Analysis of Tricyclic Antidepressants using Agilent Poroshell HPH C18

Application Note

Small Molecule Pharmaceuticals

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Introduction

Tricyclic antidepressants (TCAs) were first discovered in the early 1950s, and brought to market later that decade. Their chemical structure contains three rings of atoms. Tetracyclic antidepressants, which contain four rings of atoms, are a closely related group of antidepressant compounds.

Cyclic antidepressants were among the first antidepressants developed. While often replaced by newer drugs that cause fewer side effects, they are still a good option for many people. Cyclic antidepressants work by making more neurotransmitters such as serotonin and norepinephrine available to the brain. This helps brain cells communicate better, which in turn affects mood. Tertiary amine TCAs, such as doxepin and amitriptyline, are more potent inhibitors of serotonin reuptake than secondary amine TCAs, such as nortriptyline and desipramine [1,2,3].

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Tricyclic amines are also important as chromatographic probes. Figure 1 shows that these compounds possess high pKa values, from 9.2 to 9.6. At pH 7.0, silica silanols are in their ionized form, and basic probes such as TCAs are highly protonated. At neutral pH, TCAs undergo ion-exchange interactions with silica silanols if they are exposed to them. More peak tailing can be observed if more silanols are exposed. The tailing factor of amitriptyline is used as a measure of silanol activity [4].

In this work, we compared the performance of Agilent Poroshell HPH C18 columns and columns from another vendor for the analysis of tricyclic antidepressants.

Experimental

An Agilent 1260 Infinity LC was used, consisting of:

- Agilent 1260 Infinity Binary Pump SL, capable of delivering up to 600 bar (G1312B)
- Agilent 1260 Infinity Thermostatted Column Compartment (G1316C)
- Agilent 1260 Infinity High Performance Autosampler SL Plus (G1376C)
- Agilent 1260 Infinity Diode Array Detector (G4212A) equipped with a 10 mm path length, 1 µL flow cell (p/n G4212-60008)

The following columns were used in this study:

- Agilent Poroshell HPH C18, 3 × 100 mm, 2.7 µm (p/n 695975-502)
- Agilent Poroshell HPH C18, 3 × 100 mm, 4 µm (p/n 695970-502)
- New, high pH stable superficially porous columns, 3 × 100 mm, 2.6 and 5 µm, from another vendor

Agilent ChemStation, version C.1.05, was used to control the instrument and process the data.

The compounds examined included uracil (50 µg/mL), doxepin hydrochloride (250 µg/mL), nortriptyline hydrochloride (500 µg/mL), amitriptyline hydrochloride (250 µg/mL), and trimipramine maleate (500 µg/mL). All were bought from Sigma-Aldrich, Corp. and prepared in water. Figure 1 shows the structures and details. Sodium phosphate dibasic dihydrate and sodium phosphate monobasic dihydrate, also supplied by Sigma-Aldrich, were prepared separately at 20 mM, and titrated to pH 7.0. Acetonitrile was bought from

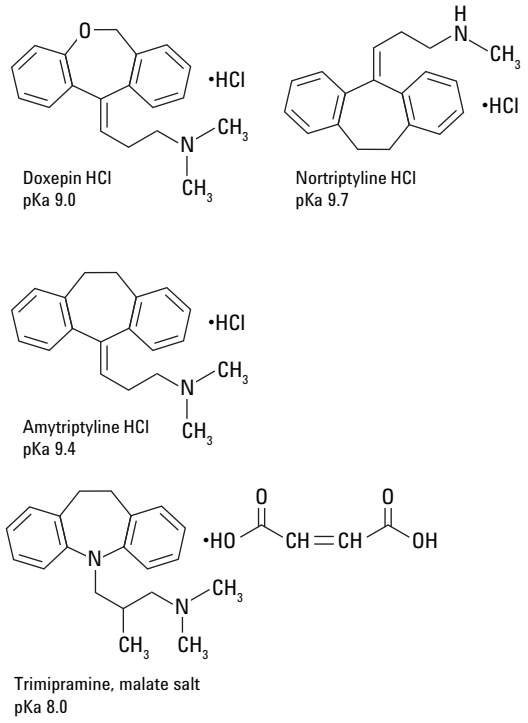


Figure 1. Tricyclic amine structures.

from Honeywell (Burdick and Jackson). Water was 0.2 µm filtered 18 MΩ from a Milli-Q system (Millipore). A mobile phase consisting of premixed 60% acetonitrile and 40% 20 mM sodium phosphate was used to evaluate the columns. Columns were heated to 25 °C, and equilibrated at 1 mL/min for 10 minutes before testing.

