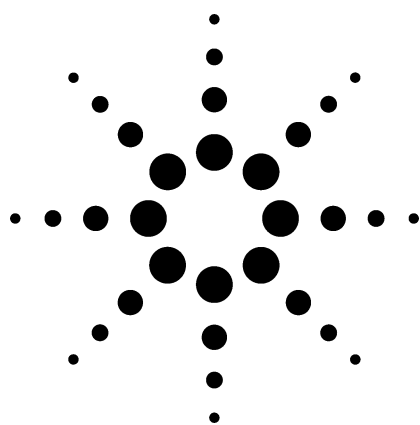




Rapid Analysis of the Beta Blocker Pindolol, using a USP Method with HPLC Columns Packed with Sub-2-Micron Particles

Application Note

Pharmaceutical

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Abstract

Pindolol is a beta blocker which helps to lower blood pressure and may possibly also alleviate depression. With potentially even more uses for this drug product it is important to develop faster analyses of it. Using pindolol and a USP method, this study demonstrates how use of short HPLC columns packed with sub-2-micron support particles (ZORBAX SB-CN (L10) columns) provide easy scalability to give faster, more-efficient HPLC separations.

Introduction

Beta blockers are a group of drugs used to treat hypertension (high blood pressure). They are named this because they block “beta receptors” on a cell membrane. There is evidence that beta blockers inhibit adrenaline from binding to receptors. Adrenaline is a hormone and neurotransmitter which, when released into the bloodstream, increases heart rate and vasoconstriction, and decreases blood clotting time. Blocking adrenaline reduces these effects, allowing the heart to work less strenuously. Pindolol (Figure 1) is thus a beta blocker helping to lower blood pressure.

Pindolol is also used to treat depression. Besides beta receptors, pindolol blocks other receptors which, when inhibited, cause an increase in serotonin in the brain. Serotonin, like adrenaline, is also a neurotransmitter. Low levels of serotonin are a symptom of depression. Again, blocking receptors increases serotonin level and possibly alleviates depression. However, clinical studies have had mixed results, indicating further investigation is needed to understand the relationship between pindolol and neurotransmitter receptors.

Ongoing Advances in the Pharmaceutical Sciences

Continuous advancement in pharmacological knowledge and new technologies often results in finding that drugs such as pindolol can treat more than one malady. These newly-discovered capabilities make drugs more popular; thus increasing need for more analyses. In turn, this creates need for more results in less time. New technologies in HPLC are part of the advancement in analytical ‘speed’: that is, in general there is a trend to faster, more efficient HPLC separations (higher throughput). This is made possible by use of smaller particles packed in shorter columns and specially designed HPLC systems to take advantage of this new column technology.

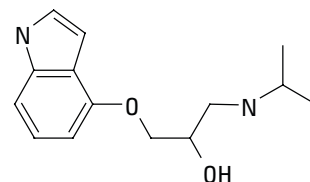


Figure 1. Chemical structure of pindolol.



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Many analytical techniques including HPLC, found in the *US Pharmacopeia/National Formulary* are revised and updated in its *Supplements*. For example, many older USP methods employing LC columns were originally designed with 10- μ m particles packed in 4.6 mm $\times$ 250 mm or 3.9 mm $\times$ 300 mm columns. Analysis times typically were 15 to 30 minutes. Many of these older methods have been revised using 5 μ m or 3.0 μ m to 3.5 μ m particles in 4.6 mm $\times$ 150 mm columns. The goal of this work was to speed up the USP method for pindolol by performing the analysis with 1.8 μ m particles packed in 4.6 $\times$ 50mm columns.

Scalability of Column Length

Scalability is the ability to change column dimensions for a particular method without causing an unacceptable change in chromatographic performance. Changing the column length and particle size of a HPLC method may not be as straightforward as it seems. Differences in silica particles, batch-to-batch variability, column manufacturing techniques, and even the HPLC instrumentation itself can influence the chromatogram perhaps producing results outside original method tolerances. All these factors may cause reluctance to try scaling or improving an existing method.

In this investigation we found the above concerns were alleviated when using ZORBAX StableBond SB-CN (USP LC column type L10) and an Agilent 1100 (reconfigured with a Rapid Resolution Modification Kit, part number 5188-5324), or an Agilent 1200 Rapid Resolution System, to scale the USP method for pindolol. The easy scalability of the ZORBAX SB-CN column series quickly improves the method by reducing analysis time and solvent usage while still maintaining sufficient resolution. These savings directly translate into increased lab productivity.

