Improved Throughput and Performance for the USP Propranolol Hydrochloride Assay using a Kinetex® 2.6 µm C8 Core-Shell Column

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The introduction of a new C8 phase to the Kinetex core-shell technology family allows for the ultra-high efficiency, speed and throughput benefits to be applied to methods requiring the use of a C8 (USP L7) column. The application of this technology to the USP assay for propranolol hydrochloride is shown to reduce analysis time while meeting the system suitability requirements for this USP monograph.

Introduction

The introduction of Kinetex core-shell columns has brought dramatic benefits to chromatographers. The ability to obtain ultra-high efficiency chromatographic separations on conventional HPLC systems with significant reductions in sample analysis time has been especially beneficial to laboratories tasked with the routine analysis of drug products. These laboratories typically have limited resources and can greatly benefit from faster separations and higher sample throughput.

Propranolol hydrochloride (propranolol) is a non-selective beta blocker mainly used in the treatment of hypertension. The USP monograph for the assay of propranolol hydrochloride specifies that a C8 (USP L7) HPLC column be used. Here we illustrate the benefits of the new Kinetex core-shell C8 column to reduce analysis time and increase productivity while meeting the system suitability requirements for the assay of propranolol hydrochloride.

Reagents and Chemicals

All reagents and solvents were HPLC or analytical grade. HPLC Grade methanol, acetonitrile, and water were purchased from Honeywell, Burdick & Jackson (Muskegon, MI). USP Propranolol Hydrochloride reference standard (RS) and Procainamide Hydrochloride reference standard (RS) were purchased from USP (Rockville, MD).

Equipment and Materials

Columns Used:
A fully-porous 5 µm C8 250 x 4.6 mm column (as specified by the monograph) was compared with a Kinetex 2.6 µm C8 100 x 4.6 mm column.

Instrumentation:
Agilent® 1100 Series HPLC (Agilent Technologies Inc., Santa Clara, CA, USA), equipped with quaternary gradient pump, autosampler, column oven, and variable wavelength detector.

Mobile Phase Preparation
To a 250 mL volumetric flask, 0.5 g of sodium dodecyl sulfate (SDS) was dissolved in 18 mL of 0.15 M phosphoric acid, 90 mL of acetonitrile and 90 mL of methanol was added, and diluted with water to the mark (250 mL) with water, mixed, and filtered (≤ 0.5 µm porosity).

Sample Preparation:
The stock solution (1 mg/mL) was prepared by dissolving an accurately weighed amount of USP Propranolol Hydrochloride RS in methanol. 5.0 mL of this stock solution was transferred to a 25 mL volumetric flask, diluted to volume with methanol, mixed, and filtered (0.45 µm). As a result, this solution contains about 0.2 mg/mL of USP Propranolol Hydrochloride RS. The resolution solution was prepared by dissolving procainamide hydrochloride in methanol to a concentration of about 0.25 mg/mL. 5 mL of this solution was transferred to a 25 mL volumetric flask along with 5 mL of the stock solution, diluted with methanol to volume, and mixed.

Propranolol HCl Assay Method:
The monograph calls for 20 µL of sample to be injected with isocratic chromatographic separation using the mobile phase as prepared above at a flow rate of 1.5 mL/min. The column temperature was maintained at ambient with UV detection at 290 nm.

HPLC Conditions

Column:	Fully porous 5 µm C8, (250 x 4.6 mm) Kinetex 2.6 µm C8, (100 x 4.6 mm)
Mobile Phase:	0.5 g SDS in 18 mL of 150 mM Phosphoric acid, 90 mL Acetonitrile, 90 mL Methanol diluted to 250 mL with water
Flow Rate:	1.5 mL/min
Temperature:	25 °C
Injection Volume	20 µL
Detection:	UV @ 290 nm
Sample:	1. Procainamide 2. Propranolol

Results and Discussion

System suitability requirements must be met before the assay of propranolol hydrochloride drug product samples can be valid. The USP assay of propranolol requires resolution to be no less than 2.0 between procainamide and propranolol, and the tailing factor for propranolol must not exceed 3.0. The monograph requires the analysis of a resolution solution to determine if the chromatographic system is suitable for use.

The assay of propranolol hydrochloride was carried out per the USP monograph using a fully-porous 5 µm C8 250 x 4.6 mm column, and the chromatogram of the resolution

solution containing procainamide and propranolol is shown in **Figure 1**. The separation was completed in less than 10 minutes with a resolution between procainamide and propranolol of about 23, which easily meets the system suitability requirement. Peak shape was also very good for propranolol and met the requirement for the USP tailing factor (no more than 3.0).