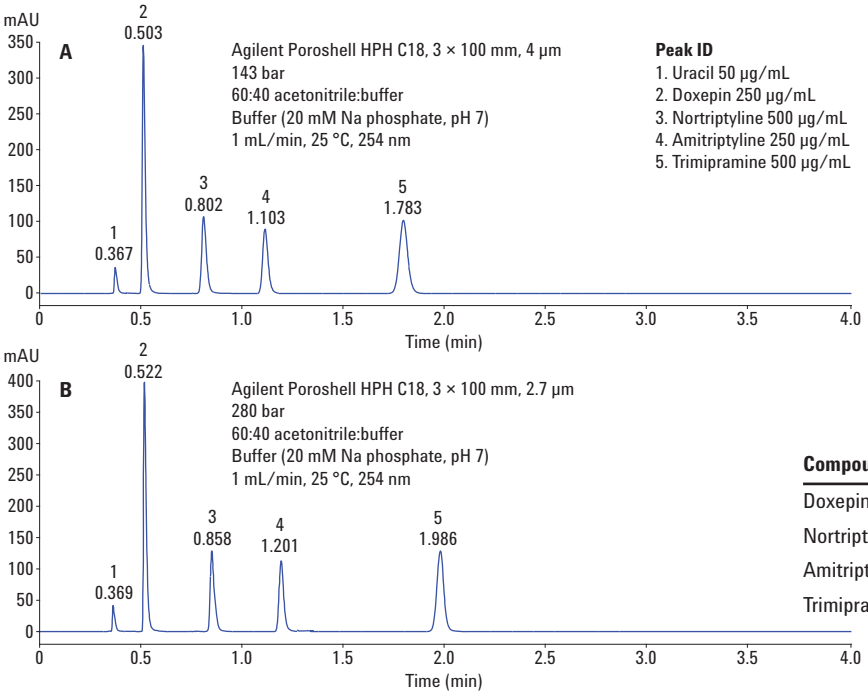
Results and Discussion

As listed in Figure 1, the pKa values of the TCAs were between 8 and 10, clearly classifying these compounds as bases. Samples containing ionizable compounds such as bases are usually best separated with mobile phases of pH 3 or below. At this pH, potential column silanol interactions are minimized. At pH 5.5 and above residual silanols on the column surface can exist as O-. These negatively charged surface silanols can have ion-exchange interactions with positively charged basic compounds. This is the most common type of peak tailing. However, many separations are performed more optimally at mid (4-8) and higher pH (>9) because sample components are not stable at low pH. Basic compounds are protonated at low pH and elute too quickly, or require band spacings that are not found at low pH.

Most chromatographers are aware that conventional silica-based columns are usually not recommended for operation at higher pH (for example, pH >8), because of potential dissolution of the silica support with accompanying column failure. Problems can arise in separations that are designed for operation in the pH 6-8 range. Silica support solubility can be significant in this pH range, so that separation reproducibility and column lifetime is less than expected. It has been documented that degradation of silica-based columns at intermediate (and higher) pH is largely a function of dissolution of the silica support, rather than a loss of bonded organic substrate due to hydrolysis [5]. It has also been found that silica support solubility in the pH 6-8 range is greatly increased in the presence of phosphate buffers, particularly at higher temperatures and higher buffer concentrations [6].

The Agilent Poroshell HPH C18 column has been shown to be stable in ammonium bicarbonate and phosphate buffer and has become a more popular column choice for applications at mid to high pH. Applications have been published using these columns in phosphate/borate buffer, triethanolamine /phosphate buffer, and phosphate, ammonium hydroxide, and ammonium bicarbonate buffer. With these columns, chromatographers can obtain the benefit of high efficiency of superficially porous columns together with long lifetime at elevated pH or temperature [7,8,9].

Figure 2 shows chromatograms from a Poroshell HPH C18, 4 µm column (A) and a 2.7 µm column (B), in a 3 × 100 mm format. These dimensions are becoming more popular as it uses less solvent than the older 4.6 mm format. Using less solvent means lower operating costs in purchasing and disposal of solvents. Columns of 3 × 100 mm are also frequently used with mass spectrometry. As shown in the summary table and the chromatogram, all peak shapes are good with minimal tailing. Peak efficiency, as measured by the trimipramine peak on the 2.7 µm column, is 15,679, and 8,616 on the 4 µm column. Both columns are easily operable on a 400 bar instrument, with 280 bar pressure on the 2.7 µm column, and 143 bar on the 4 µm column. The retention time on the 4 µm column is slightly less than on the 2.7 µm column, but the elution order and selectivity of the compounds is similar on both, indicating the scalability of the columns.



