Fast Screening Methods for Beta Blockers by HPLC with Agilent Poroshell 120 Columns

Application Note

Pharma, Biopharma, and Clinical

Author

William Long

Agilent Technologies, Inc.

Introduction

Beta blockers, or beta-adrenergic blocking agents, are a class of drugs used to treat hypertension and to manage cardiac arrhythmias after a heart attack. As beta adrenergic receptor antagonists, they diminish the effects of epinephrine (adrenaline) and other stress hormones. 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 epinephrine (adrenaline) and norepinephrine (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 in the kidney, β2-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 [1,2].

Beta blockers can be abused in sports involving little physical activity, such as archery, to reduce cardiac contraction, heart rate, and coronary blood flow. They have, therefore, been included in the list of forbidden substances by the International Olympic Committee [3].

Selectivity is the most powerful tool to optimize separations in HPLC. This parameter is changed by using different bonded phases, including C18, C8, polar embedded, and phenyl bonded phases, or by changing the mobile phase. In this work, Agilent Poroshell 120 columns and the Agilent 1260 Infinity Method Development Solutions were used to quickly evaluate method development choices for the analysis of beta blockers. The short column length and high efficiency provided short analysis times and rapid equilibration leading to fast investigations of selectivity [4].

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Experimental

The Agilent 1200 Infinity Series LC Multi-Method Solution was used. This system consisted of:

- 1260 Infinity Binary Pump (G1312B)
- 1290 Infinity Thermostatted Column Compartment (G1316C)
- 1260 Infinity High Performance Autosampler (G1367E)
- 1290 Infinity Diode-Array Detector (G4212A), equipped with 10 mm MaxiLight cartridge flow cell
- G6140 Single Quadrupole Mass Spectrometer.

The Agilent 1200 Infinity Series LC Multi-Method Solution is a highly flexible system that can be used for up to 4 (100 mm) columns. In addition, the Agilent ChemStation Method Scouting Wizard automates the setup of methods and sequences to screen the available combinations of columns, solvents, predefined gradients, and temperatures. In this work, 4 Agilent Poroshell 120 columns were used:

- Agilent Poroshell 120 StableBond SB-C18, 2.1 × 100 mm, 2.7 µm (p/n 685775-902)
- Agilent Poroshell 120 EC-C18, 2.1 × 100 mm, 2.7 µm (p/n 695775-902)
- Agilent Poroshell 120 Bonus-RP, 2.1 × 100 mm, 2.7 µm (p/n 685775-901)
- Agilent Poroshell 120 Phenyl-Hexyl, 2.1 × 100 mm, 2.7 µm (p/n 695775-912)

The TCC was fitted with a 6 position/4 port selection valve. This is a new Quick Change Valve mounted on a slide-out rail to make plumbing and maintenance more convenient. Port 1 was connected to a StableBond C18 column, and port 2 was connected to an EC-C18 column. Port 3 was connected to a Bonus-RP column, port 4 to a Phenyl-Hexyl column, and port 6 to a bypass connecting capillary.

The solvent passing into each column was heated using 1 of 4 individual low-dispersion heat exchangers. An additional 12-solvent selection valve was connected to valve position A1 on the 1260 Infinity Binary pump. Together with the internal solvent selection valve of the 1260 Infinity Binary pump, up to 15 solvents could be screened using this system. The mobile phase was methanol and 10 mM ammonium formate titrated to pH 3.8 with formic acid. Water was used as a final weak solvent and to rinse the modifiers from the columns and allow proper column storage. Ammonium formate and formic acid were purchased from Sigma. Milli-Q 18 MΩ water was used.

Methanol was used throughout as a strong solvent and was obtained from Honeywell. Temperature was controlled at 25 °C, and flow rate was set at 0.4 mL/min. Agilent ChemStation version B.04.02 was used to control the instrument and process the data.

The compounds examined included nadolol, atenolol, alprenolol, acebutalol, pindolol, propranolol, metoprolol, and labetalol, which were all were purchased from Sigma Aldrich. Structures are shown in Figure 1. The pKa of these basic compounds ranged from 8.8 to 9.7. All samples were prepared at 10 mg/mL in DMSO and were diluted in water to a final concentration of 0.1 mg/mL.