Figure 2 compares the original USP method [1] for pindolol analyzed with a 4.6 mm $\times$ 150 mm column packed with 3.5- μ m particles to an analysis performed using 1.8- μ m particles packed into a 50-mm column of the same diameter. Indole was used as the internal standard (ISTD). The bonded phase in both columns is ZORBAX StableBond SB-CN (USP LC column type L10). Notice that resolution is maintained, analysis time shortened, and sensitivity is increased in the shorter column. These three benefits are always realized when converting methods from older, larger particle technology to newer sub-2-micron technology.

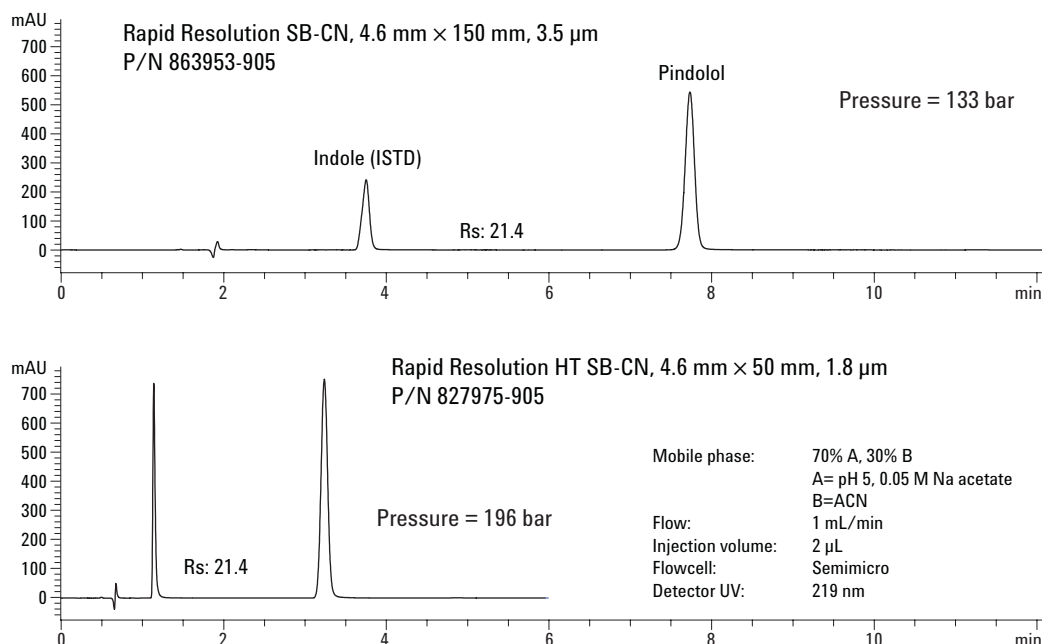


Figure 2. Scalability: Change column configuration to increase speed while maintaining resolution.

As an additional benefit: no adjustments or fine tuning of the method were necessary. Although, when converting to 1.8- μm particles, one would inherently surmise that system back pressure, ΔP , would increase due to smaller particles ($\Delta P \sim 1/d_p^2$ where d_p is average particle diameter). However, this potential increase in pressure is offset by the shorter column length and a proprietary engineered particle size distribution process which yields lower pressure than other sub-2-micron columns. In this experiment, pressure was originally 133 bar using the 3.5- μm 150-mm column; when the shorter 50-mm 1.8- μm column was employed, the pressure was 196 bar.

Determination of Impurity in Pindolol

A magnified view of the two pindolol chromatograms (Figure 3) highlights an impurity separated from pindolol. The USP monograph [1] states that no more than 0.5% each, ratio of impurity peak area to pindolol peak area, should be found and the total of all impurities cannot exceed 2.0%. Using SB-CN, an unknown impurity peak eluted after the pindolol peak. Areas of the impurity peak in the two chromatograms are the same (equivalent injections), but the peak height of the impurity analyzed by the sub-2-micron column is higher (increased sensitivity), making the impurity easier to detect and quantify. The impurity level was estimated to be about 0.2%, below the 0.5% maximum single-component impurity level stated in the USP method [1].