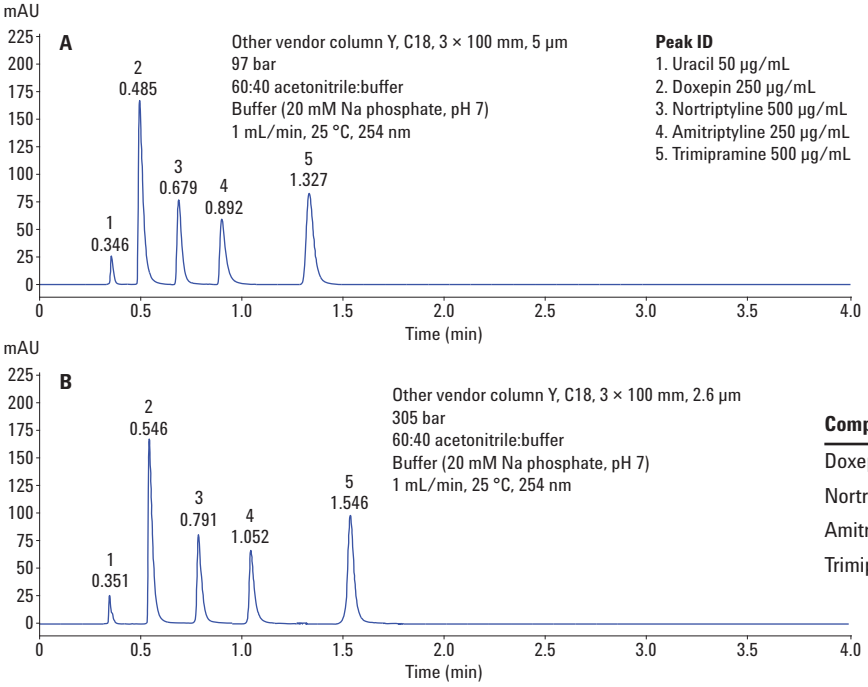
Compound	Tailing Agilent Poroshell HPH C18, 4 µm	Efficiency Agilent Poroshell HPH C18, 4 µm	Tailing Agilent Poroshell HPH C18, 2.7 µm	Efficiency Agilent Poroshell HPH C18, 2.7 µm
Doxepin	1.68	6,125	1.96	8,162
Nortriptyline	1.36	5,701	1.52	9,557
Amitriptyline	1.25	7,883	1.31	14,084
Trimipramine	1.07	8,616	1.05	15,679

Figure 2. Tricyclic antidepressant mix in phosphate buffer at pH 7 on Agilent Poroshell HPH C18 columns.

Figure 3 shows chromatograms from two high pH stable superficially porous columns from another vendor. The chromatograms show that the peak shape of the tricyclic amines was inferior to that delivered by the Poroshell HPH C18 columns in Figure 2. Because of this poor peak shape with bases, the efficiency is lower on the other vendor 2.6 µm column than on the Poroshell HPH C18, 2.7 µm column. While the pressure of the other vendor 5 µm is lower than the Poroshell HPH 4 µm column, the difference is small.

Conclusions

Agilent Poroshell HPH C18 columns have been shown to possess good durability in ammonium bicarbonate and phosphate buffers at high and mid pH making them a good choice for chromatographers. In this application note, the excellent peak shape of Poroshell HPH C18 is compared with materials from another vendor. Since peak shape influences column efficiency, the importance of good peak shape, especially when analyzing basic pharmaceutical compounds, should not be underestimated.



Compound	Tailing Vendor Y, 2.6 µm	Efficiency Vendor Y, 2.6 µm	Tailing Vendor Y, 5 µm	Efficiency Vendor Y, 5 µm
Doxepin	3.22	3,099	2.97	2,522
Nortriptyline	2.07	6,710	1.96	4,056
Amitriptyline	2.14	9,163	2.41	4,684
Trimipramine	1.29	13,657	1.6	5,968

Figure 3. Tricyclic antidepressant mix in phosphate buffer at pH 7 on superficially porous columns from another vendor.

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Printed in the USA
December 7, 2015
5991-6512EN

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