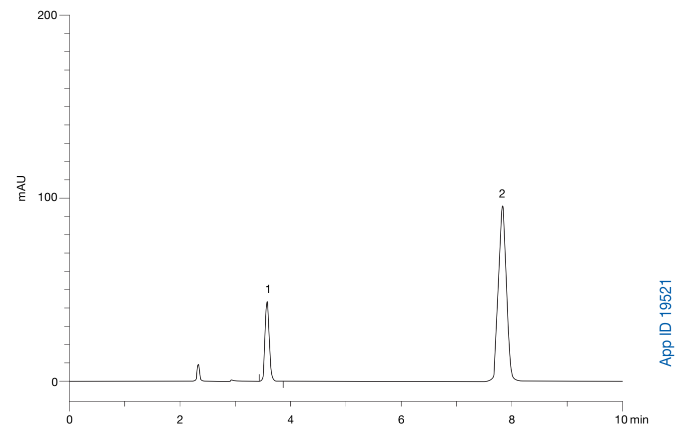
Analysis time is directly proportional to column length, so reductions in column length can result in significant decreases in chromatographic run time. However, efficiency is also directly proportional to column length, so any reductions in column length must be carefully considered to avoid loss of chromatographic resolution. A significant benefit of the core-shell particle technology is the often three-fold or greater increase in column efficiency for Kinetex® columns compared with fully porous 5 µm columns. This increase in efficiency can be utilized to reduce column length, resulting in faster separations without compromising chromatographic resolution; this easily offsets the decreased efficiency resulting from reductions in column length.

The assay of propranolol was performed on a Kinetex 2.6 µm C8 100 x 4.6 mm column using the mobile phase conditions (composition and flow rate) specified in the USP monograph **(Figure 2)** and illustrates the performance benefits provided by the Kinetex core-shell technology. Resolution between procainamide and propranolol was 14.5, well in excess of the minimum resolution of the required 2.0, and the peak shape for both propranolol and procainamide was also very good. The efficiency for the propranolol peak on the Kinetex column was 120,350 plates/meter (12,035 plates/column), almost two times greater than the efficiency on the fully porous 5 µm 250 x 4.6 mm column of 64,104 plates/meter (16,026 plates/column). The higher efficiency results in narrower, higher intensity peaks and would make determination of any low concentration impurities easier to achieve. It should be noted that this method was run using a conventional HPLC system and that higher column efficiencies, as much as 3x greater than obtained on the fully porous 5 µm column, could be obtained by reducing extra-column volume contributions (please inquire for system optimization tips).

The chromatographic separation was completed in just over two minutes. Compared with the fully-porous 5 µm 250 x 4.6 mm column, this separation was done in about 25 % of the time, representing a four-fold increase in throughput. An additional benefit was the reduction in the volume of mobile phase used. Since the separation was completed in about 2 minutes, only 2 mL of mobile phase was consumed for each assay, resulting in a savings of almost 8 mL compared with the fully-porous column.

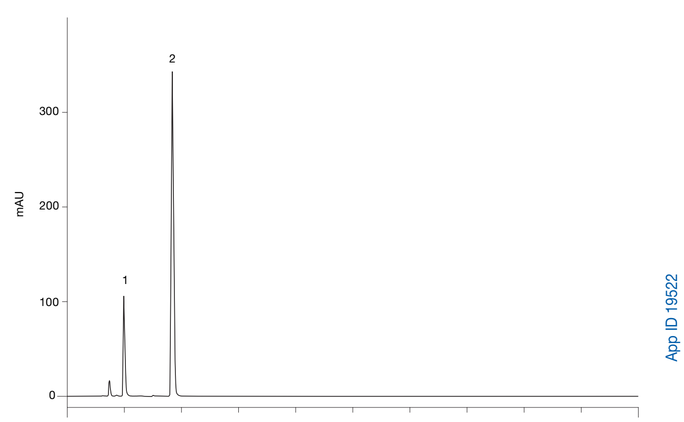
The higher efficiency provided by Kinetex core-shell technology columns can be utilized to reduce column length and deliver faster separations without compromising chromatographic performance. The shorter column length and smaller particle size are within the allowable adjustments as specified in USP General Chapter <621>.

