Separation of Beta Blockers at Low and High pH Using Agilent Poroshell HPH C18

Application Note

Small Molecule Pharmaceuticals

Author

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Introduction

Beta blockers, or beta-adrenergic blocking agents, are a class of drugs used to treat hypertension and to manage cardiac arrhythmias. As beta adrenergic receptor antagonists, they diminish the effects of epinephrine (adrenaline) and other stress hormones by blocking their binding to beta receptors on nerve endings. The first beta blocker was synthesized in 1958 by Eli Lilly Laboratories, but in 1962 the first clinically significant beta blockers, propranolol and pronethalol, were developed and used for the treatment of angina pectoris.

Beta blockers block the action of adrenaline and noradrenaline, in particular on β-adrenergic receptors, part of the sympathetic nervous system that mediates the fight-or-flight response. Three types of beta receptors are known, designated β_1, β_2, and β_3 receptors. β_1-Adrenergic receptors are located mainly in the heart and kidney; β_2-adrenergic receptors are mainly in the lungs, gastrointestinal tract, liver, uterus, vascular smooth muscle, and skeletal muscle. β_3-adrenergic receptors are found in fat cells.

There are a large number of beta blockers, and they differ in the type of beta receptors they block and, therefore, their effects. Nonselective beta blockers, such as propranolol, block β_1 and β_2 receptors, and affect the heart, blood vessels, and air passages. Selective beta blockers, such as metoprolol, primarily block β_1 receptors and, therefore, mostly affect the heart and do not affect air passages. Some beta blockers, such as pindolol, mimic the effects of epinephrine and norepinephrine and can cause an increase in blood pressure and heart rate [1,2].

The use of high pH mobile phase for the analysis of basic compounds such as beta blockers can be routine using Agilent Poroshell HPH C18 columns with 2.7 µm or 4 µm particles. These columns enable exploration of a wider pH range for method development with superficially porous particles. Columns with such particles are being increasingly adopted due to their high efficiency and speed.

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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, 4.6 × 100 mm, 2.7 µm (p/n 695975-702)
- Agilent Poroshell HPH C18, 4.6 × 100 mm, 4 µm (p/n 695970-702)

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

The compounds examined included uracil and a series of beta blockers made up in 50:50 water:acetonitrile at 1 mg/mL, then mixed in equal parts to make a solution of approximately 0.143 µg/mL atenolol, pindolol, acebutalol, metoprolol, oxprenolol, alprenolol, and propranolol. Figure 1 shows the structures and details. Ammonium formate and formic acid were prepared at 10 mM, and used to prepare a low pH buffer at pH 3. Ammonium formate was purchased from Sigma-Aldrich, Corp., and double distilled formic acid was bought from GFS. Ammonium bicarbonate and ammonium hydroxide were used to prepare a pH 10 buffer; both were supplied by Sigma-Aldrich.

Columns were heated to 25 °C, and equilibrated at 1 mL/min for 10 minutes prior to testing.

