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Accelerated Bioanalytical LC-MS-MS Using Ascentis Express Fused-Core HPLC Columns

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Fast bioanalysis requires the use of short columns packed with high efficiency particles. The use of HPLC columns packed with Fused-Core particles and sub-2 µm porous particles have led the way in these demanding analyses. They significantly enhance resolution and speed by producing either higher efficiency for the same column length or equivalent efficiency with a shorter column length. The use of ultra-high performance liquid chromatography (UHPLC) comes at a price of much higher pressure when sub-2 µm particles are employed. As an attractive alternative, the revolutionary Ascentis Express 2.7 µm Fused-Core silica particle has quickly become accepted because it is equivalent in performance to particles in the sub-2 µm range. With a very narrow particle size distribution, Ascentis Express columns employ conventional 2 µm frits and operate ruggedly at much lower pressures that are within the operating limits of conventional HPLC instruments.

The goal of this work was to demonstrate the ease of converting traditional multi-minute assays on a 50 mm Discovery® C18 column to one-minute assays using 20 mm Ascentis Express columns on an HPLC configured for LC-MS-MS bioanalysis (Agilent 1100/ABI 3200 Q Trap MS-MS). The assay integrity and quality had to be maintained in the conversion. In addition, samples were extracted from plasma based on a published method (1) and analyzed by LC-MS-MS. These extraction conditions are shown in Table 1.

Table 2 shows the compounds, their MS-MS transitions, columns and chromatographic conditions used. The slopes of each gradient were adjusted to accommodate the 20 and 50 mm column lengths. Flow rates differ because the Discovery C18 5 µm column is typically used at 0.5 mL/min in these analyses while a 1 mL/min flow rate would be more likely used with the Ascentis Express to take advantage of the higher efficiency that can be obtained at this higher flow rate.

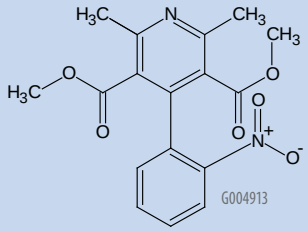
Figure 1a shows the compound mix separated in less than 0.5 minutes on the Ascentis Express HPLC column. Figure 1b shows the separation taking about 2 minutes. Excellent resolution is seen in both bases. Table 3 shows the peak width at half height measurement for both compounds. As expected the Ascentis Express phase shows much narrower peak width, indicating better efficiency for this column, even at the flow rate of 1 mL/min in a 2.1 mm column.

Table 1. Sample Preparation Procedure (1)

1. Prepare 5000 ng/mL sample in rat plasma by taking 5 µL of a mix carbamazepine and dehydronifedipine (1mg/mL each in methanol) and spike 1 mL of rat plasma.
2. Vortex 1 min, take 25 µL of plasma and 200 µL of acetonitrile to crash proteins.
3. Centrifuge at 10, 000 rpm for 1 minute. Remove 100 µL supernatant and add 200 µL of water. Vortex 1 minute.
4. Inject 2 µL.

Table 2. Experimental Conditions

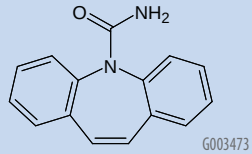
1. Compounds Used in this Study:



Dehydronifedipine

Monoisotopic Mass = 344.100836 Da

MS/MS Transition: 345.1 → 284.2



Carbamazepine

Monoisotopic Mass = 236.094963 Da

MS/MS Transition: 237.18 → 194.2

2. LC-MS-MS Conditions:

column	Ascentis Express C18, 2 cm x 2.1 mm I.D., 2.7 µm particles (53799-U)
	Discovery C18, 5 cm x 2.1 mm I.D., 5 µm particles (577507-U)
mobile phase A:	0.1% acetic acid in 95:5 water:acetonitrile
mobile phase B:	0.1% acetic acid in acetonitrile
flow rate:	see gradient table below
temp.:	40 °C
det.:	ABI 3200 Q Trap MS-MS
injection:	2 µL
sample:	carbamazepine, dehydronifedipine
back pressure:	both less than 3000 psi