Figure 1.
Resolution solution containing Procainamide and Propranolol on fully-porous 5 µm C8 250 x 4.6 mm column as specified in USP Monograph for Propranolol Hydrochloride



Column: Luna 5 µm C8(2)
Dimensions: 250 x 4.6 mm
Part No.: 00G-4249-E0
Mobile Phase: 0.5 g SDS in 18 mL of 150 mM Phosphoric acid, 90 mL Acetonitrile, 90 mL Methanol diluted to 250 mL with water
Flow Rate: 1.5 mL/min
Temperature: 25 °C
Detection: UV @ 290 nm
Backpressure: 186 bar
Sample: 1. Procainamide
2. Propranolol

Figure 2.
Resolution solution containing Procainamide and Propranolol using Kinetex 2.6 µm C8 100 x 4.6 mm column at 1.5 mL/min



Column: Kinetex 2.6 µm C8
Dimensions: 100 x 4.6 mm
Part No.: 00D-4497-E0
Mobile Phase: 0.5 g SDS in 18 mL of 150 mM Phosphoric acid, 90 mL Acetonitrile, 90 mL Methanol diluted to 250 mL with water
Flow Rate: 1.5 mL/min
Temperature: 25 °C
Detection: UV @ 290 nm
Backpressure: 348 bar
Sample: 1. Procainamide
2. Propranolol

Ordering Information

2.6 µm Analytical Columns (mm)

	30 x 4.6	50 x 4.6	75 x 4.6	100 x 4.6	150 x 4.6	SecurityGuard™ Ultra Cartridges‡ /3pk
XB-C18	—	00B-4496-E0	00C-4496-E0	00D-4496-E0	00F-4496-E0	AJ0-8768
C18	00A-4462-E0	00B-4462-E0	00C-4462-E0	00D-4462-E0	00F-4462-E0	AJ0-8768
C8	—	00B-4497-E0	00C-4497-E0	00D-4497-E0	00F-4497-E0	AJ0-8770
PFP	00A-4477-E0	00B-4477-E0	00C-4477-E0	00D-4477-E0	00F-4477-E0	AJ0-8773
HILIC	—	00B-4461-E0	00C-4461-E0	00D-4461-E0	00F-4461-E0	AJ0-8772
						for 4.6 mm ID

‡SecurityGuard Ultra cartridges require holder, Part No.: AJ0-9000.
Check for availability in your country.

2.6 µm MidBore™ Columns (mm)

	30 x 3.0	50 x 3.0	75 x 3.0	100 x 3.0	150 x 3.0	SecurityGuard Ultra Cartridges‡ /3pk
XB-C18	—	00B-4496-Y0	—	00D-4496-Y0	—	AJ0-8775
C18	00A-4462-Y0	00B-4462-Y0	00C-4462-Y0	00D-4462-Y0	00F-4462-Y0	AJ0-8775
C8	—	00B-4497-Y0	—	00D-4497-Y0	—	AJ0-8777
PFP	00A-4477-Y0	00B-4477-Y0	00C-4477-Y0	00D-4477-Y0	00F-4477-Y0	AJ0-8780
HILIC	—	—	—	—	00F-4461-Y0	AJ0-8779
						for 3.0 mm ID

‡SecurityGuard Ultra cartridges require holder, Part No.: AJ0-9000.
Check for availability in your country.

2.6 µm Minibore Columns (mm)

	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	KrudKatcher™ Ultra In-Line Filter /3pk
XB-C18	—	00B-4496-AN	00D-4496-AN	—	AF0-8497
C18	00A-4462-AN	00B-4462-AN	00D-4462-AN	00F-4462-AN	AF0-8497
C8	—	00B-4497-AN	00D-4497-AN	—	AF0-8497
PFP	00A-4477-AN	00B-4477-AN	00D-4477-AN	00F-4477-AN	AF0-8497
HILIC	—	00B-4461-AN	00D-4461-AN	00F-4461-AN	AF0-8497

KrudKatcher Ultra requires 5/16 in. wrench. Wrench not provided.

1.7 µm Minibore Columns (mm)

	50 x 2.1	100 x 2.1	150 x 2.1	KrudKatcher Ultra In-Line Filter /3pk
XB-C18	00B-4498-AN	00D-4498-AN	—	AF0-8497
C18	00B-4475-AN	00D-4475-AN	00F-4475-AN	AF0-8497
C8	00B-4499-AN	00D-4499-AN	—	AF0-8497
PFP	00B-4476-AN	00D-4476-AN	00F-4476-AN	AF0-8497
HILIC	00B-4474-AN	—	—	AF0-8497

KrudKatcher Ultra requires 5/16 in. wrench. Wrench not provided.

UHPLC / HPLC Sure-Lok™ High Pressure PEEK® Male Nut Fittings

Part No.	Description	Unit
AQ0-8503	Sure-Lok High Pressure PEEK 1-Pc Nut 10-32, For 1/16 in. Tubing, 12,000 psi (827 bar)	10/pk
AQ0-8530	Sure-Lok Fitting Tightening Tool, Aluminum	ea

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guarantee

If you are not completely satisfied with Kinetex® core-shell columns, send in your comparative data to a similar product within 45 days for a FULL REFUND.

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