

# Waters

## A Reversed-Phase HPLC Column for the Separation and Characterization of Peptides Using LC-UV-MS

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*When cytochrome C tryptic digests were characterized by LC-UV-MS, Atlantis™ dC<sub>18</sub> columns were shown to maintain excellent peak shape, resolution, and retention when the “MS-friendly” additive, formic acid, replaced TFA in the mobile phase. Atlantis™ dC<sub>18</sub> columns also offer excellent peak shape, resolution, and retention under high mass loading conditions.*

The separation and characterization of peptides derived from protein tryptic digests by reversed-phase HPLC coupled with MS have become important tools in the study of the proteome. These highly sensitive and selective analyses, however, require MS-compatible methodologies and columns to eliminate ion suppression and the subsequent loss of sensitivity by MS. Most older, UV-based separations of tryptic digests use TFA as a mobile phase additive in order to improve retention of hydrophilic peptides and enhance peak shape. However, TFA promotes ion suppression and lowers signal response when using MS. This makes transferring methods to LC-MS systems more difficult. Waters has developed a high-resolution, MS-compatible reversed-phase HPLC column that does not require TFA for the separation of peptides. Atlantis™ dC<sub>18</sub> columns have superior peak capacity and can handle higher mass loads in complex peptide separations.

### Experimental Conditions

Peptide separations were performed on a Waters Alliance® 2695 HPLC system with a Model 2996 photodiode array (PDA) detector (Waters Corp., Milford, Massachusetts). Mass spectra (data not shown) were acquired using either a Waters ZQ™ 4000 or a Micromass® Q-ToF™ 2 (Micromass, Ltd., United Kingdom). The mobile phase flow rate was split so that 0.2 mL/min was introduced into the MS and 0.55 mL/min was diverted into the 2996 PDA detector.

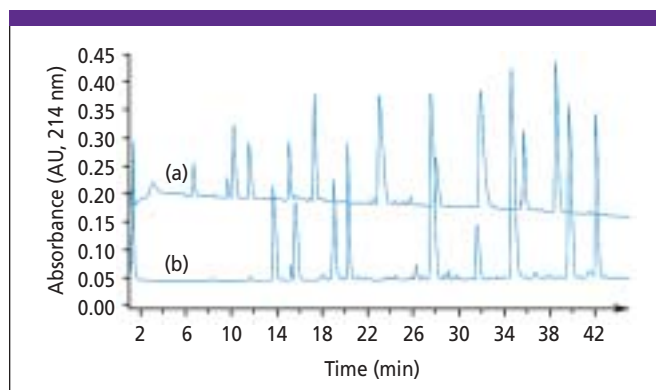
Separations were performed using a Waters Atlantis™ dC<sub>18</sub> column (4.6 mm × 50 mm, 5 μm). The mobile phases consisted of A: 0.1% formic acid in water and B: 0.1% formic acid in acetonitrile (ACN) or A: 0.020% TFA in water and B: 0.016% TFA in ACN. Gradient conditions were 0% B to 40% B in 45 min with a total flow rate of 0.75 mL/min.

### Results

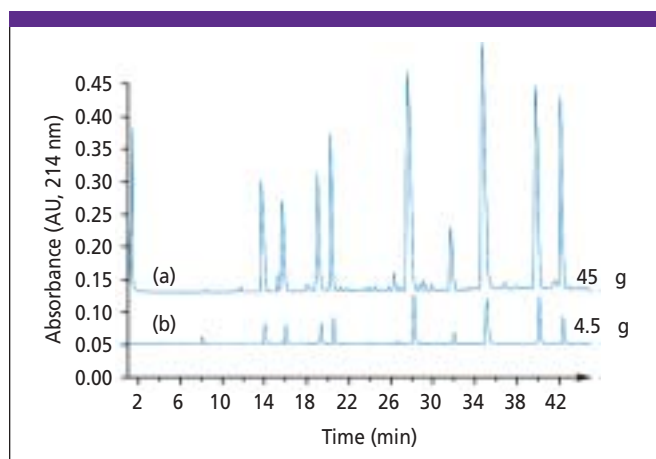
In Figure 1, separations of cytochrome C tryptic digests using mobile phases that contain 0.02% TFA or 0.1% formic acid are compared. The results show that the Atlantis™ dC<sub>18</sub> column maintains excellent peak shape, peak capacity, and retention when using the mobile phase additive, formic acid. In Figure 2, separations of 45 and 4.5 μg of the same tryptic digest are compared. The results demonstrate that an increase in mass loading by a factor of 10 does not affect peak shape, peak capacity, or resolution.

### Conclusion

These results demonstrate that Atlantis™ dC<sub>18</sub> columns retain and separate peptides using “MS-friendly” mobile phase additives such as formic acid without compromising peak shape, peak capacity, or resolution. In addition, Atlantis™ dC<sub>18</sub> columns also maintain outstanding peak shape and peak capacity under increasing mass loads. Atlantis™ dC<sub>18</sub> columns are ideal for analyzing, characterizing, and purifying tryptic digests with LC-UV and/or LC-MS-based separation methods.



**Figure 1:** Separations of 9.0 μg of tryptic cytochrome C peptides with an Atlantis™ dC<sub>18</sub> column when using formic acid (a) instead of TFA (b) in the mobile phases.



**Figure 2:** Separations of 45 μg (a) and 4.5 μg (b) of tryptic cytochrome C peptides on an Atlantis™ dC<sub>18</sub> column using mobile phases consisting of A: 0.020% TFA in water and B: 0.016% TFA in ACN.

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