

FLARE C18 Mixed-Mode Column: Tricyclic Antidepressants (TCAs)

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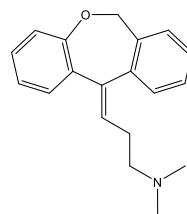
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Introduction

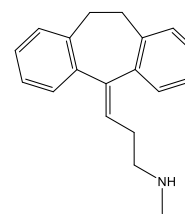
Tricyclic antidepressants (TCAs) have three rings and an amine in their structures, and are commonly used for the clinical treatment of depression.[1] Because of their basicity, they are better retained and separated in HPLC at elevated pH, which places them in their neutral state.[2, 3]

The FLARE C18 Mixed-Mode (MM), core-shell HPLC column from Diamond Analytics is made of carbon, polymer, and nanodiamond, which are stable at high pH and elevated temperature.[4-6] These material attributes of the column facilitate the retention and separation of basic analytes.[2, 4]

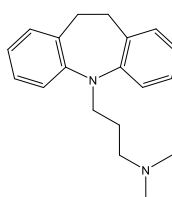
The FLARE C18 Mixed-Mode (MM) column contains amines in its stationary phase that are



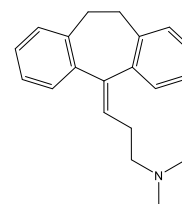
1. Doxepin



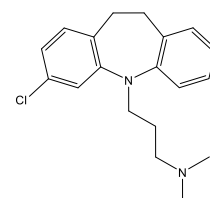
2. Nortriptyline



3. Imipramine



4. Amitriptyline



5. Clomipramine

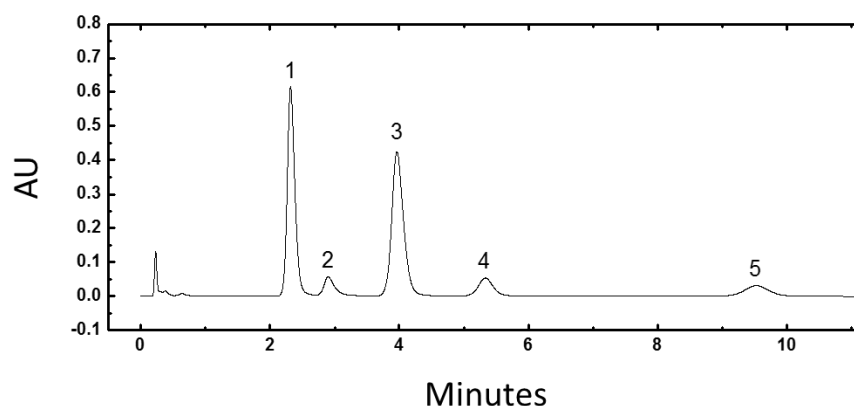


Figure 1. Chromatogram of (1) doxepin, (2) nortriptyline, (3) imipramine, (4) amitriptyline, and (5) clomipramine separated at pH 12.

deprotonated (neutral) at elevated pH, which allows basic analytes to be retained through hydrophobic interactions. At lower pH, the stationary phase is protonated (positively charged) and retains anionic compounds.[3] In this application note, TCAs are separated with the FLARE C18 MM column at pH 12.

Experimental

HPLC system: Waters HPLC 1525 Binary HPLC pump with dual wavelength UV detector (Model No. 2487)

Column: Diamond Analytics FLARE Mixed-mode column, 4.6 x 33 mm (cat # FL36-46033)

Injection volume: 5.0 µL

Elution conditions: Isocratic

Detection: UV at 254 nm

Flow rate: 1.5 mL/min

Temperature: 35 °C

Mobile phase: 10 mM phosphate buffer pH 12, ACN (70:30)

References

[1] Mercolini L., Mandrioli R., Finizio G., Boncompagni G., Raggi M. A., Simultaneous HPLC Determination of 14 Tricyclic Antidepressants and Metabolites in Human Plasma. *Journal of Separation Science*. 2010; 33:23-30.

[2] Kirkland J. J., Henderson J. W., De Stefano J. J., van Straten M. A., Claessens H. A., Stability of Silica-based, Endcapped Columns with pH 7 and 11 Mobile Phases for Reversed-Phase High-Performance Liquid Chromatography. *Journal of Chromatography A*. 1997; 762:97-112.

[3] Hung C-H., Kazarian A. A., Dadson A. E., Paull B., Nesterenko P., Linford M. R., Guidelines for Understanding the Retention Mechanism of the Diamond Analytics Flare Mixed-Mode Column.

[4] Hung C-H., Wiest L. A., Singh B., Diwan A., Valentim M. J. C., Christensen J. M., et al. Improved Efficiency of Reversed-Phase Carbon/Nanodiamond/Polymer Core-Shell Particles for HPLC Using Carbonized Poly(divinylbenzene) Microspheres as the Core Materials. *Journal of Separation Science*. 2013; 36(24):3821-9.

[5] Saini G., Jensen D. S., Wiest L. A., Vail M. A., Dadson A. E., Lee M. L., et al. Core-Shell Diamond as a Support for Solid-Phase Extraction and High-Performance Liquid Chromatography. *Analytical Chemistry*, 2010; 82(11):4448-56.

[6] Wiest L. A., Jensen D. S., Hung C-H., Olsen R. E., Davis R. C., Vail M. A., et al. Pellicular Particles with Spherical Carbon Cores and Porous Nanodiamond/Polymer Shells for Reversed-Phase HPLC. *Analytical Chemistry*. 2011; 83(14):5488-501.