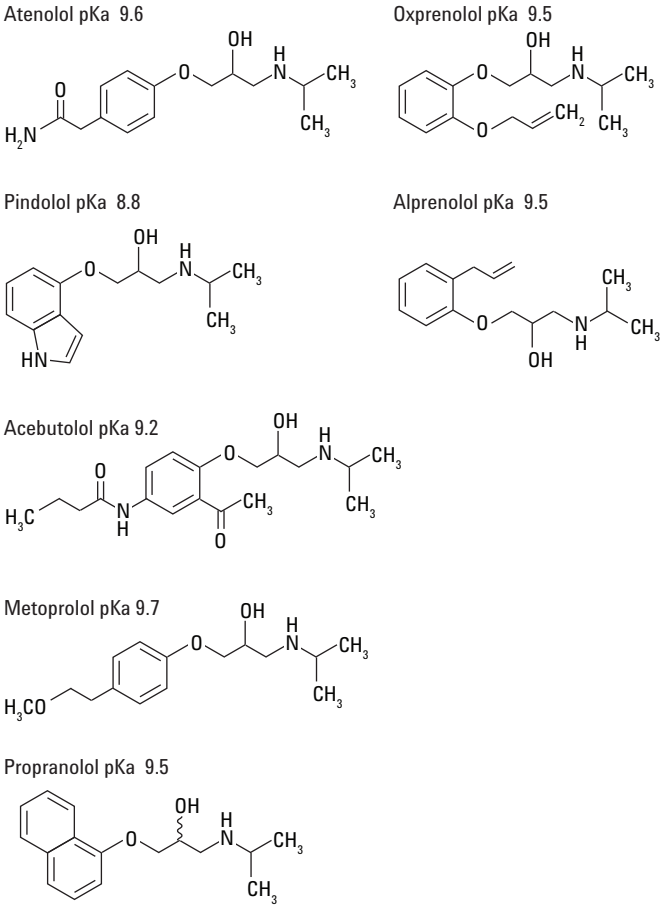

Figure 1. β-blocker structures.

Results and Discussion

Figure 1 illustrates the diverse structures of the beta blockers used. This diversity allows a wide variety of effects on various parts of the body. Beta blockers are basic compounds containing a secondary amino group in their structure.

Figure 2 shows chromatograms that can result from initial method development scanning. As shown, while an adequate separation is found, the peak shape of the later eluting peaks are not very good. As the buffer concentration in the mobile phase is reduced, the baseline decreases, and the peaks get slightly wider and shorter. Therefore, quantitation becomes more difficult. This could be corrected by putting the buffer or mobile phase modifier in the acetonitrile as well. We see identical chromatographic behavior on both the 2.7 µm Poroshell HPH C18 (A) and 4 µm Poroshell HPH C18 (B), indicating that the chromatography is transferrable.

Figure 3 shows the chromatography of the mixture at pH 10.5. In this alkaline mobile phase, the separation of the fully protonated basic compounds is better than that produced using the acidic mobile phase in Figure 2. In all cases, peak shape is superior, tailing is reduced, peaks are taller, and retention time is greater than in Figure 2. Peak pairs 6 and 7 are fully resolved under these conditions. Because of the improved chromatography, we are able to quantitate at lower levels. We see identical chromatographic behavior on both the 2.7 µm Poroshell HPH C18 (A) and 4 µm Poroshell HPH C18 (B), indicating the chromatography is transferrable.

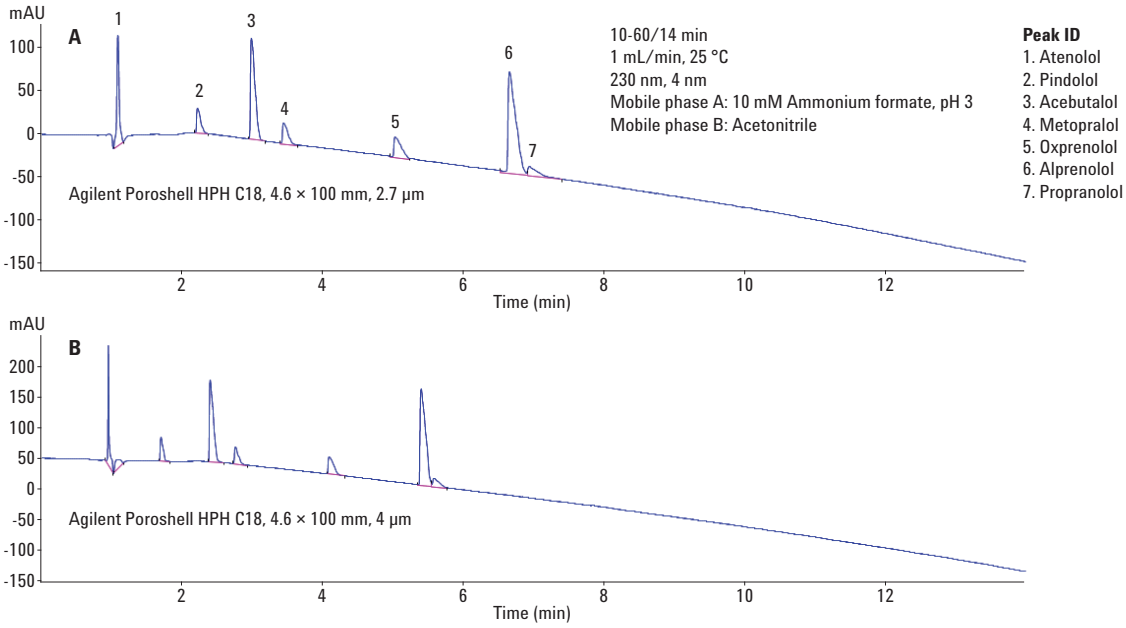

Figure 2. Low pH separation of β-blocker mix on an Agilent Poroshell HPH C18 column.

