

## Guidelines for Optimizing Performance with Ascentis® Express HPLC Columns

High performance columns with small internal volumes (shorter lengths, internal diameters < 3 mm) are being increasingly used for high speed separations, especially with specialty detection systems such as mass spectrometers. These low-volume columns generate peaks having considerably less volume than those eluting from columns of larger dimensions (e.g., 25 cm x 4.6 mm I.D.). The efficiency of separations performed in low-volume columns is highly dependent on the HPLC system having components designed to minimize band spreading. All low-volume columns perform best when used with proper attention to the following factors:

- Detector – Flow cells should be of low-volume design (preferably < 2  $\mu$ L).
- Detector – To properly sense and integrate the often very fast peaks that elute from low-volume columns, the detector response time should be set to the fastest level (~ 0.1 second) and the integration software should sample the detector signal at least 20 points per second.
- Injector – The injection system should be of a low-volume design (e.g., Rheodyne® Model 8125). Auto-samplers will often cause band-spreading with low-volume columns but may be used for convenience with the expectation of some loss in column efficiency.
- Connecting Tubing – The shortest possible lengths of connecting tubing with narrow internal diameters (at most 0.010-inch, 0.25 mm I.D.) should be used to connect the column to the injector and the detector cell. The tubing must have flat ends and should bottom out inside all fittings. Zero-dead-volume fittings should always be used where required.
- Peak Retention – As retention is increased, the volume of a peak increases, decreasing the effects on band spreading caused by components of the instrument.
- Sample Solvent – For isocratic separations, the sample should be dissolved in the mobile phase or in a solvent that is weaker than the mobile phase. For gradient separations, the sample should be dissolved in the initial mobile phase or in a solvent substantially weaker than the final mobile phase.
- Injection Volume – For isocratic separations, the volume of sample injected should be kept as small as possible (typically 2  $\mu$ L or less). Sample volumes are less critical for gradient separations, especially if the sample is dissolved in a weak solvent.