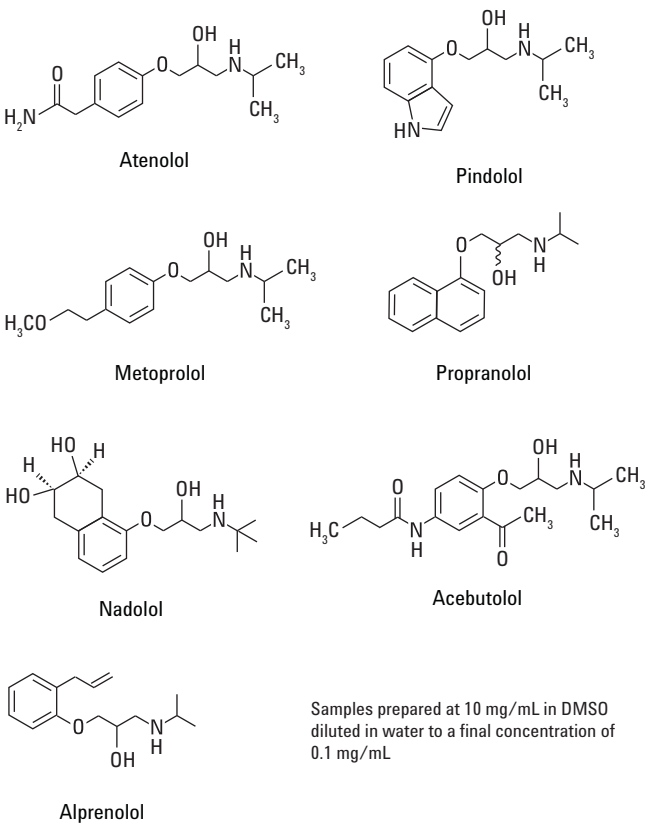

Figure 1. Structures of some beta blockers.

Column Choice to Enhance Selectivity

The columns were chosen to improve selectivity in the separation. They included a highly end capped column recommended as a first choice in method development (Poroshell 120 EC- C18); a non end capped C18 (Poroshell 120 StableBond SB-C18) that could have interaction with silanol groups, providing an alternative C18 selectivity using neutral to low pH mobile phases; a polar embedded amine column (Poroshell 120 Bonus-RP), and a phenyl-hexyl column (Poroshell 120 Phenyl-Hexyl). Phenyl bonded phases are known for their improved selectivity for aromatic compounds.

A polar-embedded group inserted into the hydrophobic C14 alkyl chain allows the Bonus-RP phase on totally porous Poroshell 120 to minimize interaction of polar samples with silanols, providing symmetrical peaks for a wide variety of applications. This phase is especially useful at neutral pH where amines can interact strongly with ionized silanols. The polar-embedded group also helps to wet the hydrophobic chains and prevents phase collapse in highly aqueous mobile phases.

Poroshell 120 Bonus-RP can be used for many of the same separations as a C18 column while avoiding some of the disadvantages of C18, such as poor wettability in high aqueous mobile phases. In addition, it is much more retentive for those molecules that can interact by hydrophobic interactions and also by H-bonding with the amide group. Compared to alkyl only phases, Bonus-RP has enhanced retention and selectivity for phenols, organic acids, and other polar solutes due to strong H-bonding between polar group (H-bond acceptor) and H-bond donors, like phenols and acids. Bonus-RP gives retention slightly less than a C18 allows for easy column comparison without the need to change mobile phase conditions. The Bonus-RP phase gives different selectivity than C18 for polar compounds. It is also compatible with 100% water.

The Phenyl-Hexyl phase has unique reversed-phase selectivity, especially for polar aromatics and heterocyclic compounds, derived from analyte interaction with the aromatic ring of the bonded phase and its delocalized electrons. Poroshell 120 Phenyl-Hexyl can be orthogonal to both C18 and Bonus-RP phases. More retention and selectivity will often be observed for solutes with aromatic electron-withdrawing groups such as fluorine or nitro groups [5,6].

Poroshell 120 Phenyl-Hexyl columns deliver unique selectivity for compounds with aromatic groups providing superior resolution for these samples. Poroshell 120 Phenyl-Hexyl can also provide optimum separations of moderately polar compounds where typical alkyl phases (C18 and C8) do not provide adequate resolution Acetonitrile tends to decrease the π–π interactions between aromatic and polarizable analytes and the phenyl-hexyl stationary phases, but methanol enhances those same interactions, giving both increased retention and changes in selectivity [7]. This does not mean that acetonitrile should not be used with a phenyl bonded phase or that it might not provide an acceptable separation, but methanol is more likely to deliver the additional selectivity that is desired from a phenyl phase.

Results and Discussion

As can be seen in Figure 2, the separation of all 7 compounds was accomplished on all columns surveyed. The Poroshell 120 EC-C18 column showed very close elution of acebutanol and propranolol and a double peak with the same molecular ion for naldolol. The double peak for nadolol was attributed to a diastereomer. The Poroshell 120 SB-C18 column delivered almost the same separation. This was not found with the Poroshell 120 Bonus-RP column, as it tended to minimize secondary interactions. The Poroshell 120 Bonus-RP column also reversed peaks 6 and 7 (propranolol and alprenolol), compared to the C18 columns. The Poroshell 120 Phenyl-Hexyl column shared this 6, 7 peak reversal and additionally reversed peaks 4 and 5 (metoprolol and acebutolol). The separation was similar to that shown by the other C18 columns but was not as retentive.