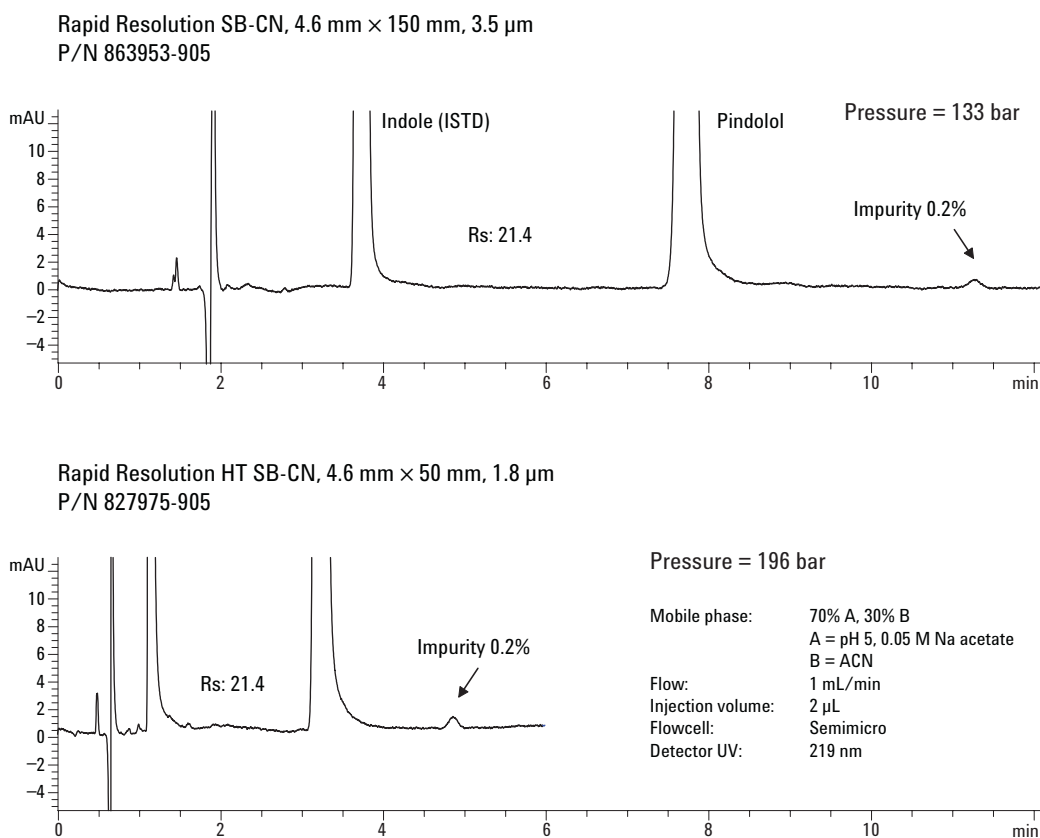


Figure 3. Scalability: Change column configuration to increase speed and sensitivity; estimation of impurity level.

Conclusions

Proven HPLC methods developed on 3.5- μ m or 5- μ m particles packed in longer columns can be scaled down by using shorter 1.8- μ m particle columns. Chromatographic benefits of this include: faster analysis time, increased sensitivity, reduced solvent costs, and maintaining the resolution of the original chromatogram. In this investigation, ZORBAX SB-CN columns were demonstrated to be scalable. Using an Agilent 1100/1200 HPLC system, simply substituting a shorter column for a longer one packed with smaller particles was all that was needed to scale down and improve the method, thereby increasing laboratory productivity. Many popular bonded phases are available on 1.8- μ m ZORBAX particles. StableBond-CN is a good example and choice for an L10 type column, especially for the USP pindolol method.

Products Used in this Application Note:

1. Rapid Resolution Modification Kit for Agilent 1100 Series Binary System, part no. 5188-5324 (for DAD).
2. Zorbax Rapid Resolution StableBond CN, 3.5- μ m, 4.6-mm $\times$ 150-mm, part no. 863953-905.
3. Zorbax Rapid Resolution HT StableBond CN, 1.8- μ m, 4.6-mm $\times$ 50-mm, part no. 827975-905.

Reference

1. United States Pharmacopeia (USP) 27-NF22, p. 1493.

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