3. Gradient Tables:

column: Ascentis Express, 2 cm x 2.1 mm, 2.7 µm
flow rate: 1 mL/min

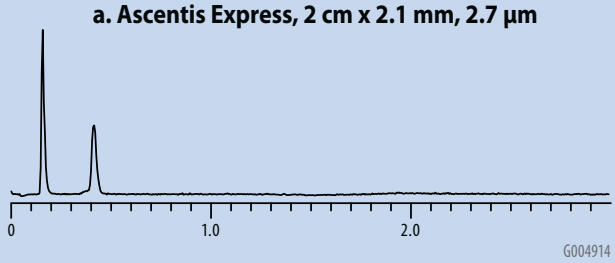
Min	%A	%B
0.00	70	30
0.75	5	95
0.85	5	95
0.90	70	30
1.00	70	30

column: Discovery C18, 5 cm x 2.1 mm, 5 µm
flow rate: 0.5 mL/min

Min	%A	%B
0.0	70	30
1.5	5	95
1.7	5	95
1.8	70	30
2.0	70	30

Figure 1. Chromatographic Comparison of Fused-Core vs. Porous Particles

a. Ascentis Express, 2 cm x 2.1 mm, 2.7 µm



b. Discovery C18, 5 cm x 2.1 mm, 5 µm

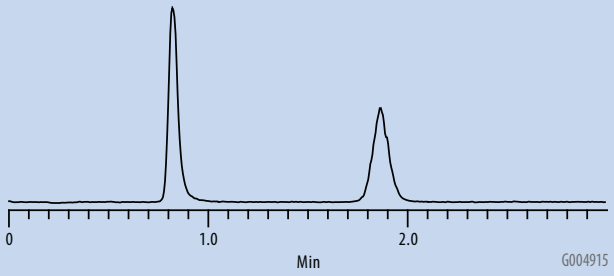
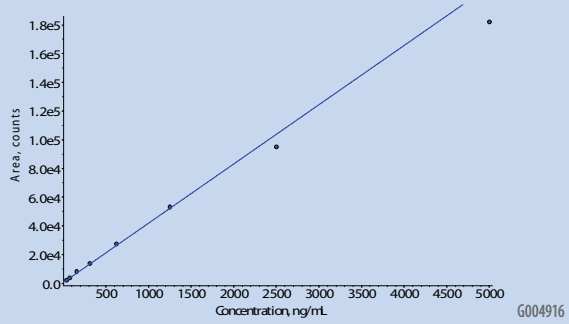


Table 3. Peak Width Comparison

Column	Flow Rate	Peak Width (carbamazepine)	Peak Width (dehydronifedipine)
Ascentis Express C18, 2 cm x 2.1 mm, 2.7 µm	1 mL/min	0.019	0.028
Discovery C18, 5 cm x 2.1 mm, 5 µm	0.5 mL/min	0.049	0.095

Figure 2. Calibration Curves

a. Carbamazepine



b. Dehydronifedipine

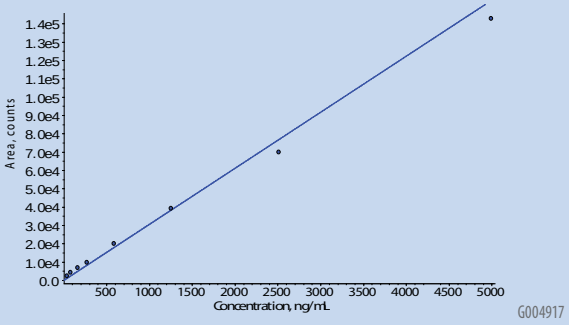


Figure 2 shows a calibration curve for the dehydronifedipine and carbamazepine. The calibration standards were prepared in plasma and extracted using the method in Table 1. Good linearity was obtained over the range studied. Calibration curves are shown using a linear plot with $1/x^2$ weighting.

The demands of increased speed and resolution are vital factors to scientists that work in the area of bioanalytical research. In this work presented here, the benefits of short Fused-Core columns have been demonstrated. The integrity and quality of the analysis was maintained in transferring from totally porous to Fused-Core particles, fast gradients were possible using traditional LC pumps and the Ascentis Express columns have been shown to be rugged and provide good results with greater than 2 years of use (1) in these types of bioanalytical assays.

Reference

1. Recent Advancements in Accelerated Bioanalytical LC/MS Using Fused-Core Columns. Richard L. Beardsley, Ethan R. Badman, Zhenmin Liang, Surendra Bansal ASMS Presentation 2009. Philadelphia, PA.

Did you know…?

Supelco's HybridSPE–PPT sample prep cartridges and 96-well plates for the removal of phospholipids are the perfect complement to Ascentis Express for bioanalytical LC-MS-MS assays. If you have an interest in these products, please complete the survey at ***sigma-aldrich.com/bioanalysis-request***

Featured Products

Particle Size (µm)	I.D. (mm)	Length (cm)	Cat. No.
Ascentis Express C18 Columns			
2.7	2.1	2	**53799-U**
2.7	2.1	3	**53802-U**
2.7	2.1	5	**53822-U**
2.7	2.1	7.5	**53804-U**
2.7	2.1	10	**53823-U**

Related Product

Particle Size (µm)	I.D. (mm)	Length (cm)	Cat. No.
Discovery C18 Column			
2.7	2.1	5	**53799-U**