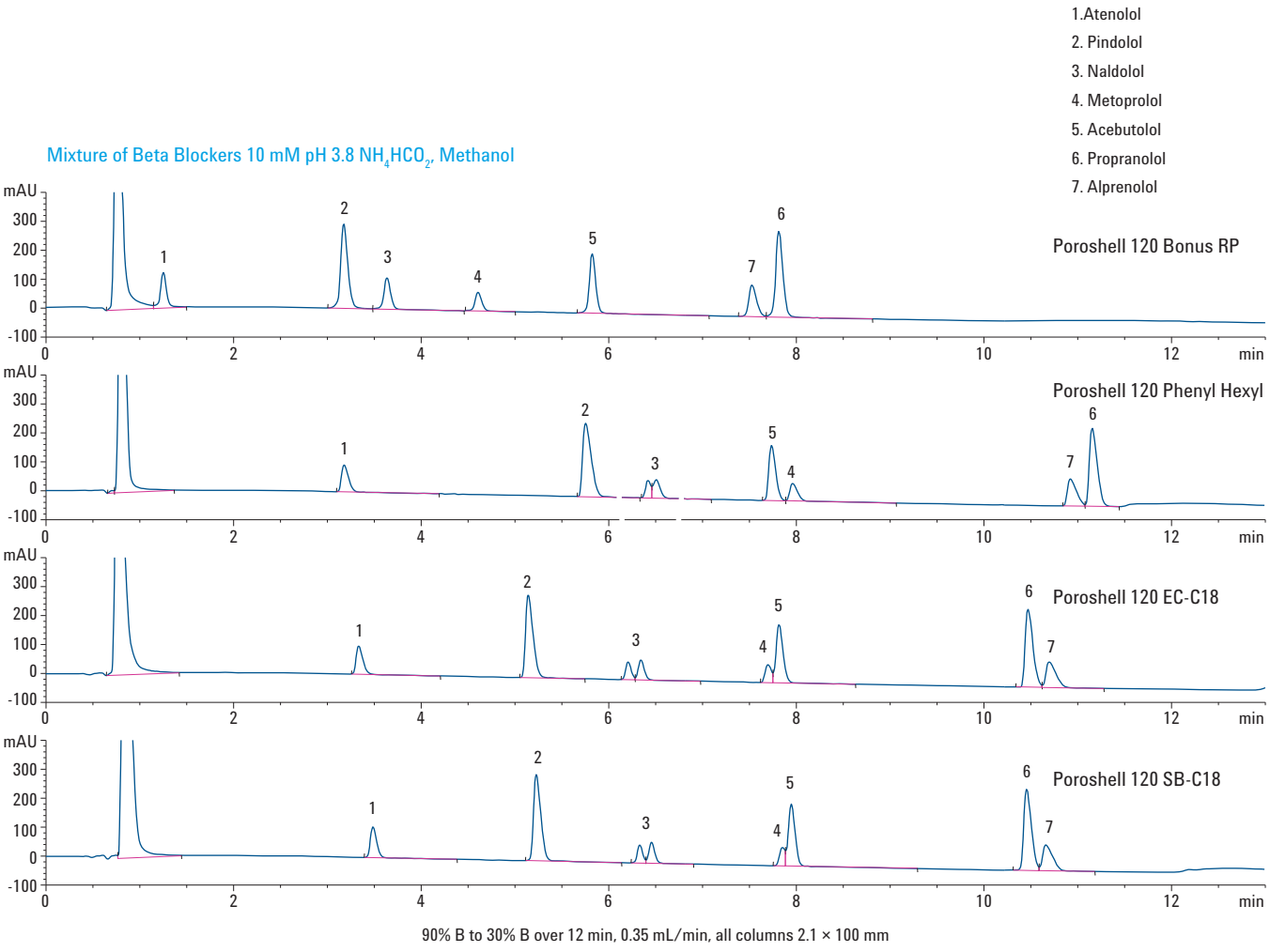

Figure 2. Separation of beta blockers using Agilent Poroshell 120 columns.

Conclusions

Analysis problems can be quickly resolved by including survey methods with generic gradients as part of the method development scheme. This work uses beta blockers as an example, and shows how phases with different selectivity can be used to optimize the separation. While the Poroshell 120 EC-C18 and SB-C18 columns provide adequate separation, using an alternative selectivity column, such as Poroshell 120 Bonus-RP, yields even better results and can be used for several thousand samples. Automatic setup of methods and sequences for the Poroshell 120 columns was straightforward using the Agilent 1200 Infinity Series LC Multi-Method Solution.

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