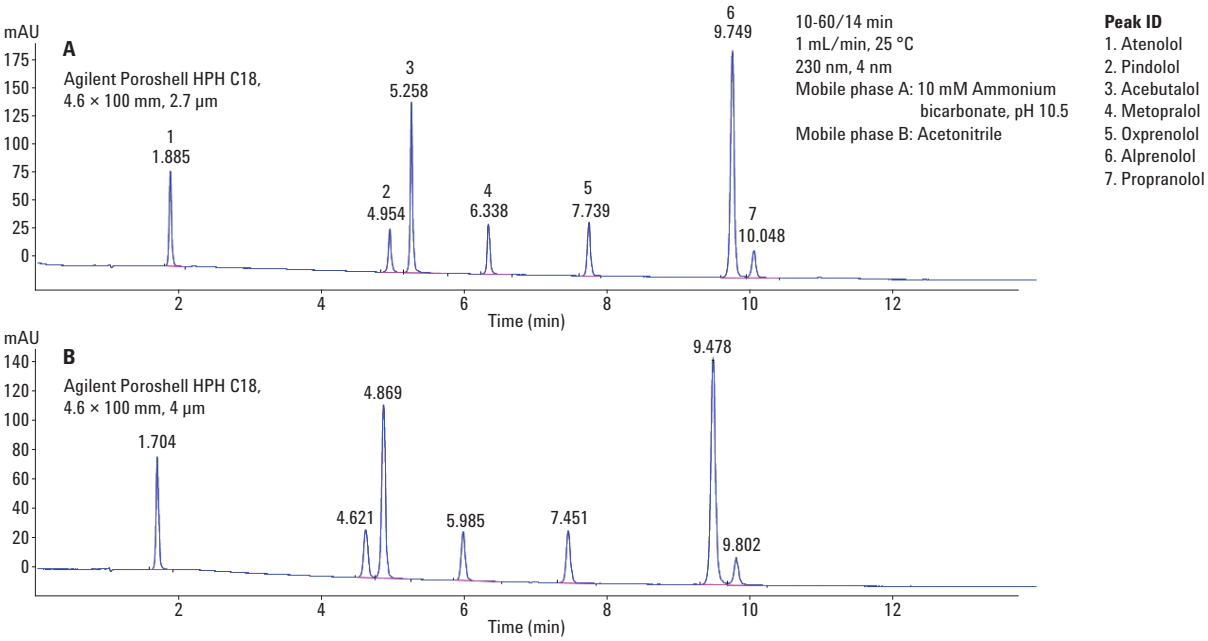

Figure 3. High pH separation of β-blocker mix on an Agilent Poroshell HPH C18 column.

Conclusions

The use of high pH mobile phase for the analysis of basic compounds can be routinely carried out using Agilent Poroshell HPH C18 columns with either 2.7 µm or 4 µm particle sizes. In both cases, high pH yields sharper and better retained peaks. In some cases better resolution is also attained. Control of pH can be used to adjust selectivity, without sacrificing column lifetime, using new high pH stable columns such as Poroshell HPH C18. Similar selectivity allows methods to be transferred between 2.7 µm and 4 µm columns as required by pressure considerations.

Using these columns, chromatographers can now explore a wider pH range in method development using superficially porous particle technology, which is being increasingly adopted due to its high efficiency and speed [3].

References

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3. Long, W. J.; Mack, A. E.; Wang, X.; Barber, W. E. Selectivity and Sensitivity Improvements for Ionizable Analytes Using High-pH-Stable Superficially Porous Particles. *LCGC* **2015**, *33* (4).

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