

## 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 µL).
- 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 (0.005 inch) 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 µL or less). Sample volumes are less critical for gradient separations, especially if the sample is dissolved in a weak solvent.