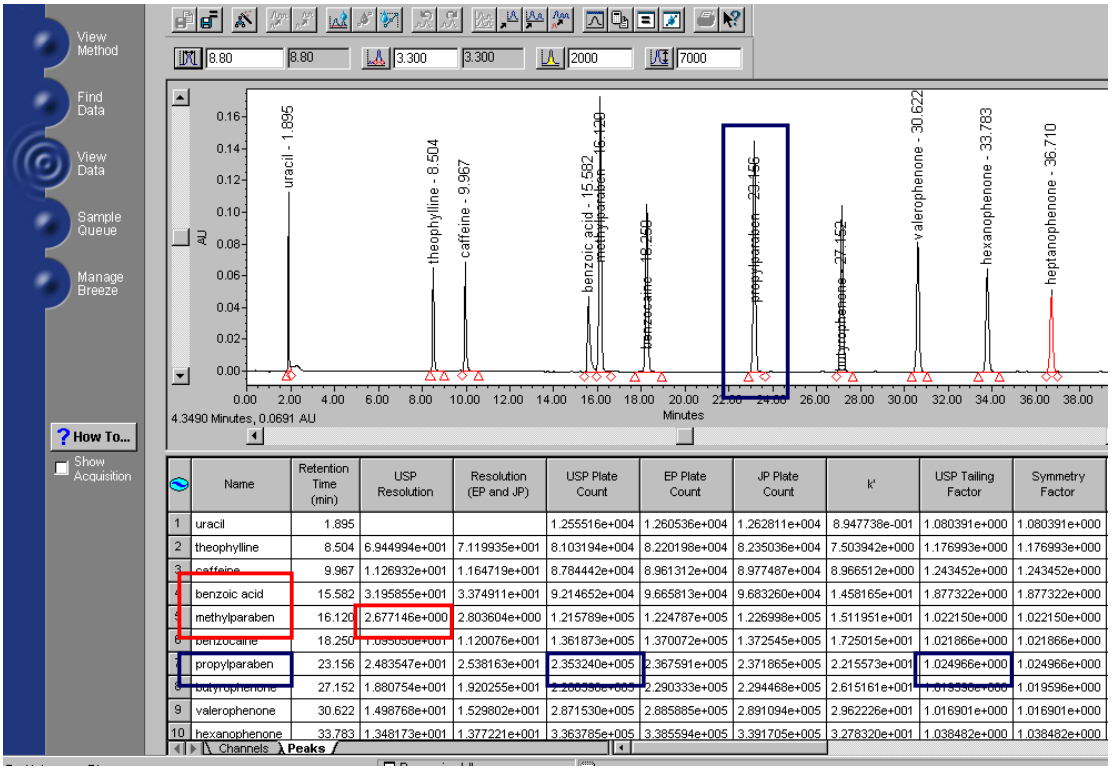
The HPLC column, pump, injector, detector, eluents, and separation conditions used for a HPLC analysis can all adversely affect the quality of the data obtained. Determining when a system "falls out of customer established performance specifications" can be difficult to ascertain. **The Performance Monitoring / Suitability features contained in Waters Breeze HPLC System Software provides useful quantitative information about system performance before, during or after the analyses of valuable samples.**

Automated monitoring of suitability parameters with Waters Breeze HPLC software (e.g., HPLC column plate count, tailing, symmetry factors, resolution, capacity factor (k') and detector noise) provides an objective measurement of system performance that can alert users to problems that could adversely affect results. Figure 1 shows the results from the analysis of a system performance test mix with a propylparaben internal standard (See Waters Performance PerSPECtive WPP34), on a Breeze HPLC system. The Suitability measurements are automatically calculated and reported for each peak in the test mixture. For demonstration purposes, we will establish that the USP resolution value between benzoic acid and methylparaben (15.582 and 16.120 min.) must be greater than or equal to 2.5. (Note: The actual Breeze Software calculated value was 2.67 for this critical pair.) The propylparaben peak (outlined in blue) served as the internal standard making it an ideal choice for monitoring HPLC column efficiency (i.e., Plate Count and Peak Tailing). A USP Plate Count of 23,532 and a USP Tailing Factor of 1.024 was automatically calculated for the propylparaben peak by the Breeze Software. These suitability values provide important information about the separation, which can serve as a benchmark for future reference.

Figure 1: Breeze HPLC System Configuration, Separation Conditions, and Chromatogram with Suitability Peaks Table Review Data Screen.

Pump: Breeze 1525 Gradient HPLC Pump
Injector: 717 Plus Autosampler
Detector: 2487 at 254 nm
Column: Symmetry®C18 (3.9 x 150 mm) 5µ at 35° C
Sample: 20 µL of System Test Mix with propylparaben

Mobile Phase:
A: 0.1% phosphoric acid in HPLC grade Water
B: 0.1% phosphoric acid in Acetonitrile
Gradient: 0 to 80% B in 40.0 minutes.
Flow rate: 1.0 mL/minute
Data: Breeze Software



	Name	Retention Time (min)	USP Resolution	Resolution (EP and JP)	USP Plate Count	EP Plate Count	JP Plate Count	k'	USP Tailing Factor	Symmetry Factor
1	uracil	1.895			1.255516e+004	1.260536e+004	1.262811e+004	8.947738e-001	1.080391e+000	1.080391e+000
2	theophylline	8.504	6.944994e+001	7.119935e+001	8.103194e+004	8.220198e+004	8.235036e+004	7.503942e+000	1.176993e+000	1.176993e+000
3	caffeine	9.967	1.126932e+001	1.164719e+001	8.784442e+004	8.961312e+004	8.977487e+004	8.966512e+000	1.243452e+000	1.243452e+000
4	benzoic acid	15.582	3.195855e+001	3.374911e+001	9.214652e+004	9.665813e+004	9.683260e+004	1.458165e+001	1.877322e+000	1.877322e+000
5	methylparaben	16.120	2.677146e+000	2.803604e+000	1.215789e+005	1.224787e+005	1.226998e+005	1.511951e+001	1.022150e+000	1.022150e+000
6	benzocaine	18.250	7.055056e+001	1.120076e+001	1.361873e+005	1.370072e+005	1.372545e+005	1.725015e+001	1.021866e+000	1.021866e+000
7	propylparaben	23.156	2.483547e+001	2.538163e+001	2.353240e+005	2.367591e+005	2.371865e+005	2.215573e+001	1.024966e+000	1.024966e+000
8	butyrophenone	27.152	1.880754e+001	1.920255e+001	2.280356e+005	2.290333e+005	2.294468e+005	2.615161e+001	1.019596e+000	1.019596e+000
9	valerophenone	30.622	1.498768e+001	1.529802e+001	2.871530e+005	2.885885e+005	2.891094e+005	2.962226e+001	1.016901e+000	1.016901e+000
10	hexanophenone	33.783	1.348173e+001	1.377221e+001	3.363785e+005	3.385594e+005	3.391705e+005	3.278320e+001	1.038482e+000	1.038482e+000

Channels | Peaks

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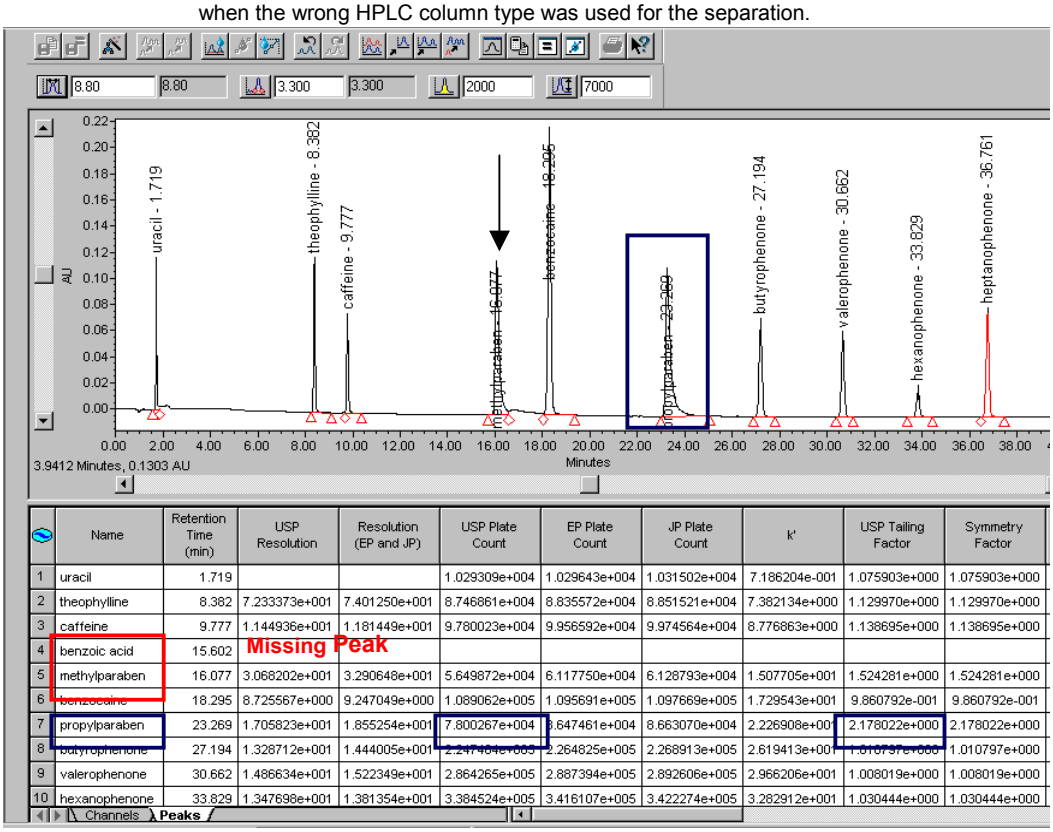
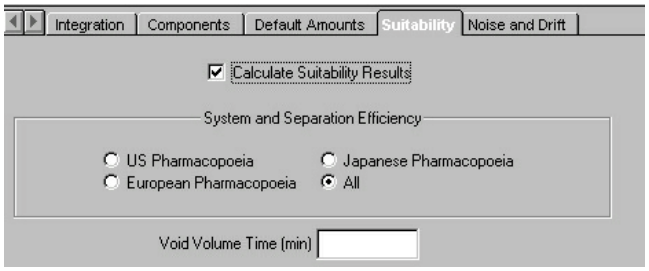
How can Breeze Software alert chemists about column or system issues? Compared to Figure 1, significantly different chromatographic results are shown in the data presented In Figure 2. Visual analysis of the chromatogram reveals that the previously resolved benzoic acid and methylparaben peaks (see Figure 1) are no longer resolved in the separation shown in Figure 2 (See arrow below). The Suitability values that are automatically calculated by the Breeze HPLC Software indicate additional changes in this separation. The USP Plate Count for the propylparaben peak decreased to 7800 from the 23,532 plates observed in Figure 1. In addition, USP Tailing of the propylparaben peak more than doubled (from 1.02 to 2.17). **Clearly, information obtained from Breeze Software's Performance Monitoring / Suitability calculations would indicate a problem with system performance BEFORE additional analyses were performed saving valuable sample, time, and money.** (Note: The observed difference between results obtained in Figures 1 and 2 was due to the discovery that a Symmetry C8, rather than the recommended Symmetry C18 HPLC column, was used in the Figure 2 separation.)

Figure 2: Chromatogram and "Failed" Breeze Software Suitability performance data when the wrong HPLC column type was used for the separation.



	Name	Retention Time (min)	USP Resolution	Resolution (EP and JP)	USP Plate Count	EP Plate Count	JP Plate Count	k'	USP Tailing Factor	Symmetry Factor
1	uracil	1.719			1.029309e+004	1.029643e+004	1.031502e+004	7.186204e-001	1.075903e+000	1.075903e+000
2	theophylline	8.382	7.233373e+001	7.401250e+001	8.746861e+004	8.835572e+004	8.851521e+004	7.382134e+000	1.129970e+000	1.129970e+000
3	caffeine	9.777	1.144936e+001	1.181449e+001	9.780023e+004	9.956592e+004	9.974564e+004	8.776863e+000	1.138695e+000	1.138695e+000
4	benzoic acid	15.602	Missing Peak							
5	methylparaben	16.077	3.068202e+001	3.290648e+001	5.649872e+004	6.117750e+004	6.128793e+004	1.507705e+001	1.524281e+000	1.524281e+000
6	benzocaine	18.295	8.725567e+000	9.247049e+000	1.089062e+005	1.095691e+005	1.097669e+005	1.729543e+001	9.860792e-001	9.860792e-001
7	propylparaben	23.269	1.705823e+001	1.855254e+001	7.800267e+004	8.647461e+004	8.663070e+004	2.226908e+001	2.178022e+000	2.178022e+000
8	butyrophenone	27.194	1.328712e+001	1.444005e+001	2.247464e+005	2.264825e+005	2.268913e+005	2.619413e+001	1.010797e+000	1.010797e+000
9	valerophenone	30.662	1.486634e+001	1.522349e+001	2.864265e+005	2.887394e+005	2.892606e+005	2.966206e+001	1.008019e+000	1.008019e+000
10	hexanophenone	33.829	1.347698e+001	1.381354e+001	3.384524e+005	3.416107e+005	3.422274e+005	3.282912e+001	1.030444e+000	1.030444e+000

Figure 3: The Suitability Selection Tab in Breeze Software is easily accessible. Choose from US, European, Japanese or All Pharmacopoeia calculations. Resolution, column plate counts, k', USP tailing, and symmetry factors are automatically calculated. Users can view the results "on screen" (as indicated in Figures 1 and 2) or by selecting a "printable" suitability report in Breeze Software.



Summary:

- The HPLC column, pump, injector, detector, eluents, and separation conditions used for an HPLC analysis can affect the quality of data obtained.
- Breeze Software's Automated Suitability calculations (e.g., HPLC column plate count, tailing, symmetry factors, resolution, capacity factor (k') and detector noise) can provide valuable information during the development of an acceptable HPLC method.
- Automated System Monitoring / Suitability calculations can also provide a direct and objective measurement of total system performance before, during, and after sample analysis. If the system fails to meet acceptable performance values, work can be halted BEFORE additional samples are run saving valuable sample